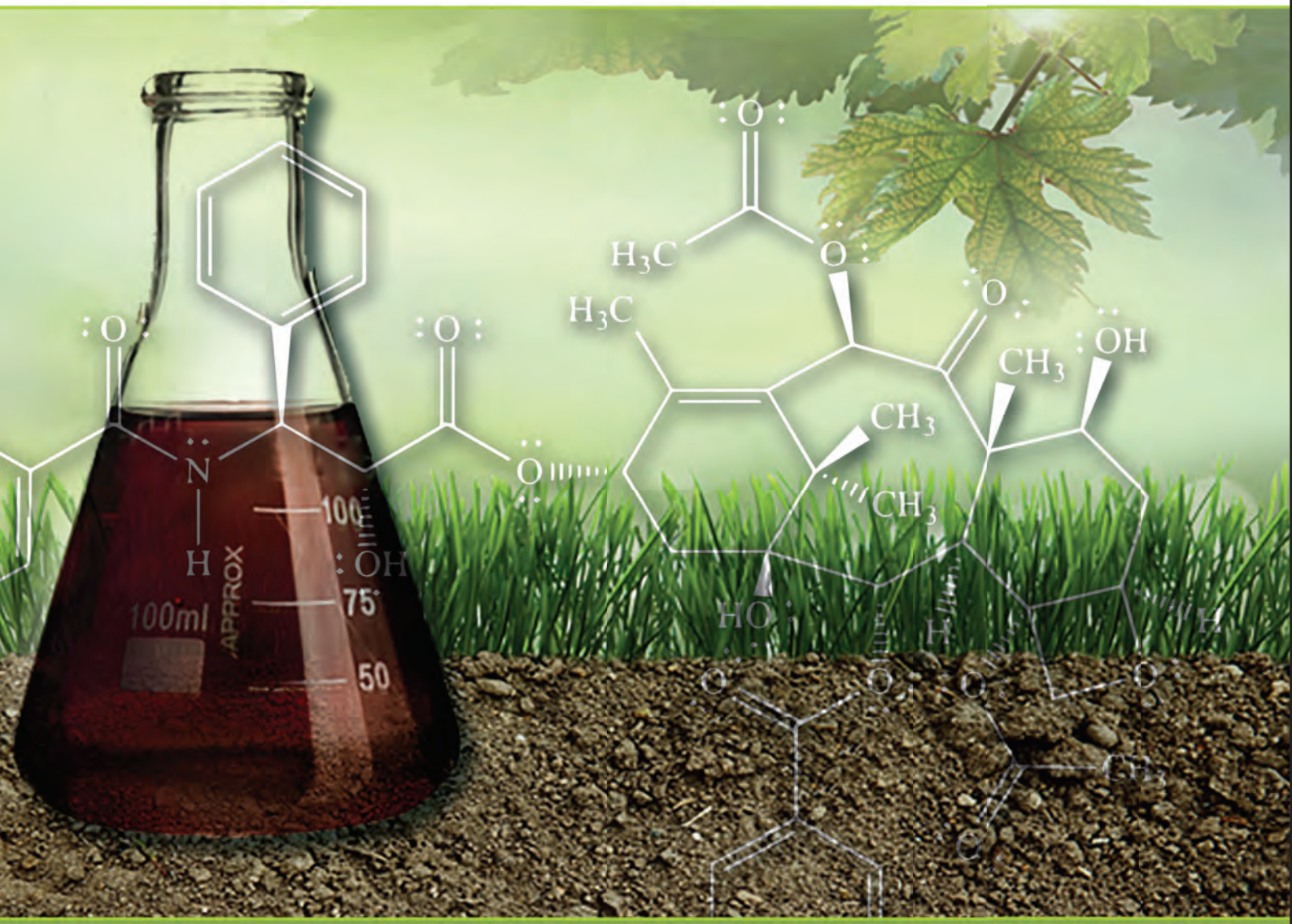
# 19th INTERNATIONAL CONFERENCE OF INTERNATIONAL HUMIC SUBSTANCES SOCIETY

## Humic Substances and Their Contribution to the Climate Change Mitigation

IHSS NATURAL ORGANIC MATTER RESEARCH

**16-21 September 2018**

Albena Resort **Bulgaria**




# BOOK OF ABSTRACTS

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## 19th International Conference

## of

## Humic Substances and their Contribution to the Climate Change Mitigation

16–21 September 2018
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Bulgarian Humic Substances Society
Sofia, 2018




















*Editors*
Prof. Dr. Ekaterina Filcheva
Members:
Prof. Dr. Maya Stefanova
Prof. Dr. Venera Tsolova
Assoc. Prof. Mariana Hristova
Assoc. Prof. Rossitza Ilieva

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*Dear participants, friends and explorers of humis substances, guests,*

On behalf of the Bulgarian Humic Substances Society and the Organizing Committee it is my pleasure to welcome all participants in the 19th IHSS conference. I cordially thank our sponsors and supporters without whom this conference would not be organized in this attractive and memorable way.

This conference will again draw attention of the world scientific community on humic substances and their immense significance for life of the Earth. Today, in the time of climate change, we will also focus our attention on their role in mitigating the negative consequences of human activity. All of us know of the indisputable evidence of phenomena (cataclysms) that have never been observed in the Earth's history, such as the rise in the temperature of the earth and the oceans, the rapid melting of the ice and the subsequent increase in the level of the water basins, their acidification and the extreme climatic storms that affecting all ecosystems and fresh water supplies. Despite a growing number of climate change mitigation policies, annual GHG emissions grew on average by 1.0 gigatonne carbon dioxide equivalent ($GtCO_2eq$) (2.2%) per year from 2000 to 2010 compared to 0.4 $GtCO_2eq$ (1.3%) per year from 1970 to 2000. Agriculture, Forestry and Other Land Use systems emit for about a quarter (~10–12 $GtCO_2eq/yr$) of net anthropogenic GHG emissions mainly from deforestation, nitrogen management and livestock (Intergovernmental Panel on Climate Change, 2017).

In the coming days, we will hear many new facts about humic substances and their modern role in preserving life on Earth, and we will be convinced of the ability of science to solve global problems.

I hope this conference to allow the participant to share ideas and experience, to facilitate establishment of new teams for investigation of humic substances and the application of new knowledge in practice.

I wish you a successful work and a pleasant stay in Bulgaria.

Presidents of the Organizing Committee and the Bulgarian Humic Substances Society

*Prof. Dr. Ekaterina Filcheva*

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# PLENARY LECTURES

# Bulgarian Humic Substances Society and an overview on humic substances research in Bulgaria

EKATERINA GEORGIEVA FILCHEVA

Institute of Soil Science, Agrotechnologies and Plant Protection "N. Poushkarov",
7 "Shosse Bankya", 1080 Sofia, Bulgaria
* E-mail: filcheva@itp.bg, efilcheva@abv.bg

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**Abstract**: The Bulgarian chapter of the International Humic Substances Society (IHSS) was established on December 2nd 1997 and officially registered in the Court on May 8th, 1998 as un independent body under the name Bulgarian Humic Substances Society (BHSS). The BHSS gathers scientists and specialists interested in humic substances (HS) of various nature and provenance, such as soil, coal, peat, sediments and organic wastes. The experts overview topics related to soil genesis, agriculture, horticulture, floriculture, ecology, geology, geography, medicine and industry. Fourth conferences with international participation were organized with main topics dedicated to the properties of humic substances, their ecological role and effect on soil fertility.

## I. Establishment and Activities of the Bulgarian Humic Substances Society

The Bulgarian chapter of the International Humic Substances Society (IHSS) was established on December 2nd 1997 and officially registered in the Court on May 8th, 1998 as un independent body named Bulgarian Humic Substances Society (BHSS). The initial number of members was 25. Nowadays 55 scientists belong to the Bulgarian Humic Substances Society (BHSS) and since 2008 some of them are also individual members of the Bulgarian chapter of the International Humic Substances Society, presently 32.

The Bulgarian Humic Substances Society enrolls scientists and specialists who are interested in humic substances (HS) of various nature and provenance, such as soil, coal, peat, sediments and organic wastes, and are involved in topics related to soil genesis, agriculture, horticulture, floriculture, ecology, geology, geography, medicine and industry. The studies concern the following main topics: a) characteristics of HS of different origin - soils, coal and sediments, composts, etc.; b) composition of soil organic matter and its implications in soil classification; c) organic carbon reserves in Bulgarian soils; d) theoretical studies on the initial humus formation in anthropogenic and artificial soils; e) development of products, methods and technologies to maintain and increase soil humus content; f) utilization of organic wastes and impact on natural HS; g) organic farming; h) management of initial humus formation using mineral and organo-mineral amendments; i) HS complexes with heavy metals and radionuclides in soils and sediments; j) development and utilization of organic and organo-mineral media for plant growth; k) development of national and international projects with the aim to maintain and increase soil humus, to utilize organic wastes, and to perform land reclamation; l) monitoring of humus state in Bulgarian soils.

The **first** Conference of the BHSS was held in Borovetz, Sofia region, 11-12 May, 2000. The papers of the first National Conference have delt with: (i) Characterisation of HS from coal, water and sediments; (ii) Bulgarian contribution to HS research; (iii) HS in soil classification and land evaluation; (iv) Development and utilization of organic and organo-mineral media for plant growth; (v) Changes in C, N and HS after different tillage systems, long-term fertilization and application of sewage sludges; (vi) HS formation during decomposition processes of plant residues and humification processes of low molecular weight organic compounds; (vii) Organic matter (OM) and the initial process of soil formation in reclaimed lands.

The Conference was jointly organized by the Bulgarian Tillage Society. There were about seventy participants; forty of them were members of the BHSS. During the first national conference of BHSS in May 2000 an overview of the research on humic substances in Bulgaria was made (Krastanov, 2000). As many as 52 papers (34 of members of BHSS) were presented at the first Conference.

The **second** conference of BHSS with international participation was held on 17-19 September, 2003 in Borovetz, Sofia region. More than 75 participants from different countries (Austria, Belgium, Bulgaria, Greece, Macedonia, Netherland and Russia) took part and have presented 52 papers, published in the *Journal of Ecological Science - Ecology and Future*, 2003.

Detailed review on "Tendencies and Perspectives of the Research on Humic substances - **World achievements and research in Bulgaria**" was published in *Journal of Ecological Science - Ecology and Future*, 2003 of the second national conference of Bulgarian Humic Substances Society with international participation held in September 2003 (Krastanov et al., 2003). Additionally, comprehensive discussions on the problems of the soil organic matter have been performed in the Working Group on Organic Matter (WGOM) at the EC. According to the already mentioned problems, the main tasks in the field of the soil organic matter study can be expressed as follows:

1. Assessment of the state of the organic carbon in the soils of the enlarged EU;
2. Highlight the multiple roles of organic matter in the soil;

3. Get a better insight in the processes resulting in organic matter depletion;
4. Formulate good management practices related to organic matter in the context of its multiple functions.
5. Recommend actions for areas where soil degradation is a risk.
6. Contribute to monitoring in the context of action.
7. Promote to research gaps and requirements in the field of organic matter.

Each one of these topics is rather broad, covering different aspects of organic matter fundamental, ecological, and agronomical research. On the basis of these tasks and on the world achievement in research of soil organic matter we try to appreciate its status in Bulgaria.

The brief overview on the fundamental research in Bulgaria shows up the theoretical model of the linkage of clay minerals and strongly bound components in the structural motive of soil adsorbent and humus stability (Raytchev, 1996; Raytchev, 2000, Raytchev et al., 2004; 2005a, 2005b; 2012).

A number of **agronomical researches** on long term experiments in Bulgaria have provided the most favorable conditions for humification of plant residues ensured by minimum and moldboard tillage (Stoinev&Dimitrov, 1998). The effect of these systems of tillage can increase remarkably if it is combined with intensive recycling of plant residues. Application of mineral and organo-mineral amendments (natural and modified zeolites, composts) has resulted in humus system stabilization and has allowed managing the humus formation processes and carbon sequestration in soils (Filcheva et al, 1998a).

**Ecological studies** were focused on the soil organic matter's role as substance, for blocking or transfering soil pollutants. An important accent was set on the organic matter solubility and eutrofication role in water basins as well as the erosion impacts the carbon pools and their redistribution in the terrestrial ecosystems.

The role of Fe $(OH)_3$ in creation of organo-mineral formations in the presence of unconventional ameliorants (coal dust, lime materials, etc.) in acidic and sodium saline soils was emphasized. These conditions were favorable for catalyzing of flocculation processes, which leaded to formation of "sandwich" charged colloids. Including $Cu^{+2}$ in these formations has decreased their toxic effect. Stabilizing of $Na_n[Fe(OH)]$ in sodium saline soils and including of Na in organo-mineral formations has blocked $Na^+$ ions inside the soil and has protected leaching of water soluble salts (Raychev, 1996; Raychev, 2000; Raytchev et al., 2012).

During the conference the effect of inadmissible practice of stubble burning in Bulgaria has been discussed. The presented results convinced the audience that straw is not a trash, but a valuable product that has to be incorporated into the soil and that the stubbles are potential source of nutrients and energy. They all support and manage biodiversity, and offer possibilities to improve soil fertility. Burning the stubbles results in decrease of soil organic matter in the humus horizon and it is well known that ***formation of humus needs a hundred of years.*** Additionally, burning the stubbles has the following negative effects on the environment: damage soil physical properties and make vulnerable soil surface to soil erosion; provoke health problems, especially for people with respiratory diseases, reduce visibility and increase the risk for transport accident, increase transboundary pollution and green house gas emissions.

Economical effect of plant residues utilization is: reduction of fertilizers application and the most important is soil preservation as **Resource of future (**Filcheva et al., 2005b**)**. In the last two decades scientists have paid special attention to the study of soil organic matter status after forest fires (Velizarova and Filcheva, 2001; Bogdanov et al., 2003; Velizarova et al., 2003; 2011; 2014).

The **third** conference with an international participation was held on the 12-16 September, 2011, Sofia and was dedicated to the studies on (i) Characterization and properties of humus substances in soils and coal, their biologic and therapeutic effect and methods for determination; (ii) Organic matter status of soils, the intensity of humus formation processes and possibilities for improving the carbon balance in soils; (iii) Composting; (iv) Humus degradation, including anthropogenic loss of humus, monitoring of critical areas; (v) study on Technosols - Organic matter supply in reclaimed Technosols in Bulgaria, Organic carbon pools in reclaimed Technosols from Chukurovo mine region, Technologies for reclamation of Coal Mining disturbed lands in the Mini Maritza Iztok region, heavy metals in the organic matter fractions without humus application (Etov and Slavova, 2011; Tsolova et al., 2011a, 2011b; Hristova et al., 2011); (iv) Composting and utilization of the composts (Beloev et al., 2011a, 2011b; Petkova et al, 2011); (vii) Evaluation of the soil humus content cultivation of different crops (Krasteva, 2011) and, spatial variability of soil organic matter in monitoring network on acidic soils (Koutev and Katsarova, 2011).

In this conference interesting research on the topic "Industrial humus substances –properties, technology and utilization", were presented by Atanassova, (2011); Valchinkova, (2011); Nankova and Nankov, (2011); Pachev et al., (2011); Savov et al, (2011). Of special concern were results on humus substances utilization.

New data on the lipids compositions in soils and lignites humic matter were presented by the members of BHSS (Atanassova et al, 2011; Stefanova et al., 2011) and subsequently published in the 3rd Conference of BHSS proceeding.

During the conference an excursion to Dobroudja Agricultural Institute in the town of General Toshevo was organized. There the main sponsor of the meeting company ROMB (Research Organization and Managing of BioProducts) demonstrated an industrial method for preparation of enriched lignite humic acids suitable for application in agriculture, floriculture as well as for bioremediation of contaminated soils with heavy metals and Mine Industry waste.

The **fourth** Conference of BHSS and Bulgarian chapter of IHSS with international participation was held on 8-10 September, 2016 in Sofia. More than 50 participants from different countries (Bulgaria, Greece, Malta and Russia) took part at

the Conference. 26 papers, 30 presentations and 23 posters, were grouped in the following main topics: (i) Organic carbon in soils and its contribution to mitigating the effects of climate change; (ii) Characteristics and functions of humic substances; (iii) Method of research, evaluation, and increase of the soil organic matter; (iv) Application of humic and "humic-like" products in agriculture and industry.

During the 4th Conference of BHSS two papers concerning chemical hallmarks of Black carbon dressed soils were presented (Tsolova et al, 2016a, 2016b). Overviews on the methods of research and evalualition and increase of the soil organic matter, utilization of composts in agriculture and its role for decreasing of heavy metals to vegetables were presented (Angelova et al, 2016; Babrikova et al, 2016; Popova et al., 2016; Stefanova et al, 2016).

From agronomical point of view during the 4th Conference an interesting comparative study on the influence of biological and conventional systems of agriculture on some characteristics of soil organic matter was presented (Nankova et al., 2016). The results provoked a thorough discussion between participants.

An interesting part of the 4th Conference of BHSS has been the topic 4: Application of humic and "humic-like" products in agriculture and industry. The main presentations have covered different topics in agriculture (Chakalov et al., 2016; Nankova et al., 2016; Pachev et al., 2016; Savov et al., 2016).

Proceedings of the 4th Conference, including 484 pages of reviewed papers under the edition of Filcheva, Stefanova and Ilieva**,** were prepared for the conference participants.

Conference scientific tour in the region of Sredna Gora Mountain was devoted to detailed description and discussions of three soil profiles and land reclamation of copper ore mine:

- Profile 1 – is classified as Brown forest soil (Bulgarian classification); Dystric Cambisol (Humic, Siltic) (WRB 2015)
- Profile 2 – is classified as undeveloped Leached Cinnamonic forest soil (Bulgarian classification); Epidystric Cambisol (Siltic) (WRB 2015)
- Profile 3 – is shallow Brown forest soil, severely eroded with rock fragments; Eutric Sceletic Litic Leptosol (Humic) (WRB 2015)
- Land reclamation in "Asarel-Medet" of copper ore mine with humic substances" with different tree species was demonstrated. "Asarel-Medet" JSC is the first and the biggest Bulgarian open pit mining company for copper and others ores processing with the practice of terrains remediation by humic substances application.

**II. An overview on humic substances studies performed by the members of BHSS**

The study on humic substances in Bulgaria started in the 60s of the previous century with the research of the Bulgarian scientist S. Krastanov. The first collection of humic acids, isolated from different soils of Bulgaria, has been prepared and main characteristics of the humic acids were published in the 70s (Krastanov, 1968; Krastanov et al, 1973). Later on studies on the characteristics of humic acids from different Bulgarian soils were published (Artinova, 1977; Filcheva, 1976a; Konishev, 1978). Statistically generalized data on the soil humic acids characteristics (O:C and H:C) gave ground to classify Bulgarian soils into two main groups - izohumic and texture differentiated soils (Krastanov and Filcheva, 2014).

Nowadays there is a global trend for a gradually replacement of chemical fertilizers by natural products. Humic substances are promising raw material as they naturally occur in high amounts in Bulgarian lignites (Stefanova et al., 1993). The pathways of their formation, content and characteristics in initial and naturally weathered coals were studied by Markova (2000, 2002). Likewise was characterized organic matter, i.e. humic substances of Hunt Peninsula, Livingston Island from the vicinity of the Bulgarian Antarctic Base (Sokolovska et al., 1996; Ilieva et al., 2003; Markova et al., 2007; 2015; Kostova et al., 2015, Sokolovska et al., 2015) and from lignite microbial biodesulphuriazation (Gonsalvesh et al., 2013), organic matter composition was analyzed in composts, sediments and litter (Filcheva and Tsadilas, 2002; Filcheva and Yorova, 2003; Filcheva and Yurukova, 2004, 2005; Filcheva et al., 2003; Tsadilas et al., 2000).

Lignites humic acids have a potential for an economically sustainable exploration in different domains, i.e. agriculture, horticulture, cosmetics, drilling industry, filter systems products, etc. For the purpose they should be properly characterized. The entire range of contemporary instrumental techniques has been used for their analyses. Soluble organic matter was predominantly characterized by GC/MS (Stefanova and Marinov, 2010; Stefanova et al., 2010) while the residue after extraction was subjected to different pyrolytic destructions (Stefanova et al., 2011). Techniques as thermochemolysis (Stefanova et. al., 2011) and reductive pyrolysis have been applied (Stefanova et al., 2003). Results have demonstrated that despite of qualitative similarity in humic acids pyrolysates quantitative data interpretation has depicted some structural peculiarities in samples studied depending on their parent materials (Stefanova et. al., 2014). It was proposed pyrolysis data for HAs of different provenances to be designed in a rectangular "spider web" diagram (Stefanova et al. 2016a, 2016b). Atmosphere Pressure–Temperature Programmed Reduction (AP-TPR) coupled "off-line" with thermal desorption gas chromatography-mass spectrometry (TD-GC/MS) could be effectively used to assign qualitatively/quantitatively organic functionalities in humic substances of different origin.

Company ROMB LTD, 2002, has imported from the Russian Confederation industrial quantity of leonardite' extracted humic substances. In collaboration with colleagues from the University of forestry, Sofia scientists have implemented a method for biological reclamation of dumps situated near "Asarel Medet JSCo", Panagyurishte (personal communications). Afterwards this method was developed in two projects in cooperation between the ROMB Ltd and

NIPRORUDA JSCo (Innovative design -73/05) and Balkan Plant Sciences Ltd. (Project № BG161PO003-1.1.06-0101-C0001). In model experiments under *Acidophilous microflora* suppression through treatment of oxidized rocks with biosoluble humic substances a possibility for bioremediation of waste piles was established. Scientists of the ROMB LTD, 2002 company have proposed methods and technologies for bioactive humic substances production. They were realized through enrichments of raw materials with physical (triboelectric separation) and biotechnological methods (Angelova et al., 2009, 2012; Chakalov et al., 2010, 2011; Popova et al., 2006; 2016). As a result, a liquid for plants treatment was produced (trade mark PLANTAGRA reg. in BFSA 0043). It is available in six modifications, incl. for field crops, vegetables, ornamental species (flowering and landscaping plants), for horticulture and organic farming. The latest product was developed in cooperation with Balkan Plant Sciences LTD. It is biodegradable by microorganism's leonardite (Project № BG161PO003-1.1.06-0101-C0001). The products of this series improve grain quality when growing corn hybrids and increase yields. It is successfully used in flower growing in a pot and hydroponic growing plants (Chakalov et al, 2008, 2011, 2012; Savov et al., 2008, 2009, 2011; Valchinkova et al, 2012).

Company ROMB Ltd. manufactures and offers accelerators for plant composting and other organic materials (Popova et al., 2008). These materials are used to develop a method for remediation of organic waste. The company, together with the Institute for Land Reclamation and Agricultural Mechanization - Sofia (Nowadays ISSAPP "N. Poushkarov), were awarded a gold medal at the AGRA' 2010 exposition.

Company ROMB Ltd produces humic and fulvic acids, capable for blending in liquid fertilizers for hydroponics of Canadian company Advanced Nutrients Ltd (personal communications). Studies for isolation of new strains able to transform leonardite into new products for agricultural and ecological application are in progress.

During 2002-2008 a study on the organic carbon stocks and first attempt to develop GPS maps on organic carbon reserves of the country were carried out. Maps were published in 2014 -2015 (Filcheva 2004a; Filcheva, 2015; Filcheva et al., 2002, 2007a, 2008; Rousseva and Filcheva, 2001; Zhiansky et al., 2009).

Recently a detailed research on the loss of organic matter due to soil erosion and erosion control practices to limit it has been published by Rousseva and Marinov, 2014.

This thorough going study on the soil organic matter, incl. micromorphology, gives the possibilities to define and track major diagnostic criteria, characterizing the state of soil organic matter, to assess the amount and degree of transformation of organic residues, the activity of soil biota, quantity and humic plasma condition. In addition with analytical information - quality and quantity of soil organic matter, the study provides more complete and realistic picture of the biogenic processes in soils. This is an important and necessary precondition for modeling, controlling and optimization of humus formation processes (Ilieva, 1986; 1989, 1994, 1995, 1998, 2003; Ilieva and Grozeva, 1994a, 1994b, 2003; Ilieva and Kolchakov, 1996; Illieva and Filcheva, 2012; Kolchakov and Ilieva, 1995; Ilieva et al., 1995; Lucot et al, 1997; Shishkov et al., 2010; Teoharov et al., 2009).

During the last couple of years our attention has been focused on the destructive and nondestructive method for soil organic matter study. Chemical, optical and spectral methods assure comprehensive view of the soil organic matter quality. Quality and quantity of humus is completely and accurately determined using destructive (chemical) methods. Optical studies provide a picture of the state and distribution of organic matter in the soil profile. Spectral characteristics in the visible and near infrared region (NIR) give supplimental information and enrich the data. Likewise, depending on the aims of the study (application) we can use different method or combination of them for quick and monitoring purposes - spectral methods for basic tasks and for specific tasks - chemical and optical methods (Ilieva and Iliev, 1997; Ilieva and Filcheva, 2013, 2014; Ilieva et al., 2016; Todorova et al, 2011).

Articles on the content and composition of soil organic matter in different crop rotations, fertilization, tillage systems and land use were published (Mitovska, 2000; 2014; Nankova, 2014; Pachev, 2000; Pachev, 2014; Filcheva and Mitova, 1997, 2002; Mitova and Filcheva, 1996; Mitovska and Filcheva, 2011; Nankova and Kirchev, 2000; Filcheva et al., 1987; Goushevilov et al., 1999; 2000; Nankova et al., 2003; Zhiansky et al., 2008). Detailed research and discussions on the transformation of plant materials and manure with different C:N and C:P, as well as mechanism of mineralization processes of organic substances and its model and humus formation processes were published in numerous papers generalized and detailed analyzed in the monograph "Soil organic matter and fertility of Bulgarian soils" (Mitovska, 2014; Jecheva, 2000; Sidibe, 1990;Mitovska et al., 1995; Petkova, 1994, 2001a, 2001b; Petkova and Mitovska, 2001, 2011). The authors have determined the rate of mineralization and humification of manure in dependence of applied fertilization norm. Data will serve as a base for evaluation of manure effectiveness on the accumulation of humus in soil. On the basis of some Bulgarian soil types the insert of organic fertilizers in the fraction of humic acids is demonstrated. The authors have expressed the opportunities for efficient utilization of all natural sources as farmyard manure, poultry manure. Organic materials valorization, incl. leaves, stems and roots of the residual production of crops and various composts of organic material, as stocks of humus in the soil was also described. Periodical application of N-containing organic materials along side with mineral fertilization ensures the preservation of the natural composition of N in soil organic matter and its mobility. It provides conditions for a positive balance of soil organic matter by keeping a balance between the processes of mineralization and humification. This good agricultural activity results in sustainable use of soil and provides stable and high quality yields. Studies on the nitrogen mineralization potential and its importance for evaluation of soil fertility were carried out by Koutev and Ikonomova, (1996) and Koutev (2014).

Soil organic matter is a great variety of mixtures - organic and organo-mineral compounds. The interim decompositional products well known as “detritus” take part as an intermediate state and it is very complicated to be separated. This is a very small part of organic residues which is difficult to be separated from the soil mass. The last achievments on detritus investigation were recently discussed in a monograph by Pachev (2016).

The major part of publications published by the members of BHSS pertained to the content and composition of soil organic matter. The research was carried out on different soil types and subtypes. The first publication on this topic was the PhD thesis of the late Prof. Krastanov (1968) and articles in different journals (Krastanov and Kabakchiev, 1979; Krastanov et al., 1972; 1973; 1985). Studies of many authors during the last decades of the previous century and recent have contributed to the research on soil organic matter characteristics of Bulgarian soils (Artinova, 1977, 1978, 1997, 2012, 2014; Artinova and Gribachev, 1983; Artinova and Moshev, 1987, 1988; Artinova and Grozeva, 1989; Artinova et al-in Press; Boyadgiev et al, 1994; Grozeva and Petrova, 1994, 1995, 1999; Filcheva, 2003a, 2003b, 2004a, 2004b, 2004c, 2004d; Filcheva and Ilieva, 2014; Filcheva and Malinova 2015a, 2015b; Filcheva et al, 1997; 2013; Sokolovska, 2002; Sokolovska et al., 2005**;** Konishev, 1978; Petrova and Trichkov, 1992; Shishkov and Kolev, 2014; Petrova and Shishkov, 1998; 2001; 2005; Shishkov and Petrova, 1999; 2001, 2003, 2006, 2007; Shishkov and Filcheva, 2000; 2009, 2010; Petrova et al., 1999; Shishkov et al., 2002, 2011, 2014; Teoharov and Filcheva, 1994, 1995, 1997, 1998, 1999; Teoharov and Petrova, 1998; Dimitrov et al., 2014; Teoharov et al., 1994, 1995; Hristov et al., 2009; 2016; Andreeva et al., 2011, 2012; Zhiansky et al., 2004; 2011)

On the base of generalized soil profiles (virgin, arable, under forest) of the main soil types and subtypes Bulgarian soils were classified (grouped) according to (i) SOM nature; (ii) SOM composition (ratio Ch/Cf - indicator for soil humus state); (iii) soil system stability (Filcheva, 2004d, 2007a, 2014, 2015). Other articles and monographs have delt with the utilization of organic matter content and composition as indicators for soil genesis and classification (Filcheva et al, 1998b; Filcheva, 2004c, 2004d, 2007a, 2014, 2015; Hlestkova, 1991; Orlov, 1985; Achkov et al., 1989; Petkov et al., 1989; Filcheva and Boyadgiev, 1995, 1997; Hubert et al, 2008).

Since 1989 studies on the initial process of humus formation in reclaimed lands in different regions in Bulgaria have been published (Bech et al., 2002, 2005, 2012; Hristov, 2000; Banov et al., 1989, 1994; Banov and Marinkina, 2000; Gencheva and Filcheva, 1995; Gencheva and Filcheva, 2000; Filcheva et al., 1998c, 2000; Ivanov et al., 2007; 2012; Markova et al., 2016; Noustorova et al, 1998; 1999; Petrova et al., 1999; Tsolova and Banov, 2012; Tsolova et al., 2014; Zheleva and Bozhinova, 2011). An overview of the technologies for reclamation of coal mining disturbed lands in the mini Maritza Iztok region was published in the proceeding of the 4$^{th}$ Conference of BHSS 2016 (Etov and Slavova, 2016).

An important research on the methodological aspects of soil organic matter has been carried out since late 70s of the previous centure (Filcheva, 1976a, 1976b; 2004c, 2007a, 2015; Raytchev and Filcheva, 1989; Filcheva and Boyadjiev, 1997; Filcheva and Malinova, 1995; Filcheva and Nikolova, 1989; Ilieva and Filcheva, 2016; Filcheva and Tsadilas, 2002; Filcheva et al., 1995; 2005a; 2011a, 2011b, 2016, 2017a; Filcheva et al, 2018 – in Press; Hegymegi et al., 2007; Krastanov et al., 1986; Petrova et al., 2003; Raytchev et al. 1993a, 1993b, 2005c; Rousseva and Filcheva, 2000; Spiege et al., 2007; Uzunov et al., 1995; Hristova et al., 2016).

As a new point of research of BHSS is the study on the biofilm formation between *Bacillus Subtilis* and *Escherihis Coli* strains as a model system for monitoring of soil process under conditions of low amount of humic substances (Ganchev, 2016).

The ecological role of humus substances for sequestration of heavy metals from polluted soils is also appreciated (Filcheva, 2004d, 2007b, 2014, 2017b). The mobility of metal-organic compounds was analyzed using different extraction procedures. The results obtained show a linear correlation between total content of heavy metals, their content in the fractions of dissolve organic matter, EDTA and $CaCl_2$ extracted amounts. Data can be used to calculate the extracted quantities of the elements in each of the fractions in the presence of one of them, and those with $CaCl_2$ and EDTA (pH 4.6 and 7) - available forms of these elements. However, each metal forms complexes with organic substances differ in their stability (Filcheva et. al., 2011a, 2011b; 2012, 2017).

In conclusion, the overview on the activities performed in Bulgaria ascertains that during the last decades many scientists have contributed to accumulate data and knowledge with a significant input to the science of humic substances and their application in agriculture, in practical and scientific aspect.

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# Can humic products substantially improve ecosystem quality and economic yield?

D. C. OLK [(a)]*, DANA L. DINNES[(a)], J. RENE SCORESBY [(b)], CHAD R. CALLAWAY [(c)], JERALD W. DARLINGTON [(b)]

(a) USDA-ARS, National Laboratory for Agriculture and the Environment, Ames, IA, 50011, USA
(b) Minerals Technologies, Inc., 622 Third Avenue, 38th Floor, New York, NY, 10017, USA
(c) Ag Logic Distributors, Conrad, IA 50621, USA
dan.olk@ars.usda.gov

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**Abstract.** Humic products have been used in cropland agriculture for several decades, but lack of widespread credibility has restricted their use to small proportions of farmers. Key knowledge gaps concern spatial and temporal variability in their field efficacy, a mechanistic explanation for plant responses to humic products, industry-wide measures for quality control of product formation and sale, and the lack of long-term field trials to evaluate soil health responses. As these gaps are closed, we believe humic products will serve multiple roles in agriculture and land management. These roles could include enhanced crop economic yield--especially in the presence of environmental stresses, improved soil health, and multiple contributions to better environmental quality, including diminished flow of N and P into the atmosphere and water bodies, oil spill remediation, and decontamination of heavy metals and anthropogenic organic pollutants. We call for greater coupling of the basic research needed to verify these concepts with field-scale application of humic products.

## Introduction

Humic products have been commercially available for several decades and have been applied to modest proportions of production fields to increase crop growth and economic yield. Market research reports generally predict ongoing growth for biostimulants as a group and humic acids specifically in coming years, driven in part by the desire to make more efficient use of mineral fertilizers in face of increasing costs. We have also noted increased discussion among agribusiness concerns of humic product performance. Yet their validity and general reliability have remained in question, drawing criticism from the research community and government agencies for a lack of factual basis. Due in part to these uncertainties, mainstream farmers have not yet adopted humic products as a conventional input.

Given the current situation, this manuscript evaluates the potential for humic products to broadly promote crop economic returns and enhance other ecosystem services. It will first summarize four key knowledge gaps that have constrained growth in humic product use and describe ongoing efforts to close these gaps. Second, this manuscript will summarize recent results from our field evaluations of humic products on fertile soils in Iowa, located within the Corn Belt of the United States. These results suggest that long-term humic product use might lead to not only increased crop economic yield but also improved soil health and further ecosystem benefits, which will be described in the final sections together with other potential ecosystem benefits suggested by independent research programs or field observations. This review is limited to humic product use in cropland agriculture and other land applications. Humic products can also be used in cosmetics, nanotechnology, petroleum oil industry, and are occasionally offered as a human health product. These uses will not be addressed here, due to a paucity of peer-reviewed publications.

## Four key knowledge gaps for widespread humic product use

For humic products to substantially improve global agricultural yields and improve ecosystem quality, they must first be adopted for regular use by mainstream farmers, which is currently not the case. Toward that objective, specific challenges must first be surmounted. Olk et al. (2018a) discussed four key knowledge gaps that have slowed growth of humic product use, and they are summarized in this section.

Spatial and temporal variability in field efficacy of humic products. Numerous studies have demonstrated significant crop responses to humic products, and they are reviewed by Visser (1986), Chen and Aviad (1990), Calvo et al. (2014), Rose et al. (2014), Canellas et al. (2015), and Olk et al. (2018a). Yet there lacks discussion of spatial or temporal variability in humic product efficacy. Even the most effective agricultural inputs vary over time and space in their promotion of crop growth, due to interactions with such factors as crop type, annual weather pattern, landscape position, soil type, tillage, and economic yield level (O'Halloran et al., 1985; Dinnes et al., 2002; Page et al., 2005; Wang et al, 2009). Interest in defining these spatial and temporal variabilities to maximize input use efficiency while minimizing environmental degradation led to the science of precision farming, which has had tremendous impact on fertilizer and pesticide management. Crop type is of obvious and paramount importance in determining optimal application rates of any input.

Published studies of humic product efficacy have addressed only a limited array of environmental conditions. Many were conducted in greenhouse or growth chamber conditions, with generally optimal temperature, light, and moisture conditions. We are unaware of any published database on crop responses to humic products under two or more

environmental/management factors simultaneously in field conditions. There has been little scientific dialogue on how environmental and management factors might alter humic product efficacy. Hence, there is the need for multi-year and multi-location field studies that maintain treatments and uniform sampling procedures and analyses across years in order to determine the consistency of humic product effect across different crops, soil types, management practices, and annual weather patterns.

Mechanistic explanations of humic product effects. A mechanistic understanding of plant responses to humic products would improve the prediction of when and where crop production would most benefit from humic products, including their interactions with other crop inputs. It would enable development of better products. The most commonly proposed explanations can be grouped into themes of soil nutrient availability, other soil properties, direct plant biostimulation, and microbial processes. All explanations must be consistent with the miniscule amounts of organic matter added to soil via recommended rates of humic product application (often less than 1 kg $ha^{-1}$) compared to the tens of thousands of kg of native humus already found in the soil. This disparity speaks against claims that humic products add to the favourable benefits already provided by soil organic matter for crop growth.

Researchers have largely focused on plant-based mechanisms for stimulating growth, as reviewed by Khristeva (1968), Visser et al. (1986), Nardi et al. (2002), Zandonadi et al. (2013), Berbara and Garcia (2014), and Calvo et al. (2014). Multiple plant processes have been affected by humic products or humic substances extracted from soil, including enzyme activity, protein metabolism, photosynthesis, respiration, and uptake of water and nutrients, and the underlying mechanisms have long been conjectured to involve hormone fluxes, the hydroxyproline:proline ratio, cell membrane permeability, electron chain transport components, free radical activity within the humic structure, and reactive oxygen species in plants (Vaughan and Malcolm 1985; Vaughan et al. 1985; Vaughan 1986; Visser 1986; Berbara and Garcia 2014; Calvo et al. 2014). More recent research has addressed activity of the ATPase enzyme (Zandonadi et al. 2013; Calvo et al. 2014; Canellas and Olivares 2014). Some arguments exist for increased Fe availability as the underlying cause (Chen et al. 2004; Aguirre et al. 2009). Limited effort has been directed at possible roles of microorganisms in plant responses, whether they are located in the soil, rhizosphere, or plant. Mechanistic studies have been conducted on plants grown under controlled conditions, though, which do not replicate the variable conditions and multiple stresses that routinely impact crop growth in field conditions.

Stress alleviation has been conjectured as a primary means through which humic products best express their benefits to plant growth (Zandonadi et al. 2013; Calvo et al. 2014). Several studies showed humic products induced greater plant resistance to saline soil (Calvo et al., 2014; Tuncturk et al., 2016; Kaya et al., 2018). However, nearly all studies were conducted in controlled conditions, while Bacilio et al. (2017) reported that growth chamber-based evidence for salinity tolerance was far less marked in a greenhouse setting and almost non-existent in the field. Anecdotal evidence exists for enhanced plant tolerance to cold. Based on the disturbing results of Bacilio et al. (2017), we call for more process-level research to be conducted on plants grown in field conditions so that the full benefits of humic products can be documented. Plant processes triggered in response to field stresses and their modification by humic products could also be observed and explained.

This situation similarly describes the search for the compound(s) in humic products that provoke crop responses. Identification of the causal compound(s) could enable both more effective products and better prediction of which crops and field settings are most suitable for humic products. One promising approach for identifying causal compounds would determine which fractions of humic products have greater biological activity and then compare their respective chemical compositions. To date, separation of humic molecules based on molecular size have failed to identify fractions that consistently better promote plant growth (Zandonadi et al., 2013). Must all humic products have the same causal compounds whether they are derived from millions-year-old leonardites, thousands-years-old peats, or instead months-old composts? Interesting challenges loom in the search for causality.

Industry-wide standards for humic product quality. The humic product market lacks universally recognized standard procedures for measuring either the humic acid / fulvic acid contents or the field efficacy of humic products. Consequently the consumer cannot ascertain for any product its concentration, source material, or variability among production batches. The consumer cannot discern effective products from molasses, lignosulphonates, or hard coal--similarly appearing substances that are rumoured to be sold as humic products and are of uncertain or negligible value to crop production.

Official procedures for measuring humic acid and fulvic acid contents are designated by some national governments. Lamar et al. (2014) report a more recent procedure that was developed in concept by members of the International Humic Substances Society on behalf of the Humic Products Trade Association (HPTA), a North American-based industry group (http://www.humictrade.org/). This method better distinguishes humic and fulvic acids from such adulterants as molasses, hard coal, and soil/sediment, but at greater cost of labor. The primary objective of any measure for humic and fulvic acid contents may well prove to merely discern genuine humic materials from adulterants or fraudulent materials, instead of quantifying slight differences among genuine products in their humic acid or fulvic acid concentrations.

No method has been proposed as a standard procedure for measuring the field efficacy of humic products. Lacking the identification of the causal compound(s) in humic products, attention has focused on rapid bioassays (e.g., plant root or shoot growth, algal, yeast, or microbial culture growth). The question exists whether any single rapid bioassay can quantitatively reproduce the capacity of each humic product to promote growth for all crops grown globally in diverse settings.

Benefits to soil health. In recent years, soil health has received increasing interest, and research funding has increased in this area. Increasing research efforts are directed at determining crop management practices that can improve soil health in economically viable manners. Soil health is widely thought to benefit from increased carbon availability in soil. Increased root growth is commonly viewed by the humic product industry as a reliable crop response to product application, and it has been reported in some published papers (Lee and Bartlett 1976; Chen and Aviad 1990; Canellas et al. 2002). If this were a general response across crop and soil types, it would constitute increased C input into the soil, and hence a potential agent for improving soil health.

Despite the potential of humic products to improve soil health, scant work has addressed this topic in field conditions. A review by Billingham (2012) found that nearly all of the studies on soil physical properties following humic product application were performed in laboratories and greenhouses, with very few set in field conditions. A major limitation to progress has been the absence of long-term field trials on humic product application. The full benefit of humic product to expanded root growth and in turn soil health would be fully expressed only after several years of continuous application to the same plots or strips.

**Our recent progress in field evaluations of two humic products.**

As collaborating researchers and vendors of humic products, we have jointly observed statistically significant increases in grain yield of maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) with humic product application in the U. S. Corn Belt, on some of the most fertile soils in the U. S. In an extensive three-year study, humic product application led to increased grain weight as measured by hand-collected maize plants for 74 of 95 (78%) on-farm fields (Olk et al. 2013, 2018b). Mechanized grain yield data were not made available from these fields. Yield component analysis showed that the yield increase was due primarily to increased ear length, especially of the shorter ears. This trend of greater ear length homogeneity suggests the primary benefit of the humic product was to enable weaker maize plants to better compete with their bigger, more vigorous neighbors within the dense stands that typify intensive agriculture. Leaf area measurements that were performed in selected fields showed a positive response to humic product application by the 9$^{th}$ leaf even with product application as recent as the 6$^{th}$ leaf, indicating a plant response within a few weeks.

In a more intensive approach, maize response to a second humic product within a farmer's field was monitored in four of five consecutive years, with measurements taken on an eroded hilltop soil, highly productive sideslope soil, and a fine-textured, carbon-rich soil at the foot of the slope. In a droughty year, mechanized grain yield increased by as much as 40% with humic product application on the eroded hilltop soil, while yield increased only by 2-3% on the sideslope soil (Olk et al., 2018d). Although the bottomland soil provided the lowest overall grain yields, its yield increases with humic product application were intermediate between those of the eroded hilltop soil and the sideslope soil. In a later year featuring nearly ideal growing conditions, with warm temperatures and timely rainfall the entire season, all soils gave similarly high mechanized grain yields, and the only yield increase was an 8% increase in the eroded hilltop soil. Despite annual variability in rainfall and grain yield levels, leaf area increased in all years with humic product application. Yield increases were again due to greater homogeneity of ear length, i.e. less frequent occurrence of short ears. Hence both soil type and annual weather patterns affected the efficacy of this humic product to increase economic yield. The similar response in leaf area across all years suggests that the humic product was positively affecting plant growth in all years, but whether this growth promotion resulted in enhanced grain yield appears to have depended on the degree of drought stress each year; grain yield increased with product application when there was drought stress for a portion of the growing season. Overall, this first research phase indicated that maize grain yield generally responded to humic products, but the magnitude of response was strongly affected by soil type and weather conditions.

In the next research phrase, we explored the hypothesis that better maize growth and grain yield with humic product application were due to enhanced root growth, enabling greater uptake of water and nutrients from soil. A general belief within the humic product industry is that humic products cause substantially greater root growth. Studies in controlled conditions are consistent with this claim ((Lee and Bartlett 1976; Chen and Aviad 1990; Canellas et al. 2002), but root responses had not yet been measured in field conditions. During three growing seasons, we measured root length and root cross-sectional area for a defined soil volume that comprised the bulk of the root systems for three adjacent maize plants. Root measurements were taken at the 8$^{th}$ and 14$^{th}$ leaf growth stages and again at an early reproductive growth stage that represented a mature root system before the onset of senescence. Both humic products were evaluated in separate fields.

In nearly all comparisons (two humic products, three seasons, two landscape positions in one field, two crop rotations in the other field), humic product application caused enhanced root growth (Olk et al., 2018c). Total root

length increased in most cases by 16-32%, and increases in total cross-sectional area were comparable. Total root length was dominated by the finest roots (<0.5 mm diameter), yet all ten root diameter size classes (ranging up to >4.5 mm diameter) showed the same proportional increases in root length with product application. Nearly all of this root growth occurred between the second sampling at the 14$^{th}$ leaf and the final sampling at reproductive growth. No consistent root response was observed at the 8$^{th}$ leaf sampling. At the 14$^{th}$ leaf sampling the only visible root response was faster emergence of nodal roots from the stem. The emerging roots were short, though, and did not contribute substantially to total root length. In summary, the root system increased with product application well after the leaf response. Therefore, we concluded that increased root growth was not responsible for greater water and nutrient uptake that fueled the leaf area response. Hence the root response was itself a side-effect of another, as yet unidentified change in plant processes following humic product application.

Visual observations in the 2012 field season demonstrated increased wind resistance by maize with humic product application. Increased lignification of the maize structure is one agent for enhanced wind resistance. Thereafter for selected fields we measured plant structural lignins and carbohydrates for stover and also for roots when available. For one humic product, the degree of lignification was positively correlated with drought incidence (Olk et al., 2018e). This held true when comparing across four growing seasons that varied in their precipitation amounts and also across two landscapes that differed in local water supply. Lignification is a normal maize response to drought (Chazen and Neumann, 1994), perhaps to maintain structural integrity despite partial loss of cellular turgor pressure. That is, this humic product strengthened the normal plant response to drought stress. One growing season featured nearly ideal growing conditions throughout the growing season, with timely precipitation. In this year, maize stover phenols decreased slightly with humic product application, while structural carbohydrates increased by 10 and 38% in two landscape positions. We interpret this divergent result as luxury production: with timely rainfall and abundant water supply, the maize plant did not need to lignify and so devoted its additional growth potential to carbohydrate accumulation. Such a hypothesis implies process-level interactions among the plant, the humic product, and environmental signals.

The maize cultivar was selected in each year by the land manager, with no input from the researchers. The effects of the second humic product on maize structural biochemistry was also analyzed in a separate field where the maize germplasm was selected by the humic product company. More robust germplasm was used in this field, and this humic product had no effect on maize structural biochemistry (data not shown), despite analogous effects as the first humic product on grain yield and root growth. These results highlight apparent interactions between humic product, plant, and environmental signals.

Other visual observations during these field studies included less curling of maize leaves during moderate drought, delayed senescence at season's end, and in cases diminished weed growth.

All these results suggest that humic products enable crops to better withstand environmental, abiotic stresses. The three-year on-farm survey demonstrated the humic product alleviated nearest neighbor (intraspecific) stress for the weaker plants. Multi-year studies in a farmer's field demonstrated several forms of increased drought tolerance by maize. Enhanced stress resistance constituted the bulk of the grain yield increases that we attributed to the humic products. Grain yield of the larger plants scarcely responded to humic product application in the on-farm survey or under nearly ideal growing conditions, even when leaf area, root growth, and plant structural biochemistry maintained their positive responses.

Hence we postulate that humic products can play meaningful roles in mainstream crop production. In the vast majority of cases, they provided an economic yield boost that was agronomically modest yet economically profitable, given the generally low cost of humic products. They do not always ensure a yield increase when favorable growing conditions enable very high yield levels, but they do offer some level of protection at low cost in case environmental stress develops during the growing season.

**Soil health**

The responses of root growth and plant structural biochemistry to humic product application as described above carry immediate implications for soil health. Carbon is a primary factor of most soil properties that are measured for soil health assessments, including nutrient mineralization, microbial activity, soil physical properties, and inorganic nutrient retention. Root carbon may be the primary source of stable carbon in soils (Balesdent and Balabane 1996; Gale and Cambardella 2000), due perhaps to the more cross-linked nature of its lignin components compared to aboveground biomass (Bertrand et al. 2006) or to it being more strongly imbedded within soil aggregates (Mendez-Milan et al. 2010). Humic-product induced lignification of crop straw or roots during droughty conditions would further strengthen the recalcitrance of crop residue-C and hence its potential stability in soil. Root system mass is generally thought to comprise at least 10% of aboveground biomass, hence dwarfing the amount of C added as the humic product itself. We expect any benefits to soil health to accrue only during several years of humic product application, as the input of stable carbon is increased only gradationally, and soils have considerable capability to degrade carbon inputs. We are currently measuring changes in several soil physical properties following four or five years of humic application in maize-soybean rotations. Measurements include bulk density, soil penetration resistance, water-holding capacity at 0.03 and 1.5 MPa, wet aggregate stability, and dry aggregate stability.

Preliminary results were gained by measuring soil penetration resistance in a farmer's maize–soybean field two years after one humic product had been applied for the six previous growing seasons. Penetration resistance was measured post-harvest in nine paired comparisons along the edge of a strip where the humic product had previously been applied. No benefit was observed at 2.5 cm increments in the uppermost 12.5 cm soil that was within the depth range of tillage performed annually in this tilled field, but subsequently at each 2.5 cm-increment from 12.5 cm down to 30 cm, soil penetration resistance was diminished by about 138 kPa within the humic product strip compared to the unamended soil immediately outside the strip (Fig. 1). The diminished penetration resistance was apparent to the instrument operator during measurement. Similar results were gained more recently in a second maize–soybean field that had received replicated treatments of the second humic product for five years. Again, no benefit was apparent at 2.5 cm-increments within the surface 10 cm that was also within the depth zone of annual tillage, but a consistent reduction in penetration resistance with two humic product treatments was measured at 2.5 cm-increments at all lower depths down to 30 or 35 cm for a finer-textured upland soil (Olk et al., 2017). We hypothesize that the greater input of root-C can be degraded in the uppermost soil depths through oxygenation of the soil that tillage introduces into both fields. At lower depths, however, the oxygen status might become limiting so that the enhanced C input of root biomass can persist in the soil, leading to a healthier soil and diminished soil penetratin resistance.

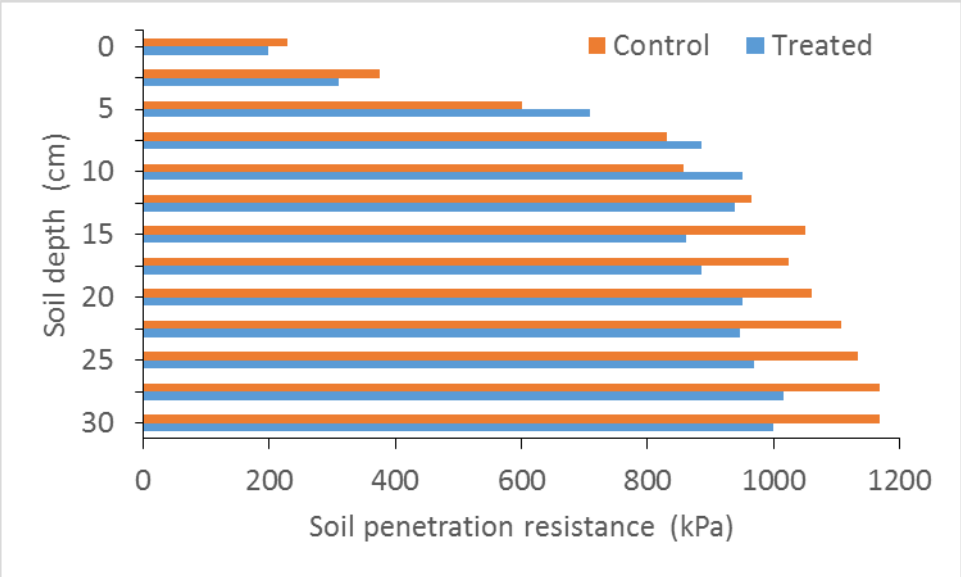



**Figure 1.** Soil penetration resistance from the 0 to 30 cm depth by 2.5-cm increments with (Treated) and without (Control) addition of a humic product.

Beyond the larger root biomass, humic product application might lead to additional carbon inputs to the soil through greater exudations from the larger root systems. Root exudates are immediately available nutrition for soil microorganisms, whose activity is pivotal to several soil health measures. Solution culture studies vary widely in estimating the amount of exuded C by plants (Uren, 2000), but it can be as high as 30% of total dry matter production by young plants (Bertin et al., 2003). No published evidence exists for field measurements of root exudates with humic product application. Yet it is reasonable to hypothesize a benefit of humic product application to soil health through enhanced root exudation. In a solution culture study with corn seedlings, Canellas et al. (2008) found that addition of a humic material caused greater root exudation of oxalic, citric, and tartaric acids compared to the unamended control. However, exudation of maleic and fumaric acids decreased while that of succinic and malic acids ceased. We are unaware of any other studies on root exudations following humic product application.

**Other ecosystem services**

Humic products may well provide additional ecosystem services beyond increased crop economic yield and soil health, although supporting evidence for some benefits consists of only anecdotal observations or theoretical extrapolations. Following are representative examples of relevant studies in recent years. This literature review is not comprehensive. Perminova et al. (2005) provided several primary studies and reviews for using humic substances to remediate polluted environments, including metals, anthropogenic organic compounds, and radionuclides.

Greater microbial activity. Several independent lines of evidence suggest humic products stimulate microbial activity. A humic product mixed with annual ryegrass (*Lolium* sp.) residue was found to accelerate fragmentation of a hardened subsoil fragipan (Murdock et al. 2017), presumably due to accelerated microbial activity. This fragipan limits the effective rooting depth of crops across about 25 million ha in the eastern U. S. Another humic product is used to

degrade oil spills on soil, and the capacity of this product to accelerate decomposition of petroleum compounds was documented in laboratory conditions (Cardenas and Lopez Jr., 2016). This same product was also used to reduce odor emissions from swine manure by 21% for barn odor emissions ($P<0.01$) and by 21% ($P=0.85$) and 60% ($P=0.001$) for land applications in field conditions and laboratory conditions, respectively (Hile et al., 2017). The explanation could possibly also be accelerated microbial degradation of odor-causing compounds. Improved livestock health, accelerated weight gain and therefor feed conversion efficiency are often attributed to the addition of humic products to animal feed, as shown for beef cattle (Cusack, 2008), finishing pigs (Wang et al., 2008), poultry (Ozturk et al., 2010), and as broadly reviewed by Islam et al. (2005) and Trckova et al. (2005). The cause is unknown, but a common speculative explanation is that the humic product bestows a competitive advantage to favourable microorganisms in the livestock gastrointestinal tract compared to pathogenic microorganisms, keeping growth-inhibiting diseases in check. Finally, some farmers apply humic products post-harvest in stover-rich fields with the intent of accelerating stover decomposition for faster recycling of stover nutrients.

If humic products were shown to consistently accelerate microbial activity, this would also constitute another possible mechanism for crop yield responses. Soil microorganisms are especially concentrated in the soil rhizosphere, and they also thrive inside plants. Microorganisms are capable of producing hormones that can stimulate plant growth (Frankenberger and Arshad, 1995). Hence, microbial responses to humic products deserve further consideration.

Better fertilizer use efficiency. Any promotion of crop growth by humic products–regardless of the mechanism–could also provide faster uptake of soil nutrients by the bigger plants. This consequence creates the potential for greater fertilizer use efficiency and diminished loss of labile nutrients via gaseous losses or leaching into groundwater and other water bodies, and reduced nutrient loss through soil erosion due to greater ground cover and quicker canopy development above the soil. Farmers often incur these losses with regular fertilizer use, and they are heightened at excessive fertilizer rates that farmers can pursue to ensure non-limiting growing conditions. Over-application of N and P fertilizers is thought to contribute both to increased global warming through nitrous oxide emissions (Shcherbak et al., 2014) and to algal blooms in freshwater lakes and rivers together with hypoxia zones in seas that ultimately receive excess nitrates and phosphates (Rabalais et al., 1996; Carpenter et al., 1998; Smith et al., 1999; Mitsch et al., 2001; Paerl, 2009). Hence a reasonable objective is that farmers would lower their fertilizer input rates if they would instead apply a humic product to ensure both greater fertilizer use efficiency and some protection against environmental stresses. We call for an array of field studies to assess crop responses to multiple rates of N and P fertilizer inputs with and without humic product application in regional sites that have been identified as critical source areas for N and P nutrient loss to water resources. Their objective would be to determine whether grain yield targets can be maintained by combining humic product application with lower fertilizer application rates that better approximate actual crop demand for each nutrient and to document changes of N and P loading to water resources, and where possible changes in N gaseous loss as nitrous oxides. These studies should be conducted across a range of crop and soil types, because N and P response functions will vary with local conditions. Such research is especially urgent in those settings where routine over-application of fertilizers has led to significant environmental degradation, such as the Mississippi River Basin that discharges into the Gulf of Mexico.

If humic product additions to animal feeds in fact lead to healthier livestock and faster weight gain as described above, this would also constitute more efficient use of inputted nutrients. Therefore, animal manures would have lower nutrient contents. Application of nutrient-depleted manures to agricultural lands could also lead to the same environmental benefits as those associated with better fertilizer use efficiency in plant production.

Heavy metal detoxification of contaminated soils and wastewaters. The binding of metals by humic substances is well documented for soil organic matter interactions with common soil cations (Tipping, 2002). A natural extension of this concept into agroecosystems would be the application of humic products or ores to remediate soils or industrial wastewaters bearing elevated concentrations of heavy metals. Humic substances appear suitable as remediation agents, given their variety and abundance of chelating groups and capacity to either solubilize or remain in colloidal form, depending on conditions (Baek and Yang, 2005).

Several studies have shown in controlled conditions that dissolved humic substances adsorb several heavy metals (including Cd, Cu, Hg, Pb, Zn) from solution (Baek and Yang, 2005; Wang et al., 2016; Kluĉáková and Pavlíková, 2017). Tang et al. (2014), Genç-Fuhrman et al. (2016), and Kantar et al. (2017) demonstrated concerns for unfavourable interactions of humic substances with other binding materials for heavy metals in solution. We are unaware of larger-scale application of this technology outside laboratory conditions. Humic product application to contaminated soil led to diminished activity of Cd (Meng et al., 2017) and of Zn, Pb, Ni, Cd and Cu, as reviewed by Pukalchik et al. (2018). Heavy metal inhibition of plant growth was alleviated following humic product or humic material application in hydroponic systems (Ondrasek et al., 2018; Ozfidan-Konakci et al., 2018). Yet Park et al. (2013) discussed the potential for humic substance binding with heavy metals to simultaneously diminish soil contamination by the heavy metal but also increase plant uptake of the heavy metal. Again, we are unaware of field-scale application of commercial humic products to remediate soils contaminated with heavy metals. Much more work remains to be done in this area,

especially at commercial application rates and in field conditions, provided humic products are economically competitive with alternative technologies for heavy metal remediation.

Remediation of anthropogenic organic pollutants. Several controlled studies have examined interactions between organic pollutants and humic substances applied in manners analogous with use of commercial products. Haderlein et al. (2001) documented significant increases in the mineralization of pyrene in contaminated soil with the addition of humic acids and composted contaminated soil. They did not postulate the underlying mechanism(s) of the humic materials. One possible explanation would be the enhancement of microbial activity as described above. In addition, physical bonding of contaminants with humic materials has also been demonstrated. In a laboratory-based study by Van Stempvoort et al. (2002), humic acid solutions bound to petroleum-derived compounds, which increased their solubilization and subsequent rates of biodegradation. Mezin and Hale (2004) examined dissolved humic acids for their potential to reduce the toxicity of 4,4'-dichlorodiphenyltrichloroethane (DDT) and chlorpyrifos pesticides for freshwater and saltwater crustaceans. The humic acid treatments had no effect on reducing the mortality of the saltwater crustacean *Americamysis bahia*, but they substantially reduced the mortality of the freshwater crustacean *Ceriodaphnia dubia*. The difference in results between the freshwater and saltwater crustacean mortality was attributed to conformational changes in the structure of the humic acids by the saltwater that impeded their binding with the pesticides. Perminova et al. (2001) studied the effects of multiple humic materials on the toxicity of pyrene, fluoranthene and anthracene for the crustacean *Daphnia magna*. They attributed reductions in toxicity with humic material addition both to chemical binding of the three compounds and to direct beneficial effects on *D. magna*. As with heavy metals, we are unaware of field applications of humic products for mitigating anthropogenic organic pollutants.

**Conclusions**

There is a clear theoretical basis for commercial humic products to contribute to several agricultural and environmental objectives. Increased crop production has received the most attention, especially in the presence of environmental stresses, but other potential benefits are animal production and environmental remediation, including reducing air and water degradation due to excessive fertilizer applications, cleaning oil spills and removing heavy metal or organic contaminants. Experiments performed under controlled conditions support the theoretical bases of such benefits, although the translation of laboratory result to field conditions is not perfect. Issues include potential differences between the chemical nature of humic materials extracted from soils for use in controlled conditions versus that of commercial products extracted from immature coals, and the disturbing results of Bacilio et al. (2017) who found diminishing benefits of humic acid application when the research was moved from the growth chamber to greenhouse to the field. Benefits observed in static laboratory conditions are often not reproduced in the more chaotic conditions of nature. Hence the lack of practical applications of humic products at industrial or farm scales presents a formidable knowledge gap. We call for greater transfer of laboratory-acquired concepts to field applications so that the true efficacy and economic value of humic product-based technologies can be properly assessed for end users.

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# Humic substances – versatile natural products: Properties & applications

MD ZAHİDUL ISLAM[(a)], MÜMİN DIZMAN[(a)], OMER FARUK TUTAR[(a)], AHMET TUTAR[(a)*]

Department of Chemistry, Faculty of Arts and Science,
Sakarya University, 54187 Esentepe, Sakarya, Turkey.
* E-mail: atutar@sakarya.edu.tr

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**Abstract.** Humic substances (HSs) are most important natural polymers in the world. The main sources of HSs, are leonardite ores, lignites and peats. Our objective is to appraise the state of art concerning these subtances: their morphology in the native solid state, methods of identification as well as the difficulties in utilizing and processing it for selected applications. HSs, which are insoluble in acidic aqueous media (Humic Acids), are used in many applications (agriculture, animal health and feeding, food, cosmetics, biomedical and pharmaceutical applications). We briefly describe the chemical modifications of HSs–an area in which a variety of syntheses have been proposed tentatively, but are not yet developed on an industrial scale. This review emphasizes recent papers on the high value-added applications of these materials in industry, medicine and cosmetics.

## Introduction

HSs are natural polyelectrolytes of major importance, first identified in 1761 (**Figure 1**). Those biopolymers are synthesized by an enormous number of living organisms; and considering the amount of organic matters produced by humification at millions years ago in the world, they are the most abundant polymers. HSs occur in nature as ordered nanocolloids forming structural components in the organic matter. HSs are also produced by a number of living organisms in the lower plants and animals, serving in many functions where reinforcement and strength are required. One of the fractions that can also be extracted from peat is humic acids, which have attracted attention for their potential use in healthcare. Humic acids belong to a group of polymers with high-molecular-weight macromolecules. Chemical and spectroscopic analyses have revealed the presence of aromatic rings, phenolic hydroxyl, ketonyl, quinone carbonyl, carboxyl and alkoxyl groups in HSs (Livens *et al*., 1991, Schulten *et al*., 1991). Although the exact composition of humic acids is unknown, it varies according to their extraction source. Chemical structure, composition, functional groups present in HSs vary markedly depending on their origin and humification processes they have undergone. Studies on humic acid chemistry have revealed that aromatic rings, phenolic hydroxyl, ketone carbon, quinone carbonyl, carboxyl and alkoxyl groups are present in their molecules.

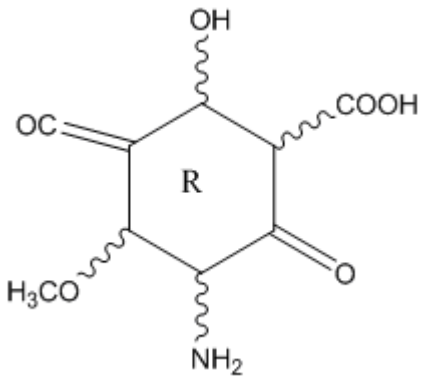



**Figure 1.** Simple representation of HSs'complex structure (R:supramolecule body).

Despite the widespread occurrence of HSs, up to now the main commercial sources of HSs have been leonardite ore and peats. In industrial processing, HSs are extracted from those sources by alkali treatment to dissolve humates. These treatments must be adapted to each HSs source, owing to differences in the ultrastructure of the initial materials. The resulting HSs need to be graded in terms of purity and color since residual humate and pigment can cause problems for further utilization, especially for biomedical products.

## Medicine

One of the fractions that can also be extracted from peat is HSs, which have attracted attention for their potential use in healthcare. The biological effects of HSs differ due to variability in their chemical structure and physiochemical properties. In Turkey, there are several drug studies using HSs in medicine in recent years. Aras, A. B., *et al.* (2014) investigated the potential neuroprotective effects of humic acids in a focal cerebral ischemia model. In their study, twenty-four rats were divided equally into three groups. A middle cerebral artery occlusion model was performed in this study where control (group II) and humic acids (group III) were administered intraperitoneally following an ischemic experimental procedure. Group I was evaluated as sham. Malondialdehyde (MDA), superoxide dismutase (SOD) and nuclear respiratory factor-1 (NRF-1) levels were analyzed biochemically on the right side of the ischemic cerebral hemisphere, while ischemic histopathological studies were completed on the left side to investigate the antioxidant status. Biochemical results showed that SOD and NRF-1 levels were significantly increased in the humic acids group (III) compared with the control group (II) while MDA levels were significantly decreased. On histopathological examination, cerebral edema, vacuolization, degeneration, and destruction of neural elements were decreased in the humic acid group (III) compared with the control group (II). Cerebral ischemia was attenuated by humic acid

administration. These observations indicate that humic acid may have potential as a therapeutic agent in cerebral ischemia by preventing oxidative stress.

Akbaş, A., *et al.* (2015) investigated the potential renoprotective effects of humic acids in a renal ischemia reperfusion model. In their study, twenty-one rats were randomly divided into three equal groups. Intraperitoneal serumor humic acids was injected at 1, 12, and 24 h. Non-ischemic group I was evaluated as sham. The left renal artery was clamped in serum (group II) and intraperitoneal humic acids (group III) to subject to left renal ischemic reperfusion procedure. Ischemia and reperfusion time was 60 min for each. Total antioxidant status, total oxidative status, oxidative stress index and ischemia-modified albumin levels were analyzed biochemically from the serum samples. Kidneys were evaluated histopatologically and immunohistochemically. Biochemical results showed that total oxidative status, ischemia-modified albumin and oxidative stress index levels were significantly decreased, but total antioxidant status was increased in the humic acid group (III) compared with the ischemia group (II). On histopathological examination, renal tubular dilatation, tubular cell damage and necrosis, dilatation of Bowman's capsule, hyaline casts and tubular cell spillage were decreased in the humic acid group (III) compared with the ischemia group (II). Immunohistochemical results showed that apoptosis was deteriorated in group III. Renal ischemia reperfusion injury was attenuated by humic acid administration. These observations indicate that humic acid may have a potential therapeutic effect on renal ischemia reperfusion injury by preventing oxidative stress.

Çalışır, M., *et al*. (2016) evaluated the morphometric and histopathological changes associated with an experimental periodontitis model in rats in response to systemic administration of humic acids. Tissues were histopathologically examined to assess the differences of osteoclast numbers, osteoblastic activity and inflammatory cell infiltration among the study groups. Enzyme-linked immunosorbent assay interleukin (IL)-1β and IL-10 levels in serum and gingival homogenates were studied. Within the limits of this study, it can be suggested that humic acid, when administered systemically as an 80 mg/kg dose, may prevent alveolar bone loss and reduce inflammation in the rat model.

In addition to this study, Çalışır, M., *et al*. (2012) investigated the effect of humic acids on oral aphthous ulcers as a potential alternative to topical antimicrobial mouthwashes. At the end of the 3rd day while lesion size decreased by approximately 60%, at the end of the first week it was almost non-existent. After application of humic acids, it was observed that the patient has no new complaint and current pain, difficulty in chewing and speaking complaints were healed up. These results showed that humic acids solution accelerates wound healing.

Meriç, G., *et al*. (2016) evaluated the efficiency of humic acids substances on removing micro-organisms from denture base materials. The most used denture-cleaning agents (Corega and Steradent) were equally effective to the humic acids-based experimental solution with respect to the ability to remove micro-organisms.

**Cosmetic and Dermacosmetics**

Humic acids have been successfully used in the dermatocosmetical practice of clinical medicine (Wollina, U., 2009). There are many indications that also a chemical component may contribute to the clinical success of cutaneous humic acids' treatment because several pharmacological effects have been found which cannot be contributed to the well established physicochemical effects. Those chemical effects demonstrate antibacterial properties (Galgóczy, L., *et al*. 2011), antiviral properties (Klöcking, R., *et al*. 2013), antimicrobial properties (Van Rensburg, C. E. J., *et al*. 2000). Examinations of the stimulatory effects of humic acids on the skin tissue have demonstrated that low molecular weight substances (<1000 Da) are responsible for the stimulatory properties. Humic acids have been found to be of particular importance when considering the biological effects of peat (Beer, A. M., *et al*. 2000).

After having identified the chemical substances responsible for the found additional therapeutic effects, it has now to be proven whether the permeation of these active hydrophilic substances through human skin is possible and may be the explanation for these additional effects. However, it is well known that the composition of humic acids in general is very complex and additionally differs depending on the source from the peat, lignite and coal. In addition, the quality and composition of the humic acids is depending on many different factors as the place of origin, the primary types of the plants of origin and a whole spectrum of environmental factors.

Furthermore, exact structure elucidation of many compounds is hampered because they are oxidized during the extraction process and in most cases multicomponent extracts of high and low molecular compounds have been analyzed. Available literature on humic acid compositions further indicates that polyphenols, polycarboxylic acids and quinone derivatives are also present in aqueous extracts which also may be able to permeate across human skin in pharmacologically active amounts (Beer, A. M., *et al*. 2003).

It has been proved that humic acids have the properties of biogenic stimulators (3 mg/kg). They stimulate the macrophage defense reaction, promote nervous tissue regeneration, stimulate tissue reparation, and produce anti-inflammatory effect in case of tissue burns and cornea diseases. They stimulate mitochondrial respiration and inhibit free radicals. By their radioprotective properties various fractions of humic acids show the evident radioprotective effect. Humic acid molecules are polydispersed (molecular mass is 1000-100000). Certain part of them penetrate the human skin by diffusion and electrophoresis (Dolidovich, E. F., *et al*. 1992).

Humidone® is a polymeric molecule formed by complexes between polyvinylpyrrolidone (PVP) and sodium humate (SH) with polyethylene glycol (PEG) (**Figure 2**). It is prepared by strong intermolecular interaction involving the formation of hydrogen bonds between the carbonyl group of PVP and hydroxyl groups and $–NH_2$ of SH (Tutar, A., *et al*. 2012).

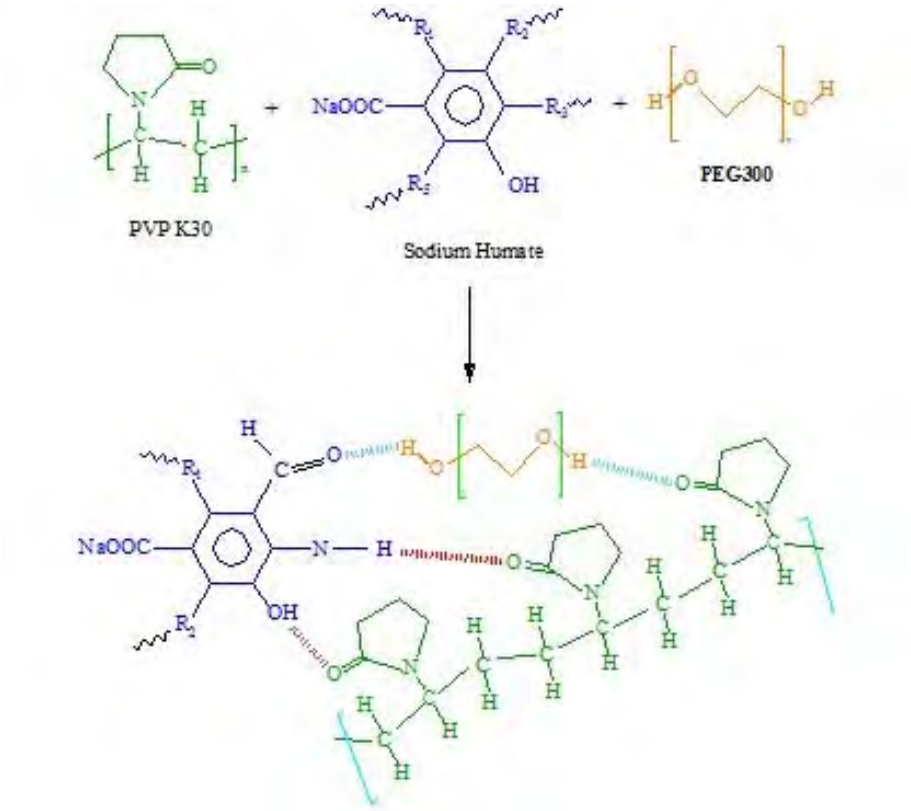



**Figure 2.** A possible model of Humidone® (R1, R2: Phenolics, carbonyls, amines, carboxylic, aliphatics).

Cosmetic or dermatological preparations which are aimed at protecting the skin care not novel in principle. In the past, there has already been various compositions in cosmetics and in dermatology whose task was to protect skin, in particular human skin. Nevertheless, there continues to be the need to improve or to modify skin protection because the environmental conditions and also living conditions and habits of users are changing. Thus, for example, the increased emission of $CO_2$ into the atmosphere brings about climate change which is linked to an increase in temperature. This has effects on the human skin, which requires increased protection and also modified protection. In the course of progress, there are contaminations of the environment, in particular of the air. These contaminations can be gaseous in the form of carbon dioxide, methane, halogenated and partly halogenated hydrocarbons and other gases or particulate, e.g. in the form of soot particles. All of these contaminations come into contact with the skin and can cause irritations ranging to severe disorders. This too renders improved and modified skin protection necessary.

Altered living conditions lead to many people, especially in western countries, spending long periods of the day in closed rooms. At cold times of the year, these rooms are heated and the room air is consequently in most cases very dry. The skin reacts to this by drying out. Improved skin protection is necessary.



(a) (b)

**Figure 3**. Treatment of pilonidal sinus diseases: (a) before Humidone®, (b) after Humidone®.



(a) (b)

**Figure 4**. Burn treatment by Humidone® : (a) before treatment, (b) after 10 days.

Klöcking, R., *et al*. (2013) analysed the UV/Vis spectra of three natural HA and three synthetic HA-like Substances to determine the appropriateness of their UV-absorbing characteristics for the product under development. Preliminary results from cultures of human U937 cells assayed for survival 24 hours after exposure to UV-B radiation show that both HA and castor oil exert a significant concentration-dependent UV-B protective filter effect similar to that of the UV-B absorbing reference substance p-aminobenzoic acid (PABA).

HSs are known for their therapeutic and cosmetic properties (Wollina, U., 2009). Various cosmetic products based on these properties exist in the market such as a beauty plaster, comprising among other ingredients, natural salt and mud.

HSs are especially known for its therapeutic properties on psoriasis and other skin related disorders. Patients, suffering from various skin diseases try to have treatments with HSs. It is the aim to overcome the problems associated with treatments with HSs by providing an easy and pleasant to use creams that while having the therapeutic benefits of them.

The incorporation of cosmetic into a leave-on cream which should be evenly spreadable, aesthetic, pleasant to use and absorbed easily to the skin, is not simple, because it involves a suspension of solid particles in an oil-in-water emulsion that contains electrolytes in a very high concentration. Such a combination has a high tendency to aggregate and to separate into phases. Still the cream must be stable and with a reasonable shelf life.

It is an easy and simple to use HSs' cream that is absorbed into the skin and in contrast to the treatments used today, it may be in prolonged contact with the skin (i.e., leave-on cream), enhancing the beneficial effects of HSs. It is also stable, does not separate to phases and has long shelf life. HSs'cream relates to an oil-in-water emulsion, suitable for applying to skin, of various viscosity ranges including milk, lotion, cream and ointment for the use of treating (healing) and preventing or delaying skin disorders and diseases. Cream composition for topical application for the treatment of skin disorders and skin diseases comprising 1-20% wt HSs as an active ingredient. HSs' composition is for use in treating skin disorders and skin diseases such as pilonidal sinus ( **Figure 3**), psoriasis, saborrehic dermatitis, xerosis, attopic dermatitis, eczema, diaper rush, skin burns (**Figure 4**) of stage I and sensitive skin. HSs' cream composition is also for use as a leave-on cosmetic cream for beautifying and enhancing the skin appearance.

Nowadays in Turkey, HSs are also used in shampoos, peelable masks, anti-aging creams, sunscreen creams, soaps, depilatory creams, toothpastes, skin moisturizing, anti-wrinkle creams, anti-pollution creams, and many personel care cosmetics.

**Food**

HSs are very important in food prosessing due to its antioxidant and mitigating effects on problems caused by some chemical additives. The effects of humic acids addition into bread (**Figure 5**) and cake (**Figure 6)** formulation at certain levels in terms of quality parameters, molding and staling were investigated. Humic acids solution (20%, v/v) was added into bread formulation at the levels of 1, 2, 3, 4%, and into cake formulation at the levels of 2.5, 5.0 and 7.5% (in water basis).

The bread and cake samples were evaluated in terms of physical parameters (weight loss, volume, index values), color (Minolta Spectrophotometer CM-3600d, Japan) and texture (Brookfield CT3 Texture Analyzer, USA) values. The products were packed in plastic bags and stored at room temperature to observe mold growth on the surface. Bread volume did not changed significantly up to 3 and 4% addition level. Molding was delayed in 1 and 2% humic acid supplemented breads, but increased in 3 and 4% added samples.

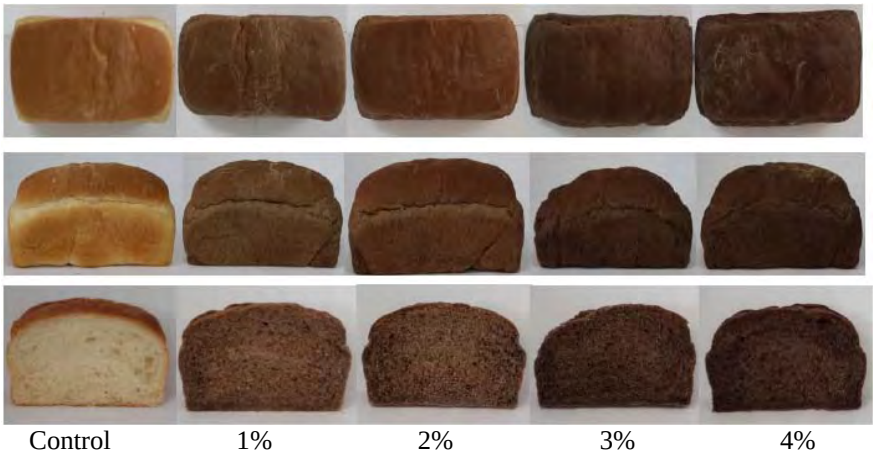



**Figure 5.** Photographs of bread samples containing humic acids.

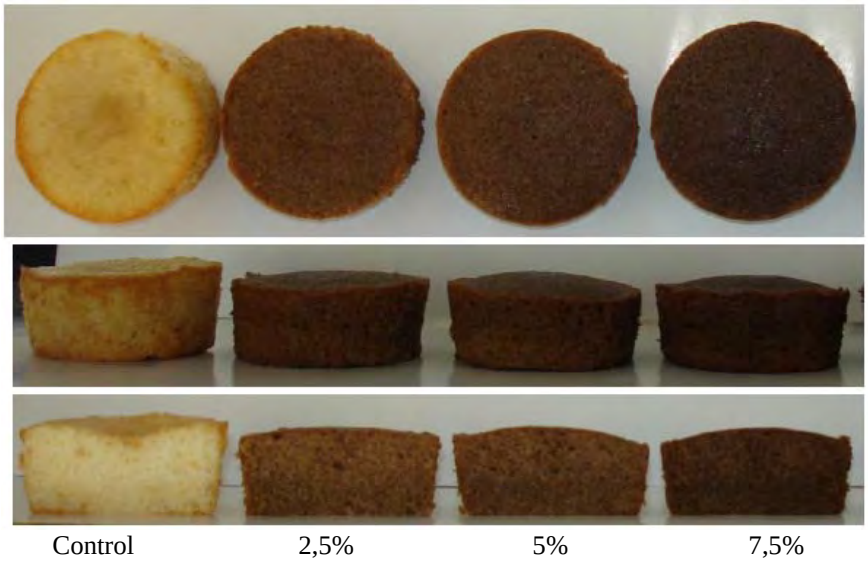



**Figure 6.** Photographs of cake samples containing humic acids.

Cake volume increased with increasing addition level of humic acids. After 12 days of storage of cakes, mold growth was only observed on the surface of 2.5% humic acids added sample. On 25th days of storage, mold growth was observed at all samples, except 7.5% humic acids added one. Because of the higher water absorption capacity of humic acids, weight loss and hardness values of products decreased with increasing addition levels. Although color of the humic acids added products were darker than that of the control sample, they were still in the acceptable range. Humic acids addition into the products did not caused any adverse effects in terms of taste and odor (Öztürk, S., *et al*. 2014).

In Turkey humic acids and fulvic acids have had a permission of the Food and Agricultural Ministry (FAM) by taking orally as food additives since 2016.

**Scale inhibitor and dissolved oxygen scavanger**

Scale build-up is a serious problem in many industrial water systems, such as cooling towers, heat exchangers, evaporators, pulping digesters, washers and in the production and processing of crude oil-water mixtures, etc. The build-up of scale deposits reduces the efficiency of heat transfer systems, interferes with fluid flow, facilitates corrosive processes and harbors bacteria. Calcium carbonate, generated in various processes, is one of the most commonly observed scale formers in industrial water systems. This scale is an expensive problem in many industries, which causes delays and shutdowns for cleaning and removal.

In particular, most industrial waters contain metal ions, such as calcium, barium, magnesium, aluminium, strontium, iron, etc. and several anions such as bicarbonate, carbonate, sulfate, oxalate, phosphate, silicate, fluoride, etc. When combinations of these anions and cations are present in concentrations which exceed the solubility of their reaction products, precipitates form until product solubility concentrations are no longer exceeded. For example, when the concentrations of calcium ion and carbonate ion exceed the solubility of the calcium carbonate reaction products, a solid phase of calcium carbonate will form.

Solubility product concentrations are exceeded for various reasons, such as partial evaporation of the water phase, change in pH, temperature or pressure, and the introduction of additional ions which form insoluble compounds with the ions already present in the solution. As these reaction products precipitate on the surfaces of the water carrying system, they form scale or deposits.

For boiler systems and similar heat exchange systems, the mechanism of scale formation is apparently one of crystallization of scale-forming salts from a solution which is locally supersaturated in the region adjacent the heating surface of the system. The thin viscous film of water in this region tends to become more concentrated than the remainder of the solution outside this region. As a result, the solubility of the scale-forming salt reaction product is first exceeded in this thin film, and crystallization of scale results directly on the heating surface. In addition to this, a common source of scale in boiler systems is the breakdown of calcium bicarbonate to form calcium carbonate water and carbon dioxide under the influence of heat.

For open recirculating cooling water systems, in which a cooling tower, spray pond, evaporative condenser, and the like serve to dissipate heat by evaporation of water, the chief factor which promotes scale formation is concentration of solids dissolved in the water by repeated evaporation of portions of the water phase. Thus, even a water which is not scale forming on a once-through basis usually will become scale forming when concentrated a multiple number of times.

Several reports have been made on natural organic molecules as scale inhibitors that are environmentally acceptable compared with conventional inhibitors. The calcium carbonate scale ability of humic acid in synthetic water was (100 mg/L) at 35°C. Results showed effective calcium carbonate scale inhibition with humic acid at a concentration of 1 mg/L. Humic acids had also a significant effect on scaling at concentration as low as 0.2 mg/L (**Figure 7**), (Chaussemier, M., *et al*. 2015).

HSs are strong antioxidants that scavange oxygen in water. Dissolved oxygen is transformed into free oxygen at $102^0$C. Free oxygen gives a damage by drilling at needle shape throughout the steel. Especially in boilers, this condition is very dangerous at high pressures by causing to exploition. HSs prevent oxygen in water by binding it with their antioxidant properties.

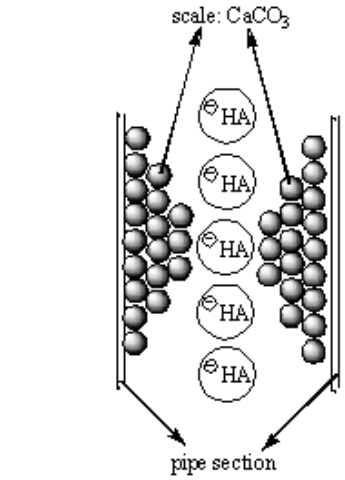



**Figure 7.** Scale inhibition by humic acids (HA).

**Cement**

HSs are widely used in cement industry in Turkey. Aggregates used in concrete production constitute the 70-80% by volume of concrete design. Crushed sand has the largest proportion in aggregate composition with its advantages on the cost of production. For this reason, the dirtiness of crushed sand is one of the most important parameters affecting the quality of concrete as well as being outstanding for chemical admixture producers on forming admixture design. In Turkey, dirtiness of aggregates creates severe problems in production of concrete. In most regions of country, it is difficult to find clean aggregates and concrete is produced with aggregates having high methylene blue value, which causes problems in production. Therefore, aggregate producers should take precautions to prevent stepping fine materials such as clay and silt in aggregates. In this sense, content and quality of fine material passing from 0.063 mm sieve such as clay, silt and very fine stone powder, directly affects the quality of concrete. Clay is a laminar or fiber formed hydrated aluminum or magnesium silicate based material that holds water and has a great ion exchange capability, having particles smaller than 0.02 mm. Activity of clay minerals depends on its characteristic patterns. It is known that some types of clay are relatively affectless and do not change aggregate performance, but other types are moist sensitive, expansive and have significant effects on aggregate performance even in small amounts. The presence of clay and silt in aggregates weakens the bond between coarse aggregates and cement paste, and increases the specific surface area of aggregates. Hence, the amount of mixing water and water/cement ratio (w/c) increases. This causes problems in strength, durability and volume stability of concrete. Another important property of clay and silt is water holding or water adsorption capability. Adsorbing water causes volume expansion and shrinkage follows this phenomenon, which increases tensile stress on surface. Clay and silt decrease the adherence by reacting with cement, and delay hydration and setting. Besides, very small amounts of fine material improve workability and impermeability of concrete. It is preferred to be in minimum because of their negative effects. Although conventional polycarboxylate based chemical admixtures reduce water requirement and provide consistency, they have limited usage because of being sensitive to dirtiness of aggregate. Some special chemical admixtures can be developed to eliminate negative effects of clay-bearing aggregates. This will be useful for producers which cannot find clean aggregates in concrete production. Kılınç, K., *et al*. (2016) studied humic acid based chemical admixtures which were developed for aggregates having high methylene

blue value. 15 cm x 15 cm x 15 cm concrete specimens were cast in laboratory conditions, then the mechanical properties of specimens were examined.

The studies about effects of humic acid on concrete are limited. Robertson and Rashid investigated the corrosive effect of humic acids on concrete in 1976. They studied on fresh water and brine water with organic substance concentration of 10 ppm. Calcium cation was released in water having 28 and 96 ppm humic acids concentrations. As a result, it was observed that humic compounds have no destructive effect on concrete structures. Besides, humic substances act as a protective layer on concrete surfaces. Prabha and Thajudeen examined the fresh and hardened concrete properties of concrete including humic acids. Humic acids were added in proportions of 0.5%, 1.0% and 1.5% by weight of cement. Maximum splitting tensile strength of cylindrical specimens and maximum flexural tensile strength of beams were observed on specimens including 0.5% of humic acid. Compressive strength of concrete with humic acid at the age of 7 days was lower than reference specimen's compressive strength. On the other hand, 28-day compressive strength was 31% higher than the reference (Prabha, K. S., *et al*. 2015).

Kang, G., *et al*. (2017) investigated strength development through a vane shear and unconfined compression tests with different water, cement, and humic acids contents. The results indicate that the strength development was governed by the cement and humic contents. Further, a threshold cement content, at which the adverse effects of humic acids on strength development are overcome, existed for a given humic acid content. Finally, a new compound was observed to form when humic acids were added to cement-treated clay.

**Renewable Energy**

HSs are tried in energy industry giving extreme important effort in Turkey. Especially they are used both photovoltaic and photogalvanic systems. Though common materials used for photovoltaics (i.e., the conversion of sunlight into electrical energy) are inorganic, there has been a tremendous effort to develop HSs' solar cells within the last three decades. The field started by the application of humic molecules (pigments), and since the development of semiconducting polymers, these materials were incorporated into humic acids solar cells resulting in remarkable improvements within the past years. The potential of semiconducting HSs to transport electric current and to absorb light in the ultraviolet (UV)-visible part of the solar spectrum is due to the $sp^2$-hybridization of carbon atoms. Due to the isomeric effect, these π-electrons are of a delocalized nature, resulting in high electronic polarizability. An important difference to inorganic solid-state semiconductors lies in the generally poor (orders of magnitudes lower) charge-carrier mobility in these materials, which has a large effect on the design and efficiency of organic semiconductor devices. Most of the organic semiconductors are hole conductors and have an optical band gap around 2 eV, which is considerably higher than that of silicon and thus limits the harvesting of the solar spectrum to a great extent.

Photovoltaic effect, production of electricity by converting photons of the sunlight, occurs in an organic solar cell by the following steps: Absorption of photons of the light in the solar cell and exciton (electron-hole pair) creation; separation of charges and carriers generation from exciton dissociation; transport and then collection of charges by respective electrodes.

HSs have a photoresponsive character and cover a broad spectrum of the visible region. The abundance of functionalities in the framework of HSs allow it to anchor onto the titania surface, which has been successfully employed as a thermally stable natural light harvesting agent. The DSSC (dye sensitized solar cells) device sensitized with HA showed 1.4% PCE (power conversion efficiency) under illumination of 1 sun. An optimum 2.4% PCE was achieved for the same device under illumination of 0.5 sun. The said efficiency was good compared with that of other reported natural sensitizers. Further, the modification in the structure of multifunctionalities of HSs may lead to an enhancement in efficiency. Hence, this work will pave the way for utilizing soil components for the generation of energy from sunlight (Vekariya, R. L., *et al*. 2016).

Photogalvanic solar cells are based on such photochemical reaction, which give rise to high energy products on excitation by a photon. These energy rich products loose energy electrochemically which lead to generation of electricity. HSs play vital role to solubilize the system by forming high molecular weight aggregates or micelles in dilute solution. HSs also enhance the electrical output of the photogalvanic cell by making process of photo ejection of electron easier if the dye and HSs are opposite in charge, the photo ejection of electron will be more pronounced and hence the efficiency of photo galvanic cell will increases. It is observed that electrical output of the cell is found to increase on increasing the concentration of HSs reaching a maximum value. On further increase in their concentration, a fall in photo potential, photocurrent and power of photogalvanic cell is obtained.

**Wastewater Treatment**

HSs are amphiphilic species whose behavior in aqueous solution suggests that they form pseudomicelles aggregates akin to the micelles familiar from synthetic surfactant chemistry. It is thought that humic pseudomicelles can be formed by both intramolecular coiling and intermolecular association, depending on the molecular weight, structural characteristics, and polydispersity of the humic acid in question. The process does not feature a critical micelle concentration. Experimental evidence indicates that metal ions enhance the detergent character of dissolved humic acids by facilitating the coiling and folding of the polymer chains. A recently conceived alternative model suggests that humic acids consist of relatively small subunits that associate through weak molecular interactions. This view appears

to run counter to certain experimental observations, but deserves careful attention. The strong association between metal ions and solid humic acids makes it possible to use an inexpensive commercial grade for the decontamination of polluted water. A continuous elution process through a column packed with humic acids allows for the removal of both heavy metals and organic xenobiotics from aqueous solution (Von Wandruszka, R., 2009).

**Conclusions**

Humic materials are naturally occurring organic compounds, which are produceed by humification process, including humic acids, fulvic acids and humus and may contain small amounts of inorganic metals. Humic acids are soluble in basic medium and insoluble in acidic medium where, fulvic acids are not only soluble in acidic medium but also in basic medium & humus is completely insoluble in both acidic and basic medium. Humic substances mainly originated from ecosphere's soils, water and sediment. The concentration, functional groups, structures of humic and fulvic acids can be varied depend on sources, location of raw materials. Molecular weight, size of humic acids molecules can also vary, as for example, soil based humic acids are larger than aquatic derived humic acids molecules (Choppin, G. R., *et al*. 1985, Stevenson, F. J., *et al*. 1982). HSs are macromolecules & contain important functional group on their skeleton, due to this reason, they are used as active chemicals for the production of a large number of benefical products in Turkey as well as many countries in the World.

**Acknowledgement**

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# Determination of humification degree in peat profiles

C. ZACCONE [(a)*], C. PLAZA [(b)], C. CIAVATTA [(c)], T. M. MIANO [(d)], W. SHOTYK [(e)]

(a) Department of the Sciences of Agriculture, Food and Environment, University of Foggia, 71122 Foggia, Italy
(b) Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas, 28006, Madrid, Spain
(c) Department of Agricultural Sciences, Alma Mater Studiorum University of Bologna, 40127 Bologna, Italy
(d) Department of Soil, Plant and Food Sciences, University of Bari "Aldo Moro", 70126 Bari, Italy
(e) Department of Renewable Resources, University of Alberta, T6G 2H1, Edmonton, Canada
* E-mail: claudio.zaccone@unifg.it

**Keywords:** organic matter, bog, natural archives, paleoenvironmental studies

**Abstract.** The humification process is one of the most intriguing aspects of humus chemistry, especially in waterlogged areas such as peatlands. Structural changes of organic matter occurring in peat during humification have been often evaluated by indirect measurements of the degree of humification. Several methods have been published and a wide variety of techniques used in humification studies in the last century, often providing contrasting results. Although peatlands cover <5% of the Earth's total land surface, they represent the largest global reservoirs of refractory organic carbon and support a unique biodiversity. They also serve as geochemical archives; in fact, ombrotrophic cores from peat bogs have been and are often used to reconstruct environmental changes and human activities over time. Consequently, understanding the extent to which bogs may serve as reliable geochemical archives is of paramount importance in order to be sure that historical information on environmental changes are effectively preserved in peat deposits over time rather than affected by humification processes. To answer such a fundamental question, and to be able to compare data, it is very important to identify what is the best humification index allowing to "quantify" the humification degree and the evolution of peat humification along vertical deep profiles. Nine bogs from four continents, showing different features including climatic conditions, botanical composition and age of formation, have been selected for this study. Four-to-five peat samples from each core have been randomly collected at different depths, and each of them tested using several physical, chemical, spectroscopic and thermal approaches in order to identify the simplest, most valid and cost-effective method to be applied in geochemical and paleoenvironmental studies.

## Introduction

The humification process, whereby plant and microbial remains are transformed into more recalcitrant humic substances (HS) through biochemical and abiotic pathways, is one of the least understood and most intriguing aspects of humus chemistry [1]. Understanding the evolution of organic matter (OM) residues into HS is of key importance in elucidating carbon (C) biogeochemistry, especially in waterlogged areas such as peatlands. In fact, because in these environments the rate of biomass production is greater than the rate of decomposition [2], they represent the largest global reservoirs of refractory organic C.

Structural changes of OM occurring during the humification process in peatlands have been generally evaluated by indirect measurements of the degree of humification. Besides traditional and more or less objective and reproducible methods, such as the von Post humification scale [3] or the visual estimation of the relative proportions of fiber structures [4], several methods have been suggested and reviewed [5-8] and a wide variety of geochemical, spectroscopic and molecular techniques have been used in humification studies, often providing contrasting results.

Ombrotrophic peat bogs are unique archives of past environmental conditions because their genesis is directly linked to the atmospheric conditions occurring during peat formation [2, 9]. For at least two centuries, peat cores have been recognized as an excellent record of environmental changes. Although hundreds of studies have been carried out in the last decades using ombrotrophic bogs in order to reconstruct past climatic, vegetational and ecological changes [10-12], as well as the historical trends of organic and inorganic pollutants due to anthropogenic activities [13-15], scientific literature is still rather controversial about the role of ombrotrophic bogs as reliable record. For example, variations in mineral matter content and composition (e.g., a peak in Pb, Hg or other heavy metals at a certain depth) within a given peat profile could not only be the result of changing rates of atmospheric dusts deposition, but also the consequence of differences in the rate of plant growth or peat accumulation, of a change in the botanical composition of peat-forming plants, as well as differences in peat decomposition, humification and mineralization degree over time [16, 17]. Therefore, understanding if all these "paleo-information" are effectively preserved in peat deposits during humification becomes an essential aspect to be tested before using bogs as archive.

Several approaches have been made to compare decomposition proxies in peat; at the same time, most studies compare a small number of humification proxies or indices, and neglect possible changes due to differences in peat-forming plants, pedoclimatic conditions and peat age. Finally, also when few humification methods were compared, this comparison was carried out among cores from the same geographical area or peats having similar features.

The main aim of this work is to identify the simplest, most valid and cost-effective humification index able to quantify the humification degree and the evolution of peat humification in geochemical and paleoenvionmental studies, i.e., along vertical, deep profiles, often consisting of hundreds of 1-to-3 cm peat slices. Identifying the extent to which

changes in a certain signal (e.g., trace element and PAH concentration) in peat reflects mineralization and humification processes rather than changing rates of atmospheric deposition or vegetational changes is of paramount importance, especially considering that ombrotrophic cores from bogs have been and are often used as a window on the past.

**Materials and methods**

**Sites and samples selection.** Nine bogs from four continents (America, *n* = 3; Asia, *n* = 1; Europe, *n* = 4; Oceania, *n* = 1) have been selected for this study [*i*) Bracebridge Inlet, Canada; *ii*) Sifton, Canada; *iii*) Harberton, Argentina; *iv*) Fleck's Loch, Scotland; *v*) Loch Laxford, Scotland; *vi*) Etang de la Gruère, Switzerland; *vii*) Posta Fibreno, Italy; *viii*) Kalimantan, Indonesia; *ix*) Kopouatai, New Zealand].

These peat bogs show different features including climatic conditions, botanical composition (from mosses to shrubs or trees), peat accumulation (from few cm to several meters) and age of formation (from few years to several millennia).

**Determination of humification proxies.** Four-to-five peat samples from each core have been randomly collected at different depths, and each of them tested using several physical (depth, density, age dating, ash content), chemical (elemental analysis and atomic ratios, extraction and determination of humified C fractions), spectroscopic ($^{13}$C CPMAS NMR, CF-IRMS, UV-Vis and molecular fluorescence) and thermal (TG-TGA) approaches in order to identify the simplest, most valid and cost-effective method to be applied in geochemical and paleoenvironmental studies.

**Results and Discussion**

Data show that, surprisingly, the C/N ratio seems to be uncorrelated with most (if not all) humification indices, and the same is true also density and $E_4/E_6$ ratio. The signature of stable isotopes is promising, but it can be used only when peat is quite homogeneous in terms of botanical composition. On the opposite, the remaining chemical (H/C, O/C, $C_{HA}/C_{FA}$, $C_{HA}$/TEC), spectroscopic (unsubstituted alkyl-C/O substituted alkyl-C, unsubstituted alkyl-C/O, N substituted alkyl-C, $sp^2$-hybridized C/O, N substituted alkyl-C, $SUVA_{254}$, $\varepsilon_{280}$, $\varepsilon_{540}$, HIX) and thermal ($Exo_1$, $Exo_2$, $Exo_2/Exo_1$) indices, instead, are quite consistent among each other.

Despite the different climatic conditions, botanical composition, depth and age of peat formation, the H/C ratio seems to be the simplest, most widely valid and cost-effective humification proxy and should find universal application to multi-proxy geochemical and paleoenvironmental studies.

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# Effects of elevated atmospheric $CO_2$ on soil organic matter: no changes in pools, but increased fluxes and accelerated cycles

YAKOV KUZYAKOV [a,b]

(a) RUDN University, Moscow, Russia
(b) Institute of Environmental Sciences, Kazan Federal University, 420049 Kazan, Russia
* E-mail: kuzyakov@gwdg.de +49-551-401-33235

**Keywords:** Elevated atmospheric $CO_2$; Belowground processes; Rhizodeposition; Carbon and nitrogen cycling; Microbial growth; Enzyme activities

**Abstract.** Atmospheric change encompassing a rising carbon dioxide ($CO_2$) concentration is one component of Global Change that affects various ecosystem processes and functions. The effects of elevated $CO_2$ ($eCO_2$) on processes belowground – in soil – are little understood because they are based on complex interactions among various ecosystem components such as net primary productivity inputs and the various living and dead soil carbon (C) and nutrient pools. Following a brief overview of the approaches to investigate the impacts of $eCO_2$, we show that all effects in soil are indirect: they are mediated by plants through increased net primary production and C inputs released by roots due to higher photosynthesis under $eCO_2$, and less water uptake. Based on literature data, we summarize the impacts of $eCO_2$ on 1) cycling of C and nitrogen (N), 2) microbial growth and enzyme activities, 3) turnover of soil organic matter and induced priming effects including N mobilization / immobilization processes, and 4) associated nutrient mobilization from organic sources,

Higher C input from plants under $eCO_2$ is compensated by faster C turnover due to accelerated microbial growth, metabolism and respiration, higher enzymatic activities, and priming of soil C and N pools. We compare the effects of $eCO_2$ on changes in pools with that in fluxes: C stocks in soil vs. C input belowground, microbial biomass vs. $CO_2$ efflux from soil and vs. various microbial activities and functions, dissolved organic matter content vs. its production, etc. Based on these comparisons, we generalize that $eCO_2$ in the atmosphere will have very small effects on these pools, but will strongly increase the fluxes. This combination – constant pools but intensified fluxes – accelerated biogeochemical cycles under elevated $CO_2$.

# TOPIC 1.
# “ENVIRONMENTAL CHEMISTRY - DYNAMICS – ROLE OF HUMIC SUBSTANCES AND NATURAL ORGANIC MATTER”

# Characterization of soil humic acids, extracted from soil of the Gronfjord area, Svalbard archipelago, by $^{13}$C NMR spectroscopy

E. ABAKUMOV [(a)*]

(a) Department of Applied Ecology, Saint-Petersburg State University, 199178, Saint-Petersburg, Russia
* E-mail: e_abakumov@mail.ru, e.abakumov@mail.ru

**Keywords:** soils, humic acids, Svalbard

**Abstract.** For the first time soils of the Gronfjord area, Svalbard achripelago, have been investigated in terms of soil organic matter content and structural characterization. Lithosols, Cambisols, Histiosols and Cryosols investigated demonstrated low degree of the humic acids aromaticity, which indicated low organic matter stabilization rate. Essential stocks of organic matter, contained in soils of this Arctic archipelago estimated to be easily decomposable in case of rapid climated changes.

## Introduction

Polar soils are considered as essential stock of organic carbon, which is now relativelly stabilized due to permafrost effect [1]. Current estimation of carbon, stored in northern circumploar soils reaches 1307 Pg [2], but these estimatiom is not precise due to the reason that evaluations are based on the very clusteded data on soil organic carbon, this caused mainly by logistic difficulties in Arctic regions investigated. Average stock of soil carbon in polar soil are essentially high in polar soil than in boreal ones [1] but the quality and structural composition of the humus of the Arctic are still underinvestigated. The aim of the present study was to evaluate by solid state $^{13}$C NMR spectroscopy the structural composition of the humic acids (HAs), isolated from the soils of Svalbard.

## Materials and methods

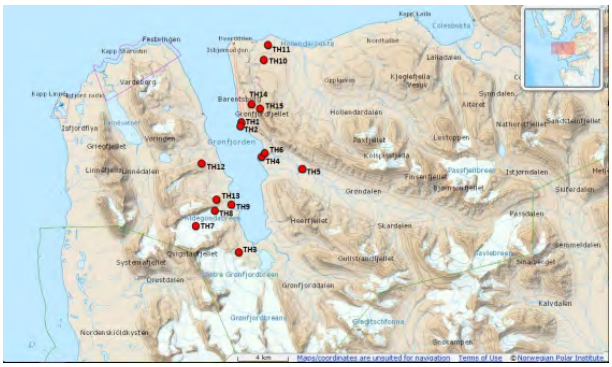



**Figure 1.** Location of soil sample plots in Grofjord area.

**Study sites.** Soils of the Svalbard archipelago were sampled during 2017 seasonal Russian Expedition to the Gronfjord regions, close to Barentsburg settlement. Lithosols, Cambiosols< Histosols and Cryosols were selected for isolation of the humic substances. Location of the sampling site is given in Fig. 1.

## Isolation and Characterization of the HAs.

Routine soil analyses were conducted according [3]. HAs from fine earth of the topsoil layes were isolated according the standart IHSS procedure [4]. Solid-state $^{13}$C NMR spectra of HAs were measured with a Bruker Avance 500 NMR spectrometer (Karlsruhe, Germany) in a 4-mm $ZrO_2$ rotor. The magic angle spinning speed was 20 kHz in all cases and the nutation frequency for cross polarization was u1/2p 1/4 62.5 kHz. Repetition delay and number of scans were 3 seconds. Groups of structural compounds were identified by their chemical shifts, [3]: alkyl carbon (10 – 45 ppm), O/N-alkyl carbon (45-110 ppm), aryl/olefin carbon (110-160 ppm), carbonyl/carboxyl/amide carbon (160 - 220 ppm)

## Results and Discussion

Soils investigated showed essential accumulation of organic carbon only in topsoil layers. Average content of carbon variates from 3 to 8 %. All samples were free of carbonates and demostrate slightly acid reaction of the fine earth. The C/N ratio were about 12-25 and were related to various organic matter decomposition rate.

$^{13}$C NMR spectra of humic substanses from soils studied (Fig. 2) were typical for published in the literature for such materials [5-8] .

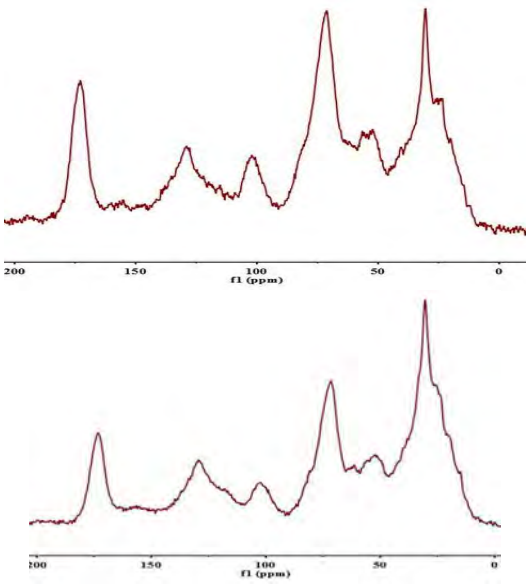



**Figure 2.** $^{13}$C NMR spectra of the HAs studied.

Average aromaticity rate for HAs invetsigated was about 17-31%. The variation was related with the type of organic horizons – the lower values was characteristic for Histic soil horizons, while the highest values were revealed for the organo-mineral horizons of Cambisols. Tha HAs demonstrate well implemented carboxylic peak and areas, related to the various types of alkyl carbon. Alkyl aliphatic organic compounds prevail over aryl ones and this indicated low degree of soil organic matter stabilisation. The same data were previsouly published for Yamal region [6], Kolyma Lowland [7] and Komi Republic. The characteristic feature of the Svalbards soils can be assighed to the increased content of aromatic carbon species for Cambisols, while the Histosols are comparable with the same soils in Komi republic [8].

By comparison with soils with Antarctic provenance [8] on the same latitudes, it will be evident that in case of Svalbard the degree of aromaticity is higher. This feature can be interpreted as result of the higher diversity of the humification precursors in Northern-Polar biome, where dozens of representatives of the higher plant could serve as humification precursors sources.

**Conclusions**

Soils of the Gronfjord areas, Svalbard archipelago, demostrate essential stock and content of organic carbon. This organic matter is characterised by low humification rate, determined firstly by the severe climatic conoditions, and secondary, by the possible organic remnantns compositions. This is the reason why the HAs are comparable with those, isolated from the most southern soils of Komi, Yamal and Kolyma. At the same time, the Cambisols demonstrate higher degree of the HAs aromaticity, wich can be related with specificity of these type of soil formation. In general, low degree of soil organic matter stabilization in investigated polar soils is estimated. Stucrtural components of the HAs are represented, mainly, by alkyl carbon, and due to this reason in case of rapid climate changes, organic matter of polar soils will be faced to fast mineralization process.

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# Spatial Variability of Humic Acids in Agricultural Soils and Implications on Soil Management and Carbon Sequestration

H. ABDELRAHMAN [(a,b)*], C. COCOZZA [(b)], A. CASTRIGNANÒ [(c)], T. MIANO [(b)]

(a) Soil Science Dept., Faculty of Agriculture, Cairo University, Giza 12613, Egypt
(b) DiSSPA, Università degli Studi di Bari Aldo Moro, Bari 70126, Italy
(c) CREA – Research Unit for Cropping Systems in Dry Environments, 70125 Bari, Italy
* E-mail: hamada@cu.edu.eg

**Keywords:** precision farming, phenolic OH, $E_4/E_6$, soil organic matter

**Abstract.** Spatial variability of soil humic acids was investigated in a 3-ha field in southern Italy. Humic acids (HAs) were extracted, according to IHSS method, from 50 georeferred sampling locations and were analyzed for their chemical and spectroscopic characteristics. Results revealed that none of HAs characteristics (total acidity, COOH, phenolic OH, and $E_4/E_6$) were correlated to soil characteristics (Soil C, N) or crop yield. Accordingly, management plans should consider the field-scale variability in order to minimize the loss of C and nutrients.

## Introduction

Recently, the assessment of spatial variation of soil properties at different scales has driven considerable interest [1, 2]. Majority of studies that investigated spatial variability focused on the variability of land use, topography, vegetation and parent material across the landscape of an ecosystem [2]. However, little attention has been paid to spatial variability on field-scale and evidently not on soil humic acids (HAs).

Humic substances are relatively stable form of soil organic matter (SOM) and are considered important component of the terrestrial C pool. However, the process of humification might be altered in response to changes in environmental conditions and variability in soil characteristics. Accordingly, the objective of this work was to assess the spatial variability of HAs on a filed-scale in relation to other soil characteristics.

## Materials and methods

### Site and sampling

Soil samples were collected from 50 georeferred sampling locations (Fig. 1) in a 3-ha field in southern Italy (41° 27' N, 15° 36' E; 90 m a.s.l.) cultivated with durum wheat (*Triticum durum Desf. cv Claudio*) – chickpea (*Cicer arietinum L. cv. Sultano*) rotation, under organic farming management. Samples were collected after crop harvest, air-dried, ground, 2-mm sieved and then stored for chemical analyses.

### HAs Extraction and characterization

HAs were extracted according the IHSS method [3] then were characterized for total acidity, carboxylic and phenolic functional groups [4] and for their $E_4/E_6$ ratio [5].

Geostatistical (kriging and co-kriging) and correlation methods were used to plot the characteristics of HAs and correlate it to soil main characteristics (soil C and N content and crop yield)

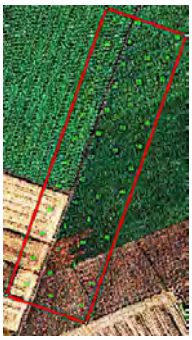


**Figure 1.** 50-georeferred sampling locations in a 3-ha field.

## Results and Discussion

### Spatial variability of soil characteristics

Total soil C ranged between 9.65 and 13.10 g.C .kg$^{-1}$ soil (Fig. 1a) while soil N ranged between 0.7 and 1.15 g.N. kg$^{-1}$ soil (Fig. 1b). The area with lower content of C and N (blue areas in the top of Fig. 1a) was associated with higher clay content (531.3 g.kg$^{-1}$ soil). This finding contradict the commonly accepted idea that larger amounts of soil C is associated with larger content of silt and clay. However, the combined effects of management, climate and other soil characteristics might have impacted SOM accumulation in soil.

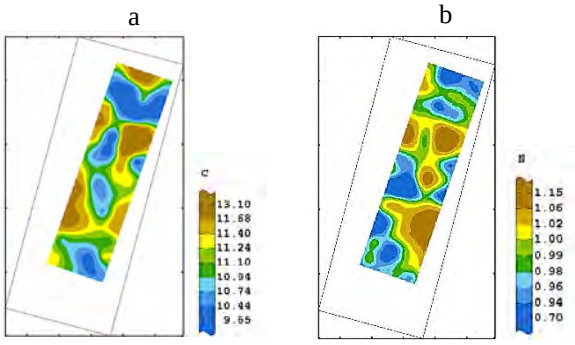



**Figure 1.** Spatial variability of (a) soil total C and (b) total N in a 3-ha field.

### Spatial variability of Humic Acid characteristics

Total acidity of HAs ranged 5.7–11.8 meq.g$^{-1}$ HA, while COOH content in HAs ranged from 3.9 to 5.5 meq.g$^{-1}$ HA. The $E_4/E_6$ ratio ranged 5.39–6.34. The $E_4/E_6$ ratio is governed by the molecular size or weight [5] and high values of $E_4/E_6$ ratio are associated with small- or low-weight molecules, whereas low $E_4/E_6$ ratio indicates a relatively large molecular size or high molecular weight. Figure 2 shows that HAs with higher $E_4/E_6$ were characterized appropriately with lower content of phenolic OH functional groups. Moreover, the HAs total acidity distribution in the field was very similar to the phenolic OH distribution (Fig 2).

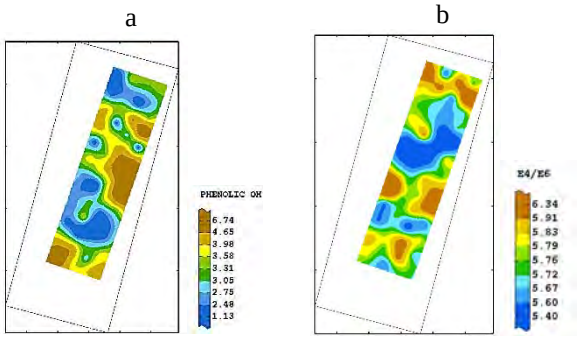



**Figure 2.** Spatial variability of (a) phenolic OH groups and (b) the $E_4/E_6$ ratio of humic acid in a 3-ha field.

Correlation between different soil components and HAs characteristics are reported in Table 1. The correlation matrix revealed that none of the studied parameters was meaningfully correlated to crop yield or other soil or HAs parameters.

**Table 1.** Correlation coefficient among different soil characteristics, HAS characteristics and crop yield

| | Soil C | Soil N | HAs-OH | HAS-TA | $E_4/E_6$ |
|---|---|---|---|---|---|
| Soil N | 0.30 | | | | |
| HAs-OH | 0.05 | -0.01 | | | |
| HAs-TA | 0.06 | -0.05 | 0.98 | | |
| $E_4/E_6$ | 0.04 | -0.18 | -0.08 | -0.08 | |
| Yield | -0.05 | 0.08 | -0.03 | -0.02 | 0.02 |

TA: total acidity, HAs-OH: phenolic OH in HAs

### Implications for management and C sequestration

It is important to underline that soil fertility is not only influenced by the quantity of organic matter, but also by its quality. Based on the spatial variability of soil parameters and HAs, zones characterized with more reactive and higher molecular weight HAs is considered more favorable for retaining nutrients and C in soil. Accordingly, applying flat rate fertilization scheme or the use of uniform management might lead to unfavorable consequences on crop production and the environment. Similar studies should be carried out in different fields and under different climatic conditions in order to understand the relationships among soil and HAs characteristics and to find out whether the findings reported here were case-specific.

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# Fourier Transform Infrared spectroscopy analysis of urea intercalated biochar

O. O. ADESANWO [(a)], J. A. FAGBENRO [(b)], M. O. AZEEZ [(a)] , O. J. OLUBISI [(a)*]

(a) Department of Soil Science and Land Resources Management,
Obafemi Awolowo University, Ile Ife, Osun State, Nigeria.
(b) Department of Crop Production, Soil and Environmental Management,
Bowen University, Iwo, Osun State, Nigeria.
* E-mail: oluwasegun557@gmail.com

**Keywords**: FTIR; Municipal biochar; Urea intercalated biochar; Organic Functional group

**Abstract.** Several studies have shown the effectiveness of biochar to improve soil physical and chemical properties. However its nitrogen content is extremely low. FTIR analysis of intercalated biochar was compared with original biochar and the result revealed appearance of so many absorption sites in regions corresponding to amides (3600-3300 $cm^{-1}$), carboxylic acid group (1750-1700), nitrile group (2260-2210 $cm^{-1}$), hydroxyl group (2950 $cm^{-1}$) which were not visible in FTIR result of original municipal biochar. The results confirmed feasibility of improving nitrogen content in biochar through intercalation.

## Introduction

The use of biochar as a sustainable technology to improve fertility of highly weathered or degraded tropical soils is gaining attention globally [1]. Biochar application enhance plant growth by improving soil physical characteristics [i.e., bulk density, water holding capacity, permeability], soil chemical characteristics [i.e., nutrient retention, nutrient availability], and soil biological properties, all contributing to an increased crop productivity [2] and reducing fertilizer requirements [1]. It has also been used to decrease soil $N_2O$ and $CH_4$ emissions through carbon sequestration [3]. The use of biochar is still at experimental stage in Nigeria. Some researchers have used it in form of charcoal with corresponding improvement of both physical and chemical properties of the soil [4]. Arable lands all over Nigeria decline in soil productivity as a result of continuous agricultural practices without application of adequate restorative measures to revitalize the soil [4]. Application of mineral fertilizer on continuous basis has led to nutrient imbalances within the soil system, leaching of $NO_3^-$ from ammonium fertilizers contaminates ground water thus constituting a threat to the environment [4]. Biochar application is a good alternative however, its nitrogen content is extremely low because it is easily lost through the pyrolysis reaction especially at higher temperature. Therefore, improving the nitrogen content in biochar needs urgent attention. Some workers tried to mix nitrogen fertilizer with biochar manually, though with positive effect but highly laborious and homogeneity of the mixture is not certain. In a bid to improve nitrogen use efficiency of some mineral fertilizers especially Urea, they were coated with neem, tar, starch etc. [5]. Apart from using organic materials, inorganic substances such as zeolite, montmorillonite and halloysite minerals have been used to coat Urea thus regulating release of N but these methods are expensive. Intercalation of N fertilizer with biochar or other organic materials have been found to improve both the N content of the soil and N use efficiency of the applied fertilizer in developed countries but the method is yet to be tried in Nigeria. This paper reports characterization study of urea intercalated biochar with the aim of establishing the feasibility of its use on Nigeria soils. The objective of this study was to establish structural differences between Municipal Biochar (MB) and Urea Intercalated Municipal Biochar (UIMB) using chemical methods.

## Material and Methods

**Preparation of Urea Intercalated Biochar.** Urea solution was prepared by dissolving 20 g of the fertilizer in 20 ml of deionized water i.e ratio of 1:1 w/v (20 g: 20 ml). The mixture was mixed thoroughly and the slurry heated on a hot plate at 105º C with constant stirring until the crystals dissolved completely. About 20 g of Municipal biochar was thoroughly mixed with the urea solution and dried in a hot air oven at 65º C till the moisture was completely evaporated. One gram of adhesive polymer (starch) was added to the mixture and air dried [6]. The product from these procedures was powdered and used as a urea-intercalated biochar fertilizer (UMBF). The following physical and chemical properties of both the biochar and urea intercalated biochar such as pH, organic matter, total N, available P and Electrical conductivity were determined.

**FT-IR Analysis.** The biochar and resultant intercalated fertilizer product was analyzed using Fourier Transform Infrared Spectroscopy (FTIR) in the range of 400 to 4000 $cm^{-1}$ with Attenuated Total Reflectance (ATR) technique at the Institute of Agriculture, Research and Training (IAR&T) Ibadan.

## Results and Discussion

### Selected properties of urea fertilizer, municipal biochar and urea intercalated biochar

Results of some chemical properties of urea fertilizer (UF), municipal biochar (MB) and urea intercalated biochar were presented in Table 1.

The pH values of 8.5, 8.0 and 8.7 were recorded for urea fertilizer, municipal biohar and intercalated material respectively. The values fall in the alkaline region thus confirming its potential as a liming material with the exception of urea fertilizer which usually leave acidic residue due to the biochemical transformation of amide to

nitrate with release of proton into the soil system. The nitrogen content of municipal biochar was very low as previously was reported [1, 2]. Great improvement in nitrogen content was observed when the biochar was intercalated with Urea fertilizer.

**Table 1.** Selected properties of Urea (UF) and Urea Intercalated fertilizer (UMBF).

| Treatment | pH (0.01 M $CaCl_2$) | Total Nitrogen (%) |
|---|---|---|
| MB | 8.0 | 3.36 |
| UF | 8.5 | 46 |
| UMBF | 8.7 | 24.5 |

The FTIR results of municipal biochar and urea intercalated municipal biochar were presented in Figures 1 and 2.

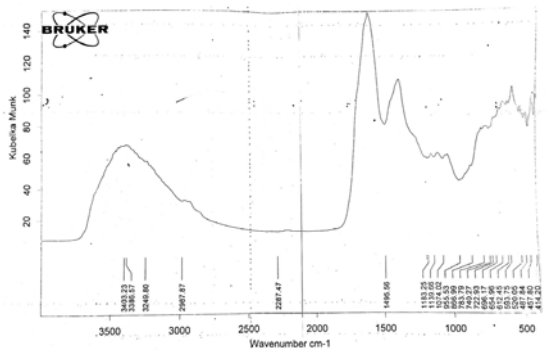



**Figure 1.** FTIR spectra (4000–400 $cm^{-1}$) for Municipal biochar.

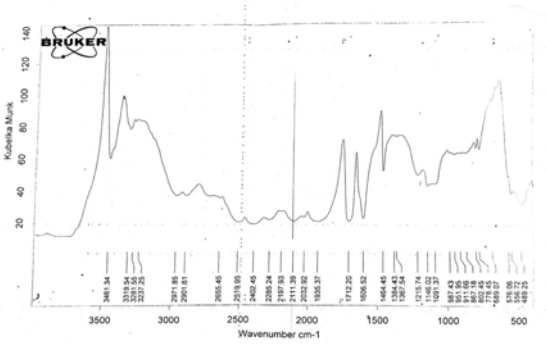



**Figure 2.** FTIR spectra (4000–400 $cm^{-1}$) for urea-intercalated municipal biochar (1:1).

The FTIR spectra clearly indicated OH stretch at wavelength between 3000 – 3500 $cm^{-1}$ [6] , the presence of aliphatic group of C – H wavelength at 2287.47 $cm^{-1}$ [6] and carbony group between 500 – 1000 $cm^{-1}$. Additionally, the FTIR spectrum of urea intercalated fertilizers revealed appearance of so many absorption sites in region corresponding to amides (3600-3300 $cm^{-1}$), carboxylic acid group (1750-1700 $cm^{-1}$), nitrile group (2260-2210 $cm^{-1}$), hydroxyl group (2950 $cm^{-1}$) which were not visible in FTIR result of original municipal biochar. The dominant peak at 1630 $cm^{-1}$ in municipal biochar spectra belongs to COOH group. It is therefore envisaged that adsorption of nitrogen on organic functional groups will slow down the release of N from the fertilizer during the plant cultivation.

The results of this study confirmed feasibility of improving nitrogen content in biochar through intercalation. In addition the presence of many functional groups in urea intercalated municipal biochar fertilizer is an indication of its potential for a slow release of N, however more studies should be carried out to ascertain its agronomic effectiveness.

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# Separation of the constituents of fulvic acids by HILIC and RP-HPLC

M. AOYAMA [(a)*], D. SAWAME [(a)]

(a) Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki 036-8561, Japan
* E-mail: aoyamam@hirosaki-u.ac.jp

**Keywords:** EEM fluorescence spectroscopy, fulvic acids, HILIC, RP-HPLC, two-dimensional HPLC

**Abstract.** In order to develop a chromatographic method to separate the consitituents of fulvic acids (FAs), the applicability of hydrophilic interaction chromatography (HILIC) using an amide-bonded silica column and reversed phase (RP) HPLC using pentabromophenyl-bonded silica column was investigated. When HILIC and RP-HPLC were applied separately, FAs were resolved into more than 70 peaks by either method. By performing two-dimensional HPLC combining RP-HPLC with HILIC, a total of 500 or more peaks were recognized. The measurments of UV-vis absorption spectra and excitation-emission matrix fluorescence spectra of the major peaks indicated that FAs were composed of the constituents with various spectroscopic features.

## Introduction

Fulvic acids (FAs) are humic substances that are soluble in both alkali and acid and play an important role in the environment, such as transport of trace metals. However, details of the chemical structure of FAs have not been clarified. Recent research has suggested that humic substances are formed due to the association between heterogeneous, relatively small molecules [1-3]. Aoyama [4] found that humic acids and FAs were separated into many constituents when hydrophilic interaction chromatography (HILIC) was applied. We improved the HPLC method by applying both HILIC and reverse-phase (RP)-HPLC and successfully separated FAs into at least 500 constituents.

## Materials and methods

**Materials.** FAs samples prepared from diffent soils and lake water samples were used.

**HILIC and RP-HPLC.** HILIC was carried out using two amide-bonded silica columns (4.6 mm I. D. × 250 mm) linked in series. The mobile phase was the mixture of acetonitrile and 0.02 M ammonium formate, and the acetonitrile concentration was linearly decreased from 85% to 65%. RP-HPLC was carried out using a pentabromophenyl-bonded silica column (4.6 mm I. D. × 250 mm). The mobile phase was the mixture of acetonitrile and 0.2% formic acid, and the acetonitrile concentration was linearly increased from 5% to 65%. Chromatograms were monitored using a photodiode array (PDA) detector and a fluorescence detector. The PDA detection wavelength was set at 265 nm and the excitation and emission wavelengths for fluorescence detection were set at 265 nm and 440 nm, respectively.

**Two-dimensional HPLC.** HILIC was performed using a preparative amide-bonded silica column (10 mm I. D. × 150 mm) and the eluents were collected to obtain the HILIC fractions. The fractions were analyzed by RP-HPLC as described above. UV-vis absorption spectra were recorded on-line during the RP-HPLC analysis over the range 200 to 700 nm using the PDA detector. The major fluorescence- detected peaks were collected manually and subjected to the measurement of excitation-emission matrix (EEM) fluorescence spectra. The spectra were recorded over the excitation and emission wavelength ranges of 250-500 nm and 300-550 nm, respectively.

## Results and Discussion

Figure 1 shows the examples of HILIC and RP-HPLC chromatograms of the FAs. When HILIC was applied to FAs, 70 or more peaks were observed with both UV and fluorescence detections (Figure 1A). RP-HPLC of FAs also gave 70 or more peaks regardless of UV detection or fluorescence detection (Figure 1B). For both HILIC and RP-HPLC, the elution profiles of the FAs varied depending on the source and extraction methods.

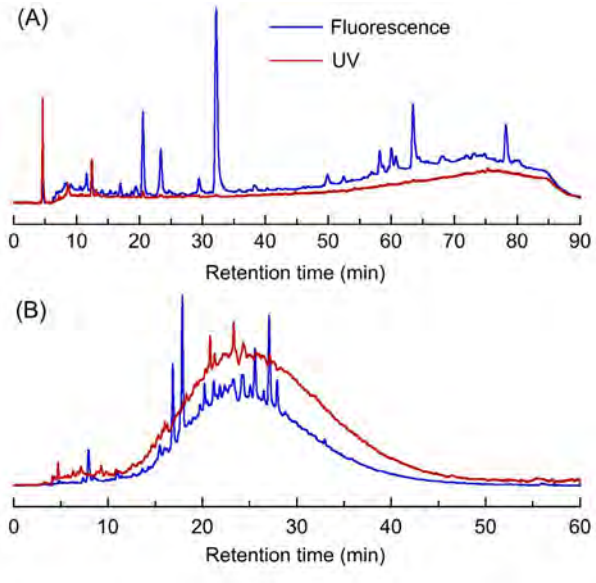



**Figure 1.** Typical HILIC (A) and RP-HPLC (B) chromatograms of FA.

Figure 2 shows a chromatogram when preparative HILIC was applied to the same FA sample as in Figure 1. The elution profile was slightly different from that when using the analytical columns. Ten fractions were collected and designated as F1 to F10 as indicated in Figure 2. The fractions F1 and F8-10 were colored brown, and in particular the F10 fraction was found to be darkly colored.

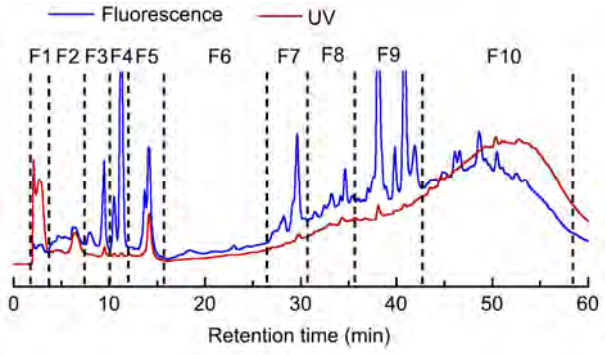



**Figure 2.** Preparative HILIC chromatogram of FA.

Each fraction was resolved into many distinct sharp peaks by RP-HPLC (selected chromatograms shown in Figure 3). For the fractions F7-10, a broad peak was observed together with sharp peaks. For all the fractions, a total of more than 500 peaks were recognized regardless of UV detection or fluorescence detection. However, the UV-detected peak and the fluorescence-detected peak were not necessarily eluted at the same retention time.

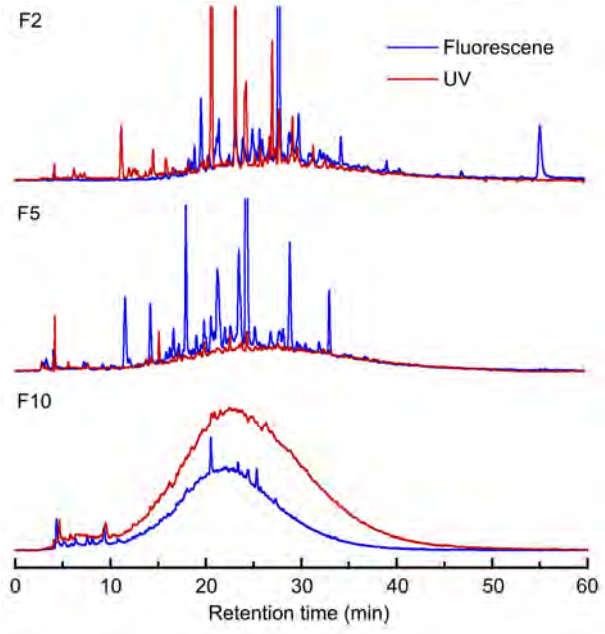



**Figure 3.** RP-HPLC chromatograms of the HILIC fractions.

The on-line measurement of UV-vis absorption spectra revealed that most of the UV-detected sharp peaks had absorption only in the UV region, whereas UV-detected broad peaks exhibited featureless spectra, with the absorbance decreasing gradually from the UV region to the visible region. This fact indicates that FAs are composed of UV-absorbing colorless substances and dark-colored substances, the latter can be regarded as “true humic substances”.

EEM fluorescence spectra were measured for the major fluorescence-detected peaks from each fraction (a total of 70 peaks) and the excitation and emission wavelengths of the fluorescence maxima were plotted in Figure 4. The excitation and emission wavelengths of the fluorescence maxima varied among the peaks, indicating that FAs consisted of molecules with different fluorophores. The molecules with fluorescence maxima at the excitation/emission wavelengths = 260-290/320-350 nm are considered to be protein-like substances.

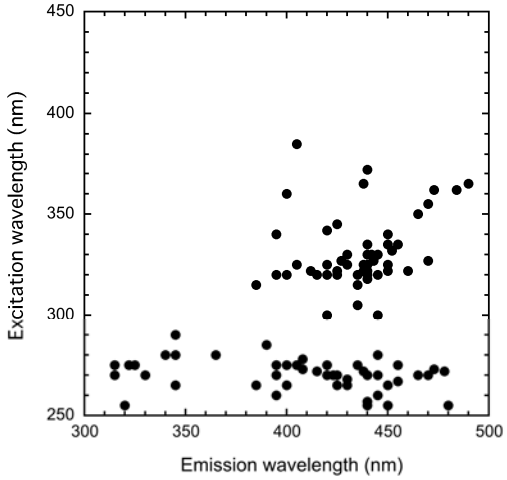



**Figure 4.** Excitation and emission wavelengths of the fluorescence maxima observed for the EEM fluorescence spectra of major fluorescence-detected peaks.

## Conclusion

The present study demonstrated the usefulness of HILIC using the amide-bonded silica column and RP-HPLC using the pentabromophenyl-bonded silica column for separating the consituents of FAs. The separation mechanism is different between HILIC and RP-HPLC. Thus, two-dimensional HPLC combining RP-HPLC with HILIC drastically improved the separation power. By applying this technique, we succeded in separating the FA into at least 500 constituents. As a next step, we aim for the comprehensive analysis of FA constituents by combining this technique with tandem mass spectrometry.

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# Characterization of soil organic Matter and PAHs concentration of Antarctic soils

D. APOSTOLOVA [(a)]*, E. FILCHEVA [(b)], A. BECHTEL [(c)], K. MARKOVA[(a)], I. KOSTOVA[(a)]

(a) Department of Geology, Paleontology and Fossil Fuels, Sofia University, 1504 Sofia, Bulgaria,
(b) Institute of Soil Science, Agrotechnologies and Plant Protection "N. Poushkarov", 1080 Sofia, Bulgaria
(c) Department of Applied Geosciences and Geophysics, University of Leoben, A-8700 Leoben, Austria
* E-mail: dapostolova@gea.uni-sofia.bg

**Keywords:** antarctic soil, PAHs, humic acids, fulvic acids

**Abstract.** Three soil samples from sites situated near to the Bulgarian Antarctic Base (BAB) have been studied for better understanding of the soil formation processes and for determining the concentration of polycyclic aromatic hydrocarbons (PAHs). The content and composition of soil organic matter show that the processes of humus formation are in a very initial stage. Twelve from totally sixteen PAHs defined by USEPA as priority pollutants are found in the studied soils. The highest concentration of PAHs is established in the first sample (1s_2016) which is located nearest to the BAB.

## Introduction

The Bulgarian Antarctic Base "St. Kliment Ohridski" (BAB) is situated at the eastern coast of the South Bay of the Livingston Island, South Shetland Archipelago. There are three more detailed investigations describing the composition, properties and some ecological aspects concerning soil sampling around BAB at Livingstone Island [1, 2, 3]. Soil organic matter (SOM) content in Antarctica is low. The sources of SOM are scarce vegetation (lichens, mosses and rarely grass) remains and bird, and penguin excrements, which play an important role on the humus formation processes.

During the last years scientists pay a great attention to human impacts on the Antarctic environment. Apart from regular human activity accidental contaminations and PAHs emission from regional sources are also possible. Since the soils are a major recipient of contamination from anthropogenic sources, there have been a number of studies focusing on various aspects of soil disturbances [4]. The low temperatures and abundant snow/rain events in some regions such as the Southern Shetlands and Antarctic Peninsula, favor the atmospheric deposition of organic pollutants and their persistence in soils, which are only free of snow/ice cover in coastal areas and for few weeks/months during the austral summer. High concentrations of polycyclic aromatic hydrocarbons (PAHs) have been measured in soil and sediment samples collected close to the research stations or where oil spills, fossil fuel combustion, or sewage discharge have been occurred [5, 6].

## Materials and methods

Soil samples from the Livingstone Island were collected during the 25$^{th}$ Bulgarian Antarctic expedition in the vicinity around BAB. The first sample (Sample 1s_2015) was collected north-east from the BAB, with 14 m elevation. The soil sample point was clear of snow, ice and sample has a gray-brown color. Mosses and lichens are observed around the sampling area. The second sample (sample 15s_2016) was taken from the Johnson Dock area, on a cliff over the sea and with 8-10 m elevation. The soil sample was dug under the grass and has a dark brown color. Thick grass vegetation, lichens, mosses and remains of mussels can be observed near to the place of sampling. The third sample (sample 19s_2016) was taken to the south-west of the base from Punta Esperides area, in the highest point with 95-98 m elevation. Many mosses and rocks are observed at the sampling site. The soil has a dark brown color. The rock bottom of the first and second soil samples are represented by sedimentary and the third by volcanic rocks.

Total organic carbon and composition of soil organic matter were determined according to the modified method of Turin (120 °C, 45 min, with catalyst $Ag_2SO_4$) and method of Kononova and Belchikova [7, 8]. Gas chromatography–mass spectrometry (GC-MS) was applied for identification and quantification of PAHs in studied samples.

## Results and Discussion

The obtained data are presented in Table 1. The results concerning content and composition of organic matter show that the three soil samples are different to a large extent. Total organic carbon content follows the descending order: 15s_2016 > 19s_2016 > 1s_2016, as already suggested by the color of the samples, reflecting differences in vegetation and elevation of the sampling site. Humic acids prevail over the fulvic acids in samples 15s_2016 and 19s_2016, especially in sample 15s_2016, where thick grass vegetation, lichens, mosses and remains of mussels were observed. In contrast, to these samples, the soil organic matter in the first sample is of fulvic type.

**Table 1.** Content and composition of soil organic matter (a-% of soil mass, b - % of the total carbon)

| Sample | Ct,% | Ch,% | Cf,% | Ch/Cf | Cres.,% |
|---|---|---|---|---|---|
| 1s_2016 | 0.96 | 0.00 | 0.22/22.92 | - | 0.74/77.08 |
| 15s_2016 | 4.12 | $1.45^a/35.19^b$ | $0.70^a/16.99^b$ | 2.07 | $1.97^a/47.82^b$ |
| 19s_2016 | 2.70 | $0.90^a/33.33^b$ | $0.36^a/13.34^b$ | 2.50 | $1.44^a/53.33^b$ |

The low molecular humic substances show a low degree of condensation. The ratios Ch/Cf and $E_4/E_6$ describe more condensed humic acids for sample 19s_2016. An interesting result was obtained for the humic substances extracted with 0.1*M* $Na_4P_2O_7$+0.1*M* NaOH and those extracted with 0.1*M* NaOH for the both soil samples – 15s_2016 and 19s_2016. Nearly half of the humic substances were extracted with 0.1*M* NaOH for the sample 15s_2016 compare to those for the pyrophosphate extract, probably due to the remains of mussels. The extracted parts of humic acids are equal for both extracts in the studied sample 19s_2016 and 20% of humic acids are found to be bound with Ca.

The twelve from sixteen PAHs defined by USEPA as priority pollutants were found in studied soils including phenanthrene, anthracene, fluoranthene, pyrene, benzo(ghi)-fluoranthene, benzo(a)anthracene, triphenylene, chrysene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene and perylene. The concentrations of all US EPA PAHs increase from 15s-2016 (0.93 μg/g TOC) to 19s_2016 (1.04 μg/g TOC), and highest values are found in sample 1s_2016 (closest to BAB) (6.38 μg/g TOC). In almost all samples PAHs comprised compounds with 2–5 rings. Five ring PAHs were not found in sample 19s_2019. PAHs of high molecular weight (HMW) (i.e., 4, 5 ring PAHs) are dominate in all studied samples.

This type of PAHs distribution is common for most fossil fuels, and was previously registered in Antarctica soils and marine sediments [9]. It is generally assumed that their origin is Special Antarctic Blend (SAB) which contains predominantly 2-3 ring PAHs [9]. Heavier PAHs are formed in emissions after incomplete combustions. Human activity which is related to combustion of fossil fuels and their products, and accidents as fire in the Brazilian Scientific Antarctic Station in 2012 lead to PAHs spreading which can migrate adsorbed on particulate matter over large distances [10], [11]. The obtained data from this study suggest that the high concentration of PAHs in 1s_2016 sample could be explained by emissions from the countries surrounding the Antarctica or by potential local sources.

To clarify the humus matter formation process and possible source of PAHs in Antarctic soils is necessary to continue this type of investigations.

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# Influence of the soil type and land use on the amount and quality of soil organic matter

G. BARANČÍKOVÁ [(a)*], J. HALAS [(a)], E. TOBIAŠOVÁ [(b)], E. GÖMÖRYOVÁ [(c)], J. MAKOVNÍKOVÁ [(a)], Š. KOCO [(a,d)], R. SKALSKÝ [(a)]

(a) National Agricultural and Food Centre - Soil Science and Conservation Research Institute, 82713 Bratislava, Slovakia
(b) Department of Soil Science, Slovak Univeristy of Agriculture in Nitra, 94976 Nitra, Slovakia
(c) Department of Natural Environment, Technical University in Zvolen, 96053 Zvolen, Slovakia
(d) Department of Geography and Applied Geoinformatics, Prešovská univerzita v Prešove, 08116 Prešov, Slovakia
* E-mail: g.barancikova@vupop.sk

**Keywords:** soil organic carbon, humic acids, soil types, ecosystems

**Abstract.** Content of soil organic carbon (SOC) and chemical structure of humic acids of soil organic matter (SOM) was studied at three soil types (Haplic Chernozem, Eutric Cambisol, Luvic Stagnosol) in three different ecosystems (forest, meadow and agro-ecosystems). Obtained data show significant differences of SOC content among ecosystems. Difference in SOM quality among ecosystems was found only in the Haplic Chernozem. Humans activity has significant effect on SOC content in all studied soil types, however SOM quality was effected by different land use in Haplic Chernozem only.

## Introduction

Soil organic matter is a very important soil parameter influencing all chemical, biological, physical properties and from this reason is a key parameter of soil quality. SOM or more precisely, soil organic carbon (SOC) is important element in terms of the carbon cycle. Terrestrial ecosystem is one of the largest reservoirs of SOC [1]. SOM is very complicated system of humic substances (HSs) which represent significant part of organic carbon in the global carbon cycle. Very important fraction of HSs are humic acids (HAs) with excellent sorption properties, which depend on their chemical composition [6]. SOC represents quantitative parameter of SOM and ratio of carbon of humic and fulvic acids ($C_{HA}/C_{FA}$), and chemical composition of HA can be considered as qualitative parameters of SOM. In natural conditions, content and quality of SOM is influenced mainly by parent material, climate and geographical parameters. However in disturbed ecosystems (intensive agriculture) mainly content, but also quality of SOM can be affected by land use and soil management. Objective of this study is to assess if natural conditions (soil type) has greater effect on content and quality of SOM than human been activity (land use).

## Material and methods

The experiment included three soil types (Haplic Chernozem, Eutric Cambisol, Luvic Stagnosol), each in the different type of ecosystems (forest (FE), meadow (ME), and agro-ecosystems (AE)). Experimental sites are located on south-western, north-eastern and central part of Slovakia and represent contrasting climate and soil conditions. The soil samples of different localities and ecosystems were sampled to a depth of 0.10 m. Organic carbon content and total nitrogen were determined by dry combustion on C, N analyser. Groups of HSs were determined according to Kononova-Beľčikova method. Humic acids were isolated according to the modified method established by the IHSS. In HA elemental analysis and $^{13}C$ NMR spectra were determined [5].

## Results and Discussions

In all ecosystems the highest content of SOC was found in Haplic Chernozem and the lowest SOC in Luvic Stagnosol (Table 1). From ecosystems point of view, the highest content of SOC was determined in the forest soils and dropped in order FE>ME>AE. The same pattern was found also for total nitrogen, because between SOC and Nt significant correlation was found (R=0.82**). Generally, higher input of plant and root residues in forest and grassland ecosystems has stabilizing effect on the SOC stock in the top soil [3].With SOC content closely related the amount of isolated HAs. The higher SOC content, the higher amount of HAs were isolated (R=0.82**). According to the soil types, highest amount of HA were isolated from Chernozem ($C_{HA}/C_{FA}>1$) and according to the ecosystems, from forest soils (Table 1). In the evaluation of the chemical structure of HAs, elemental (C, H, N) analysis is a basic chemical parameter, because it reflects degree of SOM humification. From data in Table 2, the highest amount of carbon and the lowest amount of hydrogen in Chernozem HAs, mainly in arable land, is clear. Among ecosystems, significant differences only in Chernozem HAs were found (Table 2). Very important parameter calculated from the elemental composition is the H/C ratio. A low value of H/C indicates high stability and a high condensation degree of HAs and a high humification degree of SOM [2].

The lowest value of H/C ratio and therefore the highest humification degree was determined in Chernozem HAs on AE (Table 2). Data of elemental composition are in close relation with parameters calculated from $^{13}C$ NMR spectra. The lowest amount of aliphatic and the highest amount of aromatic carbon, thus the highest degree of aromaticity ($\alpha$) were found in HA

Chernozem on AE (Table 2). As in case of elemental composition, differences in $^{13}C$ NMR values of HAs among ecosystems were determined only in Chernozem.

**Table 1.** Basic parameters of soil organic matter

| Soil type/ use | SOC (%) | Nt (%) | $C_{HA}/C_{FA}$ | $Q_{4/6}$ | HA yield (mg/100g) |
|---|---|---|---|---|---|
| 1 FE | 4.56 | 0.393 | 0.71 | 5.12 | 1.01 |
| 1 ME | 3.01 | 0.310 | 0.86 | 4.74 | 0.99 |
| 1 AE | 1.86 | 0.193 | 0.92 | 5.37 | 0.49 |
| 2 FE | 3.04 | 0.225 | 0.65 | 5.12 | 1.25 |
| 2 ME | 2.46 | 0.278 | 0.57 | 4.90 | 0.65 |
| 2 AE | 1.94 | 0.184 | 0.96 | 4.65 | 0.39 |
| 3 FE | 6.37 | 0.620 | 2.17 | 3.79 | 1.43 |
| 3 ME | 5.78 | 0.611 | 1.44 | 3.57 | 1.12 |
| 3 AE | 2.79 | 0.270 | 2.34 | 3.28 | 0.72 |

1 – Eutric Cambisol FE – forest
2 – Luvic Stagnosol ME – meadow
3 – Haplic Chernozem AE – agroecosystem

On the basis of obtained data it can be concluded that SOC content is more influenced by anthropogenic activity however in case of SOM quality soil type is more important factor. On Stagnosol and Cambisol no substantial differences in HA structure among ecosystems were found. Significant impact of ecosystem was found only in Chernozem HAs. Substantially higher aliphatic carbon in the HAs structure on meadow and forest can be present due to the higher incorporation of plant residues into ME and FE SOM compared to AE as reported by Gonzales-Perez [4].

**Table 2.** Elemental composition calculated as ash and moisture free (atomic percentage) and parameters calculated from $^{13}C$ NMR of HAs

| | C | H | N | O | H/C | $C_{ar}$ | $C_{alif}$ | (%) |
|---|---|---|---|---|---|---|---|---|
| 1 FE | 41.6 | 36.6 | 3.42 | 18.4 | 0.88 | 26.2 | 59.9 | 30.4 |
| 1ME | 39.5 | 38.9 | 3.63 | 18.0 | 0.99 | 27.5 | 58.2 | 32.1 |
| 1 AE | 39.6 | 39.5 | 3.89 | 17.0 | 1.00 | 27.8 | 58.7 | 32.1 |
| 2 FE | 40.5 | 38.4 | 3.28 | 17.9 | 0.95 | 28.4 | 56.1 | 33.6 |
| 2ME | 39.2 | 38.6 | 3.89 | 18.3 | 0.99 | 27.4 | 56.1 | 32.7 |
| 2 AE | 39.4 | 40.1 | 3.55 | 17.0 | 1.02 | 32.0 | 56.3 | 36.3 |
| 3 FE | 41.6 | 37.2 | 3.90 | 17.3 | 0.89 | 31.3 | 54.2 | 36.6 |
| 3 ME | 44.4 | 33,1 | 4.44 | 18.1 | 0.74 | 38.0 | 46.1 | 45.2 |
| 3 AE | 47.5 | 30,8 | 3.85 | 17.9 | 0.65 | 44.8 | 39.2 | 53.3 |

$C_{aliph}$ – 15-106 ppm
$C_{ar}$ – 106-157 ppm
$\alpha = C_{ar}/(C_{ar} + C_{aliph}) \times 100$ (%)

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# The effect of storage time on the aggregation processes of humic acids

R. BEJGER[a], J. CIEŚLA[b], M. SIENKIEWICZ[a], A. BIEGANOWSKI[b], D. GOŁĘBIOWSKA[a], P. NICIA[c], R. MATUSZAK-SLAMANI[a], A. GAWLIK[a]

(a) Department of Physics and Agrophysics, West Pomeranian University of Technology in Szczecin, ul. Papieża Pawła VI/3, 71-459 Szczecin, Poland
(b) The Bohdan Dobrzański Institute of Agrophysics of the Polish Academy of Sciences, ul. Doświadczalna 4, 20-290 Lublin, Poland
(c) Department of Soil Science and Soil Protection, University of Agriculture in Kraków, al. Mickiewicza 21, 31-120 Kraków, Poland
* E-mail: Romualda.Bejger@zut.edu.pl

**Keywords:** molecular weight, particles size, humic substances, mountain fens

**Abstract.** The aim of study was to determine the changes in the size distribution of humic acids (HA) suspensions at different pH conditions during their longtime storage. Dynamic Light Scattering method was used for determination of particles size distribution in the HA dispersions after 0, 24, 72, 192, 360, 504 and 672 hours. The strong association of HA at the pH 5.56 was observed. With increasing alkalization this process was inhibited. At the pH 8.20 disaggregation dominated in samples. The storage time had an influence on the rate of these processes.

## Introduction

Humic acids (HA) are very important component of natural environment, especially soil. They modify the soil structure due to creation of the organo-mineral complexes. The amphiphilic molecules of HA possess the net negative electrical charge, so that the colloidal organic matter actively participates in the exchange and transport of cations. On the other hand, the molecules of humic acids exhibit affinity for the hydrophobic substances due to the presence of aliphatic and aromatic groups [1].

There are many theories related to HA behavior and structure in solution [2, 3] One of them considers the HA as a linear macromolecular polyelectrolyte which form molecular aggregates with a random coil conformation in solution [4, 5]. The second one defines the HA as supramolecular assemblies of small molecules held together by weak interactions – hydrophobic forces and hydrogen bonds [5, 6]. Other characterizes the HA as micelles or "pseudomicelles" which can be proceed by both intramolecular and intermolecular mechanisms in aqueous solution [1, 7]. „Pseudomicelles" are the results of rearrangement of single polymer chain which leads to exposition of hydrophilic parts (e.g., carboxyl groups) of molecule to the aqueous surroundings with the isolation of hydrophobic portion in the center [1]. However, in practice the point of view represented by different authors depends on the kind of HA studied and the experimental conditions [8]. There is possible a lot of different kind of interactions in the HA molecule, e.g. between compounds of HA, compounds and solvent, between different macromolecules as well as intermolecular interactions, between polyions and counterions [9]. Colloidal stability of HA is strongly influenced by the sample composition, pH, presence of multivalent cations, ionic strength of solution and temperature [8, 9, 10].

Many different techniques are involved in the investigations of HA dispersions. There are e. g. gel permeation chromatography/size exclusion chromatography (GPC/SEC) [8], ultrafiltration [8], dynamic and static light scattering [11], voltammetry [9], conductometry [10], electrokinetic technique [11] and others. Despite the many available research techniques and well identified role of HA in the natural environment, the size, conformation and aggregation process of these substances are still poorly understood.

The aim of study was to determine the changes in the size distribution of HA and their two molecular weight fraction suspensions at different pH conditions during their longtime storage.

## Materials and methods

Mountain fen soil samples were collected from surface layer from Babia Góra National Park areas in Polish Carpathians. HA separation was made on the following procedure approved by IHSS [12]. HA were fractionated into two molecular fractions using ultrafiltration technique (Amicon, Model 8400). The hydrophilic cellulose membranes (Millipore) used, had nominal molecular weight cut-off 30 kDa.

HAN-F (non-fractionated), HA>30kDA and HA<30kDa samples were diluted in Michaelis buffer solutions of pH: 5.59, 7.17 and 8.20. Carbon content in the solutions was 0.005 $g \cdot dm^{-3}$. Samples were stored in a refrigerator ($4^oC$). Before the measurements they have been conditioned for one hour in the dark at the temperature of $20^oC$.

Dynamic Light Scattering (DLS) method was used for determination of particles size distribution in the HA dispersions. Measurements were performed using the ZetaSizer Nano ZS apparatus (Malvern Ltd., UK) which is equipped with the He-Ne laser (633nm). The back scattering technique ($173^oC$) was applied [13].

Measurements were done by the use of the "Size & Zeta potential" capillary cell in three replications with 12 sub runs at the $20^oC$.

The HS suspensions were mixed vigorously before measurements. Particles size distribution was determined

after 0, 24, 72, 192, 360, 504 and 672 hours from preparation.

To show the changes of samples composition during the storage the participation of three size-classes in the particles population: with diameter ranged from 10-100 nm, 100-1000 nm and 1000-6000 nm, respectively, were analysed for all kinds of HA suspensions.

**Results and Discussion**

In HA>30kDa at the pH equal to 5.59 the participation of particles with the size ranged from 100 to 1000 nm decreased, whereas the percentage of particles bigger than 1000 nm increased during the time of samples storage. At the end of experiment the particles with the diameter higher then 1000 nm dominated in the system but also the particles class of 10-100 nm was appeared (about 4%). At the pH equal to 7.17 was dominated by the 100-1000 nm class of particles. Its participation decreased up to minimum value at 72 hours but then it increased to 100% at the end of experiment. Simultaneously, the participation of the biggest particles decreased. At the highest value of pH the percentage of the middle-size particles decreased from 100% to 40 % after 72 hours. This contribution was stable for the next 288 hours then the 100% of the 100-1000 nm class was obtained.

The composition of HA<30kDa at the pH equal to 5.59 was flexible during the experiment. In the samples during the time of storage the participation of the biggest particles changed from 9% at start time to 0 at the end of experiment. Simultaneously, the participation of the smallest particles increased from 3 to 16 %. At the pH of 7.17 the participation of the fraction of middle-size particles was equal to 94% after 672 hours and did not change in respect to the start-time data. At the end of experiment the contribution of the biggest particles decreased to 0 (where it was 7% at time t=0) whereas the smallest particles appeared (5%). At the pH equal to 8.20 the particles higher than 1000 nm were noticed only after 24 and 72 hours. The samples were generally composed of the middle-size particles. The participation of this class of size was changeable in time but it decreased (from 95% at time t=0 to 82 %) at increasing percentage of particles lower than 100 nm (from 4.8% to 18%).

In the case of HAN-F, at the pH equal to 5.59 after 72 hours the smallest particles disappeared. Simultaneously, the participation of the middle-sized particles was decreasing at the increasing contribution of the particles higher than 1000 nm. The maximum amount of the biggest molecules was obtained after 360 hours, then it decreased up to 0.9% at the end of experiment. The middle-sized particles dominated in the sample after 672 hours.

At the pH equal to 7.17 the changes of the particular fraction contributions were similar like for the samples at lower pH. The maximum value of participation of the biggest particles was obtained also after 360 hours, but it was equal to 49%. After 672 hours their amount was at the level of 23 % and the participation of the middle-sized particles was equal to 77%. In the alkaline environment (pH=8.20) the contribution of particular size fractions were changeable. Generally, at the end of experiment the sample contained higher amount the particles with the diameter lower than 100 nm (21%) than it was noticed at the start time (2%).

**Conclusions**

The HA suspensions are complicated systems according to the processes dissolution, precipitation, dissociation and association which occur there.

At the pH 5.56 the strong association of HA was prevailed. With increasing alkalization this process was inhibited. At the pH 8.20 disaggregation dominated in samples.

The time, molecular fraction, pH conditions had an influence on the rate of these processes.

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# Humus content in Chernozems and Kastanozems (chestnut soils) from specially protected areas in Rostov region, Russia

O. BEZUGLOVA [(a)*], O. CHERNOVA [(b)]

(a) Academy of Biology and Biotechnologies, Southern Federal University, Rostov-on-Don, 344090, Russia
(b) Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences,Moscow, 119071 Russia
* E-mail: lola314@mail.ru

**Keywords:** humus content, Protocalcic Chernozems, Haplic Kastanozems, protected areas, arable and virgin soils

**Abstract.** The study of soils in protected and reserved areas indicated that they could serve as reference soils while conducting monitoring observations for both arable lands and those with a different type of land use. Standing mode contributes to humus recovery in Chernozems and Kastanozems in virgin and long-standing soils, weighted average indicators of humus content (for 25-cm soil depth) appear to be 0.4–3.0% higher than in arable soils.

## Introduction

In the soil cover monitoring system, the focus is placed on determining indicators of anthropogenic impact on soils. Besides, it seems essential not only to make a list of indicators under control but also to confirm the target values for comparing them with similar anthropogenically altered soil samples. As a starting point, it seems reasonable to use characteristics of the least affected soils (reference soils) that include soils from protected landscapes in steppe zone. The most comprehensive indicator of soil fertility is humus content in the soil. For this reason, it was chosen as the main indicator of fertility for reference soils.

## Materials and methods

In the study area Chernozems and Kastanozems (according to WRB, 2015) [3] represent the zonal soil types**.** Full-profile cuttings were made in typical relief elements in several protected landscapes in Rostov Region. The soils include Chernozems (Haplic and Protocalcic) and Kastanozems (Haplic and Sodic). In addition, 15 topsoil samples (0-25 cm) were selected within a 100 m radius area from the sections in order to verify spatial variation of indicators. The total organic carbon content was analyzed by wet combustion of the organic matter with a mixture of potassium dichromate and sulfuric acid [4]. It used a modification of the Tjurin-Simakov [1].

## Results and Discussion

The study soils collected from the protected areas revealed increased humus enrichment of topsoil horizons compared to similar arable soils. In virgin and long-standing chernozems, weighted average indicators of humus content (for 25 cm depth) appear to exceed those of arable soils by 0.4-3.0% (Table 1). Hereby, it is significant to emphasize that only uneroded and undeflated arable soil samples with noticeably lower humus content were selected to be compared with soils collected from the protected areas.

The process of maximum enrichment with organic substance occurs in Haplic Chernozems under full-age rarefied broad-leaved forest («Fominskaya Dacha» Protected Landscape).

Humus content in topsoil horizons found under the forest is 2.2-3.1% higher than that of agricultural soils of the adjacent areas. This fact could result from more than a hundred-year-long lack of anthropogenic damaging effect on soil cover in the protected area because the age of oak trees in the places of cuttings made is over 100 years. Under the forest, there are additional factors contributing to increasing humus content including alteration of the content of plant litter and the way it enters the soil, as well as variation of parameters of hydrothermal regime affected by forest vegetation. Protected landscapes with steppe vegetation even those not affected by tillage are likely to have been used periodically as hayfields and pastures, therefore the positive impact of standing regime on restoring humus condition of chernozems is vividly observed here, however, to a lesser degree than that under the forest. The comparison of arable Protocalcic Chernozems studied in 1983, with the soil sample taken from the cutting made in the spot with approximately the same coordinates on the territory of «Khoroly» Protected Landscape in 2015 showed that humus content in the top soil has increased by over 1.5% over the last 20 years of the standing regime. However, according to statistical evaluations conducted for the entire province, protected area chernozems humification exceeds the values for uneroded arable chernozems by as little as 0.4%.

**Table 1.** Humus content in virgin and arable Chernozems in Rostov region (0-25 cm)

| Horizon | Virgin Chernozems of Protected Areas | | Arable Chernozems (2) | |
|---|---|---|---|---|
| | μ | Borders of Typical Values | μ | Borders of Typical Values |
| Haplic Chernozems in South-Russian Province «Fominskaya Dacha» Protected Landscape | | | | |
| Ad (Апах.) | 7.8 | 6.4–8.4 | 4.7 | 4.2–5.3 |
| Protocalcic Chernozems pre-Caucasian Province, «Razdorskiye Sklony» Protected Landscape | | | | |
| Ad (Апах.) | 5.6 | 4.6–6.0 | 4.6 | 4.4–5.0 |

| | | | | |
|---|---|---|---|---|
| Protocalcic Chernozems pre-Caucasian Province «Khoroly» PL, «Raznotravno-tipchakovo-kovylnaya steppe» Protected Landscape | | | | |
| Ad (Апах.) | 4.6 | 4.1–5.0 | 4.2 | 3.8–4.7 |
| Protocalcic Chernozems pre-Caucasian Province, «SFedUni Botanical Garden», Roston-on-Don Protected Landscape | | | | |
| Ad (Апах.) | 4.7 | 4.3–5.0 | 4.6 | 4.4–4.8 |
| Haplic Kastanozems Don Province, «Rostovskiy» Nature Reserve | | | | |
| Ad (Апах.) | 4.1 | 3.6–4.6 | 2.8 | 2.6–3.0 |
| Kastanozems Sodic, «Rostovskiy» Nature Reserve | | | | |
| A | 2.3 | 2.0–2.5 | 2.1 | 1.7–2.4 |

However, it seems significant to emphasize that higher humus enrichment of virgin and standing chernozems compared to arable lands does not always result in so vivid difference in humus reserves, since loosening of the soil by roots of natural vegetation reduces the bulk volume of surface horizons. Unfortunately, the lack of the factual data (mainly, bulk weight evaluation) does not allow us to accurately assess the range of possible profile application of these characteristics for the purposes of monitoring so far.

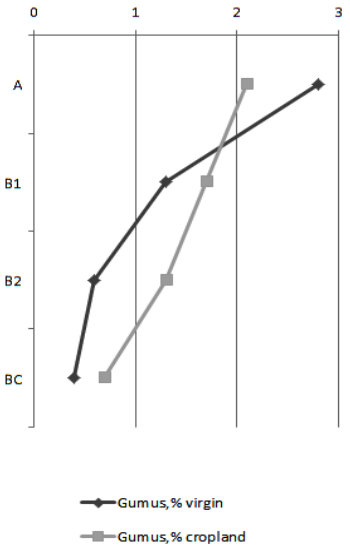



**Figure 1.** Profile distribution of humus in Solonetz.

We did not succeed in identifying any significant difference in humus stored in topsoil horizons (0-20 and 0-50 cm) in studied long-standing and virgin soils from similar arable lands. Only in Haplic Chernozems located under the forest ("Fominskaya Dacha" Protected Landscape), significantly higher humus reserve is detected in 20 cm deep soil layer (per 15-35 t/ha or per 20-30%) compared to arable Haplic Chernozems of the same region.

Humus content in Ad horizon in virgin Haplic Kastanozems averages 4.1 %. According to the findings [2], humus content in arable soils only equals to 2.6-3.0%. Humus content decrease accounts for 1.3%. The comparison of humus content in Kastanozems Sodic reservoir with similar arable lands provides the growing body of evidence of slow humus restoration in these soils. For the last 20 years, since the Rostov nature reserve was established, humus content has increased insignificantly (by 0.2 %), but reliably.

In Solonetz Chestnut top soil of 0-20 cm, humification value appeared to be 2.8%, while it was 2.1% in the similar arable soil. Decreasing humification made up 0.7%. However, there is no critical decrease in profile humus content in Solonetz soils under tillage, as it appears to be typical for these soils in their native state (Fig.1).

Reclamation activities are likely to contribute to this effect, particularly, soil turbulence practices when three-tiered plowing is used. It is likely, that a wide range of typical values in B1 and B2 horizons, and even in BC horizon, could be related to it, i.e. the horizons that appear to be affected by gypsuming when the technique of three-tiered tillage is used. In the arable Solonetz soils, where such techniques were not applied, humus content in the above-mentioned horizons appears to correspond to humus level of Solonetz in their virgin state. In the soils with turbulence techniques implemented, humus content in lower horizons is considerably higher due to increasing entry of organic residues resulting from overall fertility growth.

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# Molecular characterization of cloud water samples collected at the puy de Dôme (France) by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

A. BIANCO [a,b], M. VAITILINGOM[d], E. NICOL[c], N. CHAUMERLIAC[a], L. DEGUILLAUME[a], M. BRIDOUX [b]*

(a) Laboratoire de Météorologie Physique (LaMP), Université Clermont Auvergne (UCA), France
(b) CEA, DAM, DIF, F-91297 Arpajon, France
(c) Laboratoire de Chimie Moléculaire (LCM), CNRS, Ecole Polytechnique, Université Paris-Saclay, 91128 Palaiseau, France
(d) Laboratoire de Recherche en Géosciences et Energies (LaRGE), Université des Antilles
* E-mail: maxime.bridoux@cea.fr

**Keywords:** Cloud, organic matter, Ultra-high-resolution mass spectrometry, FT-ICR, atmospheric chemistry

**Abstract.** Cloud droplets contain dynamic and complex pools of highly heterogeneous organic matter, resulting from the dissolution of both aerosol particles and gas-phase soluble species, and are constantly impacted by photochemical and biological transformations. Cloud samples characterized by different air masses and physicochemical properties were collected at the puy de Dôme station in France, concentrated on a strata-X solid-phase extraction (SPE) cartridge and directly infused using electrospray ionization in the negative mode (ESI-) coupled with ultrahigh-resolution mass spectrometry (UHRMS). Here, the molecular heterogeneity of cloud DOM at the puy de Dôme station in France will be presented and compared to previous studies conducted on other atmospheric aqueous media.

## Introduction

Organic compounds are released into the atmosphere from both natural and anthropogenic sources, such as volatile organic compounds (VOCs) and primary organic aerosols (POAs). These compounds have substantial effects on air quality, atmospheric chemistry, and climate forcing.

Cloud droplets can be composed of a myriad of organic compounds that are efficiently transferred from (i) the gas phase and (ii) from the soluble fraction of aerosol particles that also serve as cloud condensation nuclei (CCN). These organic compounds are subsequently modified substantially by chemical and biological transformations occurring during the lifetime of the cloud [2]. A large proportion of the organic matter in clouds is also composed of relatively high molecular weight organic compounds that can contain a substantial fraction of heteroatoms (N, S, O), such as the so-called humic-like substances (HULIS) found in aerosols [4].

Herein, we present the negative mode FT-ICR MS characterization of two cloud samples collected at the puy de Dôme station (PUY) (France) with respect to their physicochemical parameters to demonstrate that cloud DOM composition contains integral information on emission sources and processes. Our results are compared to previous studies conducted on other atmospheric aqueous media. This approach provides a means to study at the molecular level a large, otherwise inaccessible proportion of the DOM in clouds and to characterize the heterogeneity of the DOM. This study is the first comparative investigation of the molecular composition of DOM from different ecosystems using ultrahigh-resolution mass spectrometry.

## Materials and methods

**FT-ICR analysis of cloud water samples.** Cloud water samples were prepared for analysis by ultrahigh-resolution FT-ICR mass spectrometry by using the method described by Zhao et al. [5]. High-resolution mass spectrometric analysis was performed using an FT-ICR mass spectrometer (Bruker) equipped with an electrospray ionization (ESI, Bruker) source set in negative ionization. FT-ICR mass spectra were processed using Composer software (Sierra Analytics, Modesto, CA).

## Results and Discussion

### Molecular characterization of cloud DOM

The mass spectra for cloud water samples are complex, with a high number of isobaric ions. An example of the isobaric complexity, exhibiting as many as 27 molecular formulas within 0.20 a.m.u., is shown in the inset of Fig. 1.

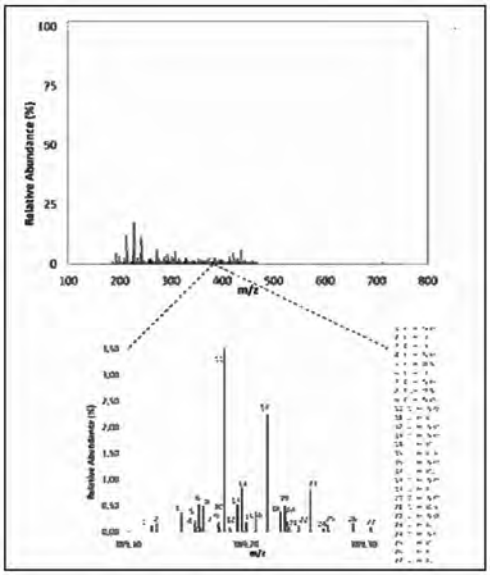



**Figure 1.** Reconstructed mass spectrum of the identified molecular formulas in J1.

After blank subtraction and quality assurance filtering, a relatively high number of molecular formulas were assigned (5260 and 2897 for J1 and J2, respectively).

The assigned formulas were grouped into four subgroups based on elemental composition (Table 1).

**Table 1.** Properties of both cloud samples collected

| Sample | J1 | J2 |
|---|---|---|
| Date | 01/06/2016 | 02/07/2016 |
| Sampling time | 14h50-19h20 | 2h00-8h00 |
| pH | 4.6 | 6.1 |
| Number frequency | 5260 | 2894 |
| O/C | 0,34 ± 0,20 | 0,40 ± 0,19 |
| H/C | 1,51 ± 0,36 | 1,59 ± 0,30 |

Samples J1 and J2 contain CHNO compounds with high DBE values (i.e., 14–19) located in the aromatic region (O/C < 0.3 and H/C < 1.0) of the VK diagram (Fig. 2).

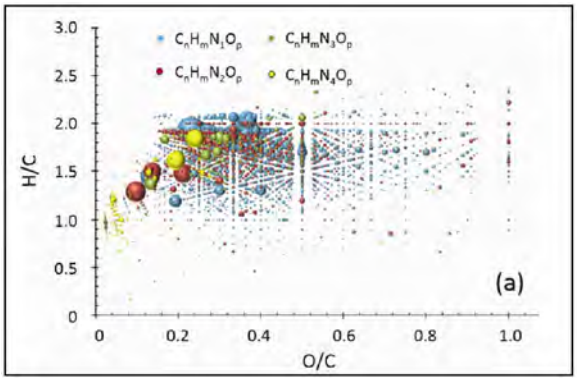



**Figure 2**. Isoabundance Van Krevelen diagram for CHNO compounds in sample J1.

The three most populated regions in both samples correspond to lignins/CRAMs, aliphatics/ proteins and lipids. These are followed by unsaturated hydrocarbons in sample J1 and by carbohydrates in sample J2.

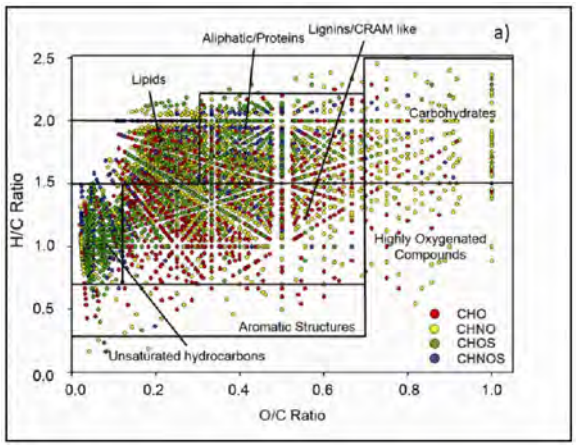



**Figure 3.** Isoabundance van Krevelen plots for sample J1. Subgroups are reported using different colors (CHO in red, CHNO in yellow, CHOS in green and CHNOS in blue).

**Comparison with other atmospheric aqueous media: rain, fog and aerosol particles**

O/C ratios reported in this work (0.37) are similar to those reported for fog water and for biogenic aerosols but are lower than the values measured for aerosols sampled in the atmosphere and for aerosols produced artificially in environmental chambers, as reported in Fig.4.

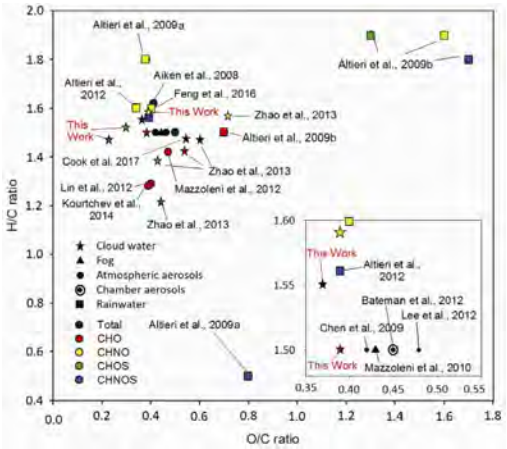



**Figure 4.** Comparison of O/C and H/C ratios of water soluble organic compounds in different atmospheric media: cloud waters is indicated by stars, rainwaters by square, atmospheric aerosols by circles, chamber produced aerosols in double circle and fogs by triangles.

Although the O/C ratio is in the lower range of the reported DOM values, the H/C ratio is within the same range of values as those reported for fog water, natural aerosols, and aerosols produced in chambers.

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# Application of the coupled techniques in thermal analysis (thermogravimetry-mass spectrometry-infrared spectroscopy) to study the structure and thermal properties of humic and fulvic acids

P. BOGUTA [(a)*], Z. SOKOŁOWSKA [(a)], K. SKIC [(a)], K. SZEWCZUK-KARPISZ [(a)], M. CYBULAK [(a)], A. TOMCZYK [(a)]

(a) Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland
* E-mail: p.boguta@ipan.lublin.pl

**Keywords:** humic acids, fulvic acids, coupled thermogravimetry, chemical structure

**Abstract.** Thermal analysis coupled with mass and FTIR spectrometry was used for analysis of the structure, chemical and thermal properties of humic and fulvic acids. The parameters obtained on the way of analysis related to three temperature ranges characteristic for stability of different structures: 40–220°C; 220–430°C and 430–650°C. Statistical analysis showed that selected parameters calculated from the obtained results correlated with chemical properties of the samples determined by classic methods. This indicated the usefulness of coupled analysis to comprehensive characteristics of humic substances.

## Introduction

Humic substances (HS) belong to one of the most important components of soils. They determine the sorption and buffer capacity showing key role in the retention of nutrients. Their valuable properties are strongly determined by its chemical structure: aromatic units, aliphatic chains, functional groups and heteroatoms. In the consequence, studies of the structure of different fractions of humic substances are very important topic in the meaning of selection of the product with the best properties.

The main problem in studying above issue is high complexity of the HS structure and the fact of constantly progressing chemical transformations. The description of the structure often requires the use of many independent techniques, which extends the analysis time.

Thermal analysis has been known for many years. However, in recent years, this technique has been combined with modern chromatographic and spectroscopic methods, which allows analyzing the structure of an identically prepared sample using several techniques at the same time. Combining thermogravimetry (TG) with differential scanning calorimetry (DSC), infrared spectroscopy (FTIR) and quadrupole mass spectrometry (QMS) could enable a more comprehensive analysis of HS samples. Using real-time, simultaneous analysis in a TG-DSC-QMS-FTIR system and under pyrolysis conditions it can be analyzed both chemical units of structure and stability of these units. A limited number of investigations have dealt with the relationships between measured parameters in thermogravimetric-coupled techniques and parameters calculated from other methods, whereas parameters obtained from thermogravimetric-coupled techniques could constitute a good indicator of HS structure and properties.

Therefore, the main aim of the studies was attempt of comprehensive description of the structure, chemical properties and thermal stability of the humic (HA) and fulvic acids (FA) from different soils, using TG and DSC analysis coupled with QMS and FTIR spectroscopy under $N_2$. An attempt was made to perform statistical analysis between parameters calculated from TG-DSC-QMS-FTIR and parameters determined via other methods that characterize humus compounds. Moreover, TG-DSC-QMS-FTIR parameters were determined for temperature ranges, describing the individual thermal process/reaction [1].

## Materials and methods

**HAs and FAs.** Initial research materials were 4 chemically different soils. The samples were collected from region of south-west Poland from A-horizons of the soils. HAs and FAs fractions were isolated on the way of alkaline extraction method recommended by International Humic Substances Society (IHSS) [2]. Lyophilized, hydrogen forms of HAs and FAs were analyzed in terms of chemical properties like for example:

- Elemental composition: C, H, N, O
- Atomic ratios
- Optical parameters obtained from UV-VIS spectroscopy: $E_2/E_4$, $E_2/E_6$
- Content of oxygen functional groups: carboxylic and phenolic: COOH and OH [1].

**Coupled thermal analysis.** Thermal decomposition of FA and HA samples with evolved gas analyses (EGA) were carried out using the coupled TG-DSC-QMS-FTIR system:

- Netzsch STA 449 F3 Jupiter thermogravimeter (TG and DSC)
- Netzsch Aeolos mass spectrometer (QMS)
- Bruker Tensor 27 infrared spectrometer.

All modules of the above system recorded signals simultaneously in real time during the temperature increase from 40 to 800°C under $N_2$ atmosphere at a heating rate of 10°C $min^{-1}$. TG module measured the changes of sample mass, DSC – changes of heat in the function of temperature. 3D FTIR spectra were recorded in function of temperature/time, wavenumber and absorbance whereas mass spectra were obtained as ionic current of different m/z ratios [1].

**Statistical analysis.** Statistical analysis of the obtained data was performed using t-student test. Correlation coefficients were studied between parameters determined from thermal analysis and from conventional methods at the $p = 0.05$. Shapiro-Wilk test was used for test of data normal distribution.

**Results and Discussion**

The obtained results showed significant differences in the chemical structure of HAs and FAs. DSC curves revealed the presence of a number of thermal transformations which allowed distinguishing three main temperature ranges related to decomposition of three groups of structures with varied energy of chemical bonds [1]:

- R1: 40-220°C
- R2: 220-430°C
- R3: 430-650°C

Weight loss (TG characteristics) in the entire temperature range was higher for FAs, indicating a greater number of labile structures in contrast to more recalcitrant HA structures. The weight loss at temperature intervals R1, R2 and R3 revealed distinct differences within the FAs and HAs in terms of:

- Physically adsorbed water (R1)
- Volatile and labile functional groups (R2)
- Recalcitrant and refractory structures (R3) [1].

QMS and FTIR modules enabled chemical identification (by masses and by functional groups, respectively) of gaseous species evolved during thermal decomposition at R1, R2 and R3. Release of physically adsorbed water at R1 was accompanied by water releasing at R2 due to dehydration of oxygen-containing functional groups and at R3 – due to intermolecular condensation reactions. Decomposition of labile structures included carboxylic, alcoholic, aldehydes, amides, amines and phenol groups, polysaccharide C–O bonds and simple aromatics. Recalcitrant structures were attributed to long chain hydrocarbons, N-heterocyclic and aromatic compounds (also from additional aromatization of aliphatic chains) [1].

Parameters calculated from QMS (areas of m/z=16, 17, 18, 44), DSC (maximum od DSC) and TG (weight loss) at R1, R2 or R3 demonstrated statistical relationships with some chemical properties of the HAs and FAs for example:

- Content of N, O and COOH
- Parameters: $\Delta\log K$, $E_2/E_6$, $E_2/E_4$

This indicated high potential of the used coupled techniques for monitoring the chemical changes of humic substances [1].

All above findings showed that TG-DSC-QMS-FTIR coupled technique can comprise useful tool for comprehensive assessment of FAs and HAs properties regarding their various origin [1].

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# Interaction between Quantum Dots (CdTe) and humic substances

W. G. BOTERO [(a)*], A. M. B. SILVA [(a)], J. C. C. SANTOS [(a)], A. D. M. CAVAGIS [(b)], L. C. OLIVEIRA [(b)], K. R. COSTA[(a)]

(a) Department of Chemistry, Federal University of Alagoas, 57.160-000, Maceió, Alagoas, Brazil
(b) Department of Physics, Chemistry and Mathematics, Federal University of São Carlos, 18.052-780, Sorocaba, São Paulo, Brazil
* E-mail: wander.botero@arapiraca.ufal.br

**Keywords:** Quantum Dots, Humic substances, Fluorescence emission spectroscopy

**Abstract.** Quantum Dots (QDs) consist of semiconductor nanocrystals, with potentially toxic metals in their structures. These materials are in the spotlight of the nanotechnology field and the lack of information on their behavior in environment makes especially relevant studies concerning their interactions with humic substances (HS). Thus, this work evaluated the interaction between QDs of CdTe and humic substances from Rio Negro (Amazonia) and peat (Sao Paulo), under different pH and ionic strengths. The results revealed that HS containing more aromatic groups (from peat) present higher interaction with the quantum dots.

## Introduction

Nanomaterials consist of relatively new inputs and suffer constant innovations, which reflects in the lack of information about their behavior in different environmental compartments, beyond concerns about their toxicities. In this scenario, stand out the Quantum Dots (QDs), formed by semiconductor nanocrystals, typically constituted in an inorganic nucleus, surrounded by a passivation layer [1].

QDs of CdTe and other chalcogenides are particularly useful due to their bandgap affinities across the visible region, including high fluorescent quantum yield, broad absorption / narrow emission and high photostability, as well as established control in obtaining monodisperse samples having the same size, shape and surface chemistry, characteristics that make them the most applied species [2]. Once in the environment, little is known about the interactions of these metals with humic substances, which may influence the passivation or release of Cd (potentially toxic metal) into the environment. In this way, this work evaluated the interaction between QDs of CdTe, coated with mercaptopropionic acid (MPA), mercaptosuccinic acid (MSA) and glutathione (GLU), and humic substances, extracted from Rio Negro (Amazonia) and peat (São Paulo), under different pH and ionic strengths.

## Materials and methods

**Synthesis of CdTe QDs.** The synthesis of CdTe QDs, using three different stabilizing agents: mercaptopromonic acid (MPA), mercaptosuccinic acid (MAS) and glutathione (GLU) was carried out according to Weng, 2006 [3].

**Characterization of the QDs.** The absorbance, concentration and size of the QDs were determined in a 1800 UV-VIS spectrophotometer (Shimadzu) in the range of 300 to 700 nm wavelength.

**Extraction of humic substances.** The humic substances were extracted from Rio Negro, Amazonia (HSRN) waters and from peat samples collected in São Paulo (HSP), according to the methodology recommended by the IHSS [4].

**Interactions between QDs and HS.** The intrinsic fluorescence emission spectra of the synthesized QDs, in the presence and absence of HS were obtained from spectrofluorimetric titrations of QDs ($7x10^{-7}$ mol $L^{-1}$) and HS (0-80 mg $L^{-1}$), at the pHs 6, 7 and 8 (phosphate buffer), using $\lambda ex$ = 350 nm.

## Results and Discussion

### QD Characterization

Figure 1 shows the absorption pattern presented by the CdTe nanoparticles:

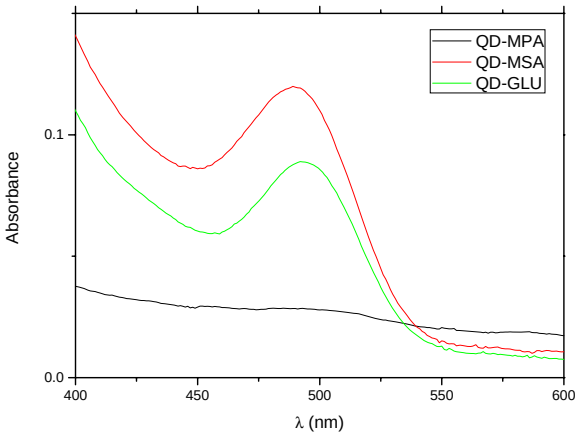



**Figure 1.** Passive absorption spectrum of CdTe QDs stabilized with MPA, MSA and GLU.

By means of the absorption spectrum (Figure 1) and the empirical method developed by Yu *et al.*, 2003 [5], the medium size and concentration of the synthesized QDs were calculated using the position of the first excitation peak, which are condensed in Table 1.

**Table 1.** Wavelength values of the first excitation absorption (max), medium size of the nanocrystals (D) and concentration of nanocrystals in solution (C).

| QD | $\lambda_{máx}$ (nm) | D (nm) | C x $10^{-5}$ (mol $L^{-1}$) |
|---|---|---|---|
| QD-MPA | 525 | 2.13794 | 1.419 |
| QD-MAS | 489 | 2.00889 | 6.802 |
| QD-GLU | 492 | 2.10649 | 4.561 |

It was observed that the structures have similar diameters and the corresponding spectra refer to the emission in the green region of the electromagnetic spectrum.

**Interactions between QDs and HS.**

The results of HSRN and HSP interactions with CdTe QDs (MPA-QD, MAS-QD and GLU-QD) by spectrofluorometric titrations, in the absence and presence of HS matrices, at different concentrations, indicated that the presence of HSRN and HSP promotes the suppression of QDs intrinsic fluorescence (Figure 2 and 3). This suppression increases proportionately with HSRN and HSP concentrations (Figures 2 and 3). This spectral behavior suggests the formation of a non-fluorescent QD-HS complex, allowing only the free nanomaterials particles to exhibit fluorescence, thus diminishing the fluorescence intensity observed.

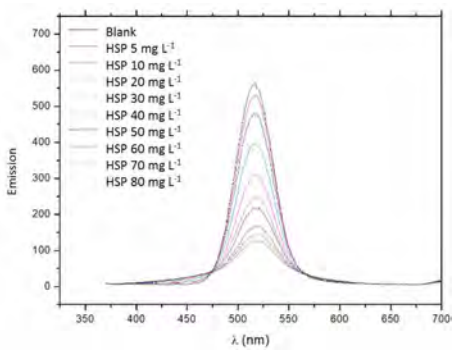



**Figure 2.** Spectral profile of spectrofluorimetric titration, at pH 8, of CdTe-MAS with different concentrations of HSP.

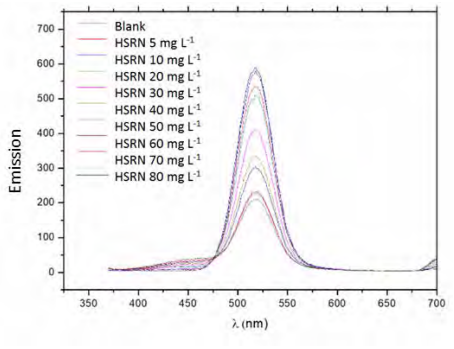



**Figure 3.** Spectral profile of spectrofluorimetric titration, at pH 8, of CdTe-MSA2- with different concentrations of HSRN.

Similar results were observed for the other CdTe QDs (MPA-QD and GLU-QD) at different pH values. In order to better illustrate and understand the results, the Stern-Volmer constant (Ksv) and binding constant (Kb) were determined and the results are described in Table 2. These results indicate that the system which demonstrated higher suppression efficiency and, consequently, indicates greater interaction with QDs was HST. In a similar study, developed by Hyung and Kim, 2008 [6], on the interaction of natural organic matter (NOM) and carbon nanotubes, the authors observed proportionality between the aromatic carbon content of NOM and the interaction with nanotubes, corroborating the results of the present study, in which HSP presented higher content of aromatic groups in their structure (HSP 62% of aromatic groups and HSRN with 49% of aromatic groups).

**Acknowledgment**. This research has been co-financed by National Council for Scientific and Technological Development (CNPq) and Research Foundation of the Alagoas State (FAPEAL).

**Table 2.** The Stern-Volmer constants (Ksv) and binding constants (Kb) related to the interaction between HS and QDs, at the pHs 6, 7 and 8

| | | | QD-MPA | QD-MAS | QD-GLU |
|---|---|---|---|---|---|
| HSP | pH 6 | **Ksv** | $2.56 \times 10^{3}$ | $6.21 \times 10^{2}$ | $4.97 \times 10^{2}$ |
| | | **Kb** | $1.900 \times 10^{3}$ | $1.66 \times 10^{2}$ | $1.40 \times 10^{2}$ |
| | | **Kb*** | $2.45 \times 10^{4}$ | $2.74 \times 10^{3}$ | $8.38 \times 10^{1}$ |
| | | ***n*** | 1.38 | 1.29 | 0.81 |
| | pH 7 | **Ksv** | $2.51 \times 10^{2}$ | $7.83 \times 10^{2}$ | $2.29 \times 10^{2}$ |
| | | **Kb** | $1.17 \times 10^{3}$ | $2.40 \times 10^{2}$ | $1.00 \times 10^{2}$ |
| | | **Kb*** | $6.44 \times 10^{3}$ | $4.88 \times 10^{3}$ | $1.22 \times 10^{3}$ |
| | | ***n*** | 0.77 | 1.36 | 1.32 |
| | pH 8 | **Ksv** | $3.31 \times 10^{2}$ | $9.52 \times 10^{2}$ | $5.69 \times 10^{2}$ |
| | | **Kb** | $5.00 \times 10^{2}$ | $1.60 \times 10^{3}$ | $3.70 \times 10^{2}$ |
| | | **Kb*** | $7.51 \times 10^{2}$ | $8.58 \times 10^{3}$ | $1.04 \times 10^{2}$ |
| | | ***n*** | 1.14 | 1.43 | 0.81 |
| HSRN | pH 6 | **Ksv** | $3.32 \times 10^{2}$ | $6.65 \times 10^{2}$ | $4.86 \times 10^{1}$ |
| | | **Kb** | $2.15 \times 10^{3}$ | $6.00 \times 10^{2}$ | 7.50 |
| | | **Kb*** | $2.06 \times 10^{1}$ | $3.30 \times 10^{2}$ | $5.01 \times 10^{2}$ |
| | | ***n*** | 0.56 | 0.91 | 1.08 |
| | pH 7 | **Ksv** | $3.17 \times 10^{2}$ | $1.38 \times 10^{3}$ | $5.38 \times 10^{1}$ |
| | | **Kb** | $1.3 \times 10^{3}$ | $8.00 \times 10^{2}$ | $5.25 \times 10^{1}$ |
| | | **Kb*** | $1.17 \times 10^{2}$ | $5.26 \times 10^{3}$ | $5.75 \times 10^{3}$ |
| | | ***n*** | 0.84 | 1.22 | 1.41 |
| | pH 8 | **Ksv** | $2.41 \times 10^{2}$ | $1.75 \times 10^{3}$ | $6.34 \times 10^{1}$ |
| | | **Kb** | $1.25 \times 10^{3}$ | $3.00 \times 10^{3}$ | $1.00 \times 10^{2}$ |
| | | **Kb*** | $1.26 \times 10^{4}$ | $1.32 \times 10^{3}$ | $1.66 \times 10^{4}$ |
| | | ***n*** | 1.54 | 1.82 | 1.56 |

Kb* Determined by linear regression

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# Electrochemical and structural effects of microbial reduction on peat humic acids in the solid state

C. BRAVO [a,b] *, R. TONIOLO [a], M. CONTIN [a], M. DE NOBILI [a]

(a) Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, via delle Scienze 208, 33100 Udine, Italy
(b) Department of Life Sciences, University of Trieste, Via Licio Giorgieri 5, 34128 Trieste, Italy
*E-mail: carlo.bravo@phd.units.it

**Keywords:** TEA; reduction; humic acids; anaerobic respiration

**Abstract**. Under anaerobic conditions, humic acids (HA) may act as terminal electron acceptors, allowing facultative anaerobic bacteria to obtain energy from anaerobic respiration. Structural and electrochemical changes due to biological oxidation and reduction were investigated on HA extracted from a peat sample, incubated under controlled aerobic and anaerobic conditions. Colorimetric analysis showed the increase of the electron donating capacity (EDC) of HA after biological reduction. Coherent structural changes were confirmed by FTIR. This experiment showed that transfer of electrons from microbially reduced moieties is slow and most of their EDC persists under oxic conditions.

## Introduction

In natural wetlands and submerged soils, facultative anaerobic bacteria can use humic substances as terminal electron acceptors (TEA) during anaerobic respiration [1, 2].

The fact that humic substances can reversibly sustain redox cycling was demonstrated for humic substances analogues and extracted HA in solutions [3]. Nevertheless, structural changes of natural HA brought about by biological reduction in their natural solid state were never investigated.

In this work, biological oxidation/reduction by aerobic/anaerobic microorganisms, of peat HA in their natural solid state was performed by subjecting a peat sample to either intense oxidative or reductive conditions. Structural changes were investigated by FTIR spectroscopy while electrochemical effects were characterized and quantified by cyclic voltammetry and colorimetry.

## Materials and methods

Samples from a Lithuanian sphagnum peat (100 g) were incubated for 90 days at 25°C under either fully aerobic (insufflation of air) and anaerobic (submerged and under $N_2$) conditions. To boost biological activity, 3% fresh plant residues and 1 g of either an aerobic or anaerobic soil inoculum were added just before incubation. HA were extracted in an anoxic glovebox following the procedure recommended by the IHSS.

Cyclic voltammograms (CVs) were recorded by a 430A CHI electrochemical analyzer in a 0.1 M phosphate buffer (pH 7.0) solution using a 3 mm diameter glassy carbon (GC) disk working electrode, an Ag/AgCl reference electrode and a Pt wire auxiliary electrode. The cathodic and anodic vertex potentials were fixed at $E_h$ = -0.1 V and +0.7 V (scan rate 10 mV $s^{-1}$). CVs were collected in the presence of either ABTS (60 µM) or HA (0.2 g $L^{-1}$), and in the presence of both 60 µM ABTS and HA (0.05 to 0.5 g $l^{-1}$) [4].

Mediated Electrochemical Oxidation (MEO) measurements were carried out, under anoxic atmosphere and continuous solution stirring, in a bulk electrolysis cell containing a macro GC WE polarized to E = 0.423 V, an Ag/AgCl reference electrode and a Pt wire auxiliary electrode (separated by a porous glass frit). After oxidation of a 0.20 mM ABTS solution (0.1 M phosphate, pH 7), small aliquots of HA (0.4 mg), were spiked into the electrochemical cell. EDCs were quantified by integration of oxidative currents.

FTIR spectra were recorded with a Spectrum 100 FTIR (PerkinElmer) spectrometer over an interval from 4000 to 500 $cm^{-1}$.

## Results and Discussion

Figure 1 shows the results of MEO experiments.

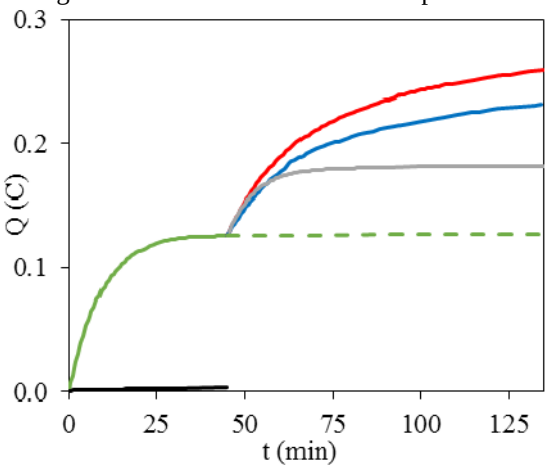



**Figure 1.** Oxidative charge responses to spike of ABTS (green trace) and to spikes of HA aerobically (blue trace) and anaerobically (red trace) incubated peat and model antioxidant Trolox (grey line). Black trace represents HA charge response in the absence of ABTS.

While for the model antioxidant Trolox (grey line) the stable end-point is reached within a short time (ca. 10 min), for HA the electrons transfer to the ABTS mediator could not be considered completed within the time allowed for the experiment. This may be attributed to the complex nature of HA, where many functional moieties (e.g. polyphenols and quinones) can exert their redox

activity with different kinetics. However, the EDC measured 60 minutes after addition of HA was significantly stronger (2.90 $mmol_{e^-}$ $g_{HA}^{-1}$) for HA extracted from the anaerobically incubated peat compared to that of HA extracted from the aerobically incubated peat, but the latter still displayed a measurable reductive capacity (2.35 $mmol_{e^-}$ $g_{HA}^{-1}$). This residual EDC of HA exposed to oxic conditions can be explained considering that peat formed in anoxic environments and in its natural state its components can be assumed to be in reduced conditions. Albeit subject to oxidation during excavation and further on during aerobic incubation, some residual ECD may persist in the absence of a suitable electron acceptor.

CVs of HA extracted from oxidized and reduced peat (Fig.2) were both featureless, when recorded in the absence of a suitable mediator [4] and showed catalytic oxidative currents during anodic scanning in the presence of ABTS. Compared to the oxidized HA, the reduced HA presented a not significantly different voltammetric response (see insert in Fig. 2).

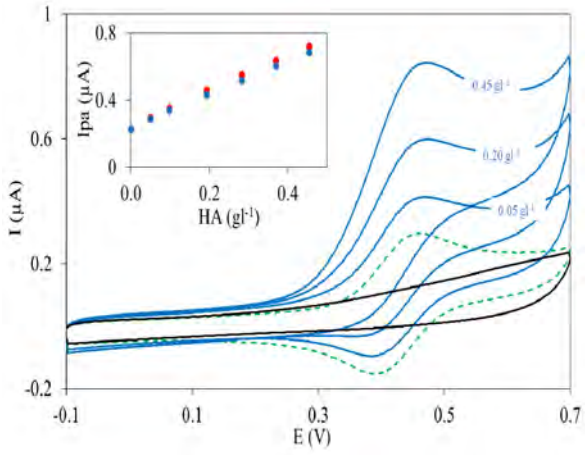



**Figure 2**. CVs of solutions containing only HA from oxidized peat (black trace), only mediator ABTS (dotted green line) and both HA and ABTS (blue traces) at different concentrations of HA. **Insert:** correlation of the oxidative peak current ($Ip_a$) with the concentration of HA extracted from aerobically (blue) and anaerobically (red) incubated peat.

FTIR spectra of HA exhibited the typical major peaks reported for humic acids (Fig. 3) [5]. However, their relative intensities (R), changed (Tab. 1). As could be expected, HA from non-incubated peat displayed intermediate structural characteristics between aerobically incubated and anaerobically incubated HA. In particular, ratios between intensities at 1720/1025 and 1650/1600 $cm^{-1}$ are respectively related to the reduction of C=O to COOH and to quinones. These ratios were lower in the anaerobically incubated peat HA.

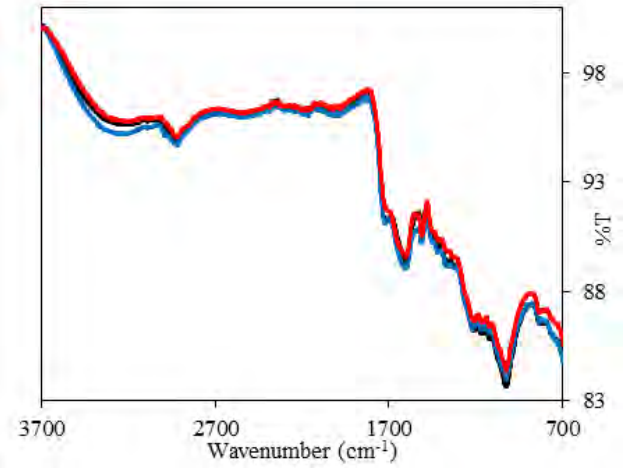



**Figure 3.** FTIR spectra of HA extracted from non-incubated (black trace), aerobically (blue trace) and anaerobically (red trace) incubated peat.

**Table 1**. Intensity ratios of selected absorption peaks in FTIR spectra of HA.

| | $R_{1600/1720}$ | $R_{1720/1025}$ | $R_{1490/1720}$ | $R_{1650/1600}$ |
|---|---|---|---|---|
| $HA_{red}$ | 1.81 | 0.42 | 1.05 | 0.47 |
| $HA_{non\text{-}inc}$ | 1.28 | 0.59 | 0.37 | 0.75 |
| $HA_{ox}$ | 0.77 | 0.91 | 0.33 | 0.92 |

This experiment showed that, when exposed to the activity of anaerobic microorganisms even in their natural solid state, HA undergo chemical-physical and structural changes that are coherent with their role as TEA. Reduced moieties, however, appear to be able to transfer electrons only slowly, whereas HA may retain their antioxidant properties for long periods even under oxic conditions.

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# The estimation of changes on carbon stocks in lime applicated maize cultivation upland soil by using net ecosystem carbon budget analysis

S. R. CHO [(a)], J. G. LEE [(b)], J. S. TAK [(b)], J. Y. LIM [(b),] P. J. KIM [(a,b)*]

(a) Department of Applied Life Science (BK 21$^+$ program), Graduate School, Gyeongsang National University
(b) Institute of Agriculture and Life Sciences, Gyeongsang National University
* E-mail: pjkim@gnu.ac.kr

**Keywords:** liming, soil respiration, net ecosystem carbon balance, calcium carbonate

**Abstract.** Liming is a common agricultural practice worldwide, used for increasing productivity in acid agricultural soils. The effect of liming on soil carbon (C) stock changes wasn't studied well. To evaluate the effect of liming on C accumulation changes, selected liming material as $CaCO_3$. The treatments were installed different application levels of $CaCO_3$ as 0, 0.5, 1 and 2 Mg ha$^{-1}$. The $CO_2$-C flux was increase with increased rate of $CaCO_3$. The two year's NECB results were different but, the NECB was still highly decreased. Conclusively, Stover should be recycled to increase C stocks.

## Introduction

Mineralization of organic matter is a key process regulating the cycling of nutrients in soil [1]. Among the many nutrient which incloud in organic matters, soil organic carbon (SOC) can play an important role in increasing crop productivity, improving soil fertility, converting atmospheric carbon dioxide ($CO_2$) into stable carbon (C) pools and thus mitigating climate change [2]. $CO_2$ is realeased from the soil through soil respiration, which includes three biological processes, namely microbial respiration, root respiration and faunal respiration [3].

## Materials and methods

**Investigation plot installation.** Investigation plots were installed in a typical temperate upland soil at the agronomy field of Gyeongsang national university, for two years. The initial soil properties were pH 5.6 (1:2.5 with $H_2O$), total carbon content and Nitrogen as 23.7 g C kg$^{-1}$, 2.4 g N kg$^{-1}$. The electrical conductivity (EC) 3.1 dS m$^{-1}$, and exchangeable calcium ($Ca^{2+}$) 5.2 cmol$^+$ kg$^{-1}$. Four different levels of $CaCO_3$ (0, 500, 1000, and 2000 kg ha$^{-1}$) were installed as treatments. Each experimental plot (100 m$^2$) was established with randomized block design with 3 replications.

**Maize cultivation.** Mazie (Zea mays L.) seedlings (two weeks old) were transplanted on early June 2016 and 2017 with a spacing of 30 cm x 30 cm. All the experiment treatments were applied chemical fertilizers with same levels of N-$P_2O_5$-$K_2O$ (186-35-74kg ha$^{-1}$). Maize was harvested in early September and ears, stover and grain were manually separated, oven-dried at 60$^0$ for 72 h. and their yield was measured.

**Evaluation of net ecosystem carbon budget (NECB).** The soil carbon stock changes were estimated analyzing NECB(4). NECB = ∑Input - ∑Output = (NPP + fertilzer + $CaCO_3$) - (Mineralized loss + harvested removal). Where the NPP (net primary production) of Maize (ear, stover, root, grass, rhizodeposit), fertilizer, and $CaCO_3$ were evaluated as C input source, and mineralized C loss and harvested removal were as C output source.

## Results and Discussion

### Soil temperature and moisture content

As shown as Fig. 1 the soil temperature was highest in cropping season because the cropping season was summer, and lowest in fallow season because of the atmosphere temperature become lower than cropping season. And soil moisture content was high in cropping season, because of in Korea during cropping season is summer and during summer season, the precipitation is high.

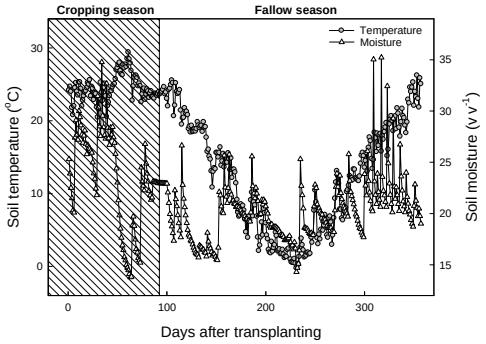



**Figure 1.** The changes of soil temperature and moisture contents during whole experimental period.

### Net primary production (NPP)

As shown as Table 1, each factors of NPP were increased with application levels of $CaCO_3$. And most of the NPP was about 75% occupied in aboveground biomass.

**Table 1.** The changes of net primary productivity

| Application (Mg ha$^{-1}$ $CaCO_3$) | Net primary productivity (NPP, kg C ha$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Grain | Stover | Root | Rhizodeposit | Grass | Total |
| 0 | 2048 | 3440 | 464 | 878 | 523 | 7353 |
| 0.5 | 2254 | 3681 | 425 | 950 | 545 | 7858 |
| 1 | 3042 | 3887 | 756 | 1109 | 560 | 9355 |
| 2 | 3239 | 4009 | 861 | 1160 | 582 | 9851 |

### Total carbon input

Same as NPP value, total carbon input also increased with increasing rate of $CaCO_3$ application levels, because NPP value has highest mass of the total carbon input. This result is shown in Fig. 2.

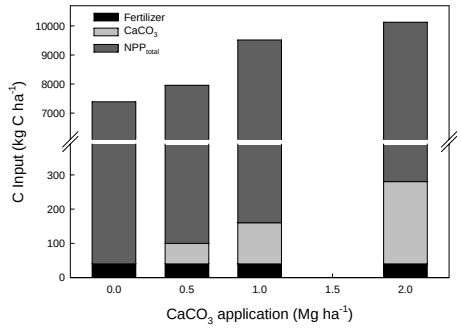



**Figure 2.** The changes of total carbon input.

### Total output

Among the categories of total output, mineralized carbon flux was measured by $CO_2$ emission. During cropping season, $CO_2$ emission rate was higher than fallow season, but total amount of mineralized carbon flux was higher at fallow season (Fig. 3). This result caused by the period of each season. The fallow season was longer than cropping season. The total carbon output was increased with $CaCO_3$ application level (Fig. 4).

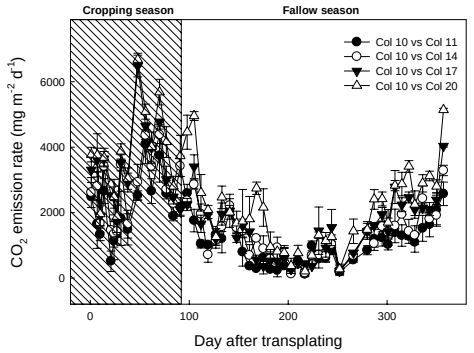



**Figure 3.** The changes of $CO_2$ emission rate in whole experimental period.

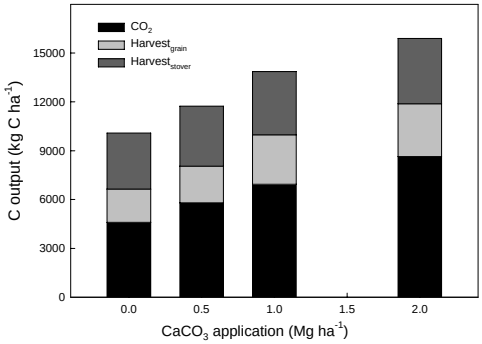



**Figure 4.** The changes of total carbon output.

### Net ecosystem carbon budget (NECB)

With total input and output value, NECB was calculated as Fig. 5. The NECB value was decreased with increasing rate of $CaCO_3$ application level. This result means total carbon ouput value was bigger than total input value.

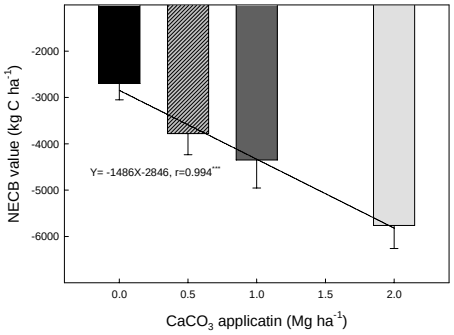



**Figure 5.** The changes of NECB value.

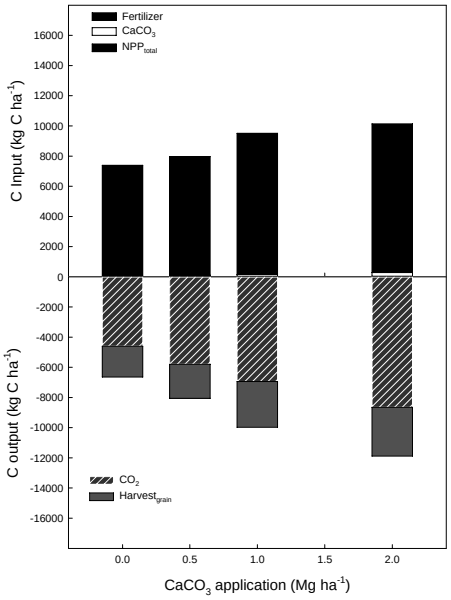



**Figure 6.** The total carbon input and output value without stover mass.

Conclusively, soil organic carbon will deplete in lime applied soil without other organic carbon inputs. Among the total carbon output parts, harvested stover mass was highest. Therefore, to keep or raise soil organic carbon content, plough stover back to soil could be another alternative choice (Fig. 6).

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# Interaction of humic acid and wheat-straw-biochar native radicals with pesticides

I. ĆWIELĄG-PIASECKA [(a)*], M. JERZYKIEWICZ [(b)], A. MEDYŃSKA-JURASZEK [(a)], E. JAMROZ [(a)]

(a) Institute of Soil Science and Environmental Protection, Wroclaw University of Environmental and Life Sciences, 50-375 Wroclaw, Poland
(b) Faculty of Chemistry, University of Wroclaw, 50-383 Wroclaw, Poland
* E-mail: irmina.cwielag-piasecka@upwr.edu.pl

**Keywords:** humic acids, biochar, radicals, pesticides

**Abstract.** This paper compares the types of indigenious radicals occuring in the humic acid (HA) derived from arable soil and a wheat-straw biochar (BC) obtained during a flash pyrolysis. Electron paramagnetic resonance (EPR) spectrosocpy was used to detect the radicals and to monitor the effect of addition of different classsess of polar pesticides on the spin concentration. In both sorbents organic radicals were detected - semiquinone type in HA and aliphatic and PAH radicals in BC. Addition of pesticides to BC resulted in a substantial enhancement in carbon-centered radical formation.

## Introduction

Well characterized semiquinone radicals are known to be an indispensable structural component of the humic acids (HA). These stable free radicals are capable of binding certain organic pollutants due to their electron donating and accepting properties [1]. This property can be monitored by the decrease or increase of semiquinone radicals concentration using electron paramagnetic resonance (EPR) spectroscopy.

A carbon-rich product originating from the thermal decomposition of biomass under the limited oxygen conditions, namely biochar (BC), has received a considerable attention in terms of its ability to capture organic xenobiotics [2, 3]. Many different mechanisms have been postulated to explain sorption-desorption behaviour of various pollutants towards BC [4, 5]. Still, little is known on the role of the native radicals, formed during the biomass pyrolysis, on the interaction of organic contaminants with biochar. Hence, in the present work, we studied and compared the nature and the quantity of the indigenous, native radicals present in HA and a wheat-straw BC using the EPR spectroscopy. Additionally, the interaction of five polar pesticides (2,4-D, MCPA, carbaryl, carbofuran and metolachlor) with native HA and BC radicals was investigated.

## Materials and methods

### Organic sorbents and pesticides for the studies.

Humic acids for the study was extracted by the standard IHSS method from the topsoil horizon of arable Gleyic Phaeozem. Biochar was produced from a wheat-straw in a flash pyrolysis process at 550˚C. Investigated organic xenobiotics belonged to three pesticide classes: carbamates (carbaryl and carbofuran), phenoxyacetic acids (2,4-D and MCPA) and aniline derivatives (metolachlor). Basic structural and spectroscopic properties of both sorbents and characteristic of pesticides under study are presented in our previous paper [5].

### Reaction of the HA and BC with pesticides.

Prior to the EPR analysis we conducted the sorption experiment allowing HA and BC to interact with the studied pesticides. To the investigated sorbents (HA or BC) a 15 mg $L^{-1}$ pesticides solutions in 10 mM $CaCl_2$ were added. The mixtures were equalibrated for 24h, centrifuged and filtered through the 0.45-µm membrane. The solid remnants of the humic acid and biochar were washed with distilled water. Afterwards the samples were freeze-dried. Additionally, the control samples containing only HA or BC and a 10 mM $CaCl_2$ solution were prepared.

### EPR measurements.

EPR measurements were conducted at frequency close to 9.7 GHz (X-band) at room temperature using a Bruker ELEXYS E500 spectrometer. Microwave power was 20 mW, magnetic field modulation 100 kHz and modulation amplitude of 0.5 G. Leonardite HA (IHSS) was used as a standard in quantitative EPR measurements [6].

## Results and Discussion

### EPR Characterization

The experimental EPR spectra of native radicals in HA and BC samples (Figure 1) were the base to establish g-parameter values and line widths, thus to identify analyzed radical types (Table 1). Regardless of the chemical factor to which HA was exposed, the obtained experimental g parameter (g= 2.0030 -2.0033) remained in the region typical for the indigenious semiquinone radicals in a condensed ring system [6, 7]. The results obtained for the biochar samples (Table 1) suggest the presence of two types of radicals: aliphatic (g = 2.0025–2.0026) and presumably simple, 1-5 rings aromatic hydrocarbons (g = 2.0026–2.0028). It is in accordance with the results obtained by Trubetskaya et al. [8], who also detected aliphatic carbon-centered and polycyclic aromatic hydrocarbon (PAH) radicals of a relatively narrow line width (4–5.6 G) in a high-temperature wheat-straw biochar.

Line widths of the presented experimental BC spectra were in a range of 3.95- 4.4 G, suggesting the formation of a disorganized carbon with unpaired electrons associated with aromatic delocalized π system [8], which was additionally confirmed by the value of g-parameter close to that of a free electron.

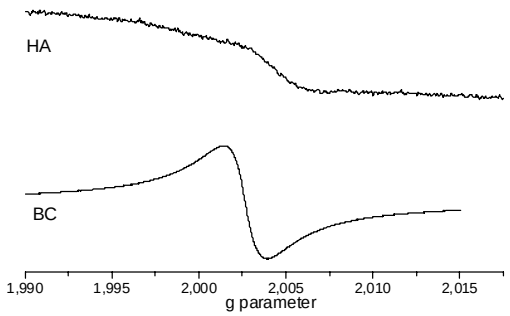



**Figure 1.** EPR spectra of radicals detected in humic acid (HA) and wheat-straw biochar (BC) samples.

### Quantitative EPR measurements

EPR spectroscopy was also used to determine the concentration of radicals detected in the HA and BC. Humic acid samples contained lower amount of radicals than the biochar. Addition of different pesticides to the native HA resulted in a slight increase (in case of 2,4-D and MCPA) or a decrease (carbaryl, carbofuran, metolachlor) of radical concentration (Table 1). Observed quenching of radicals in case of nonionic pesticides under study (carbaryl, carbofuran, metolachlor) may suggest some coupling reactions occuring in the investigated mixtures.

**Table 1.** Concentrations, g-factor and line width of free radicals (FR) in untreated humic acid and biochar samples and samples with the addition of pesticides

| Sample | FRs concentr. ($10^{18}$ spins/g) | g–parameter | Line width (Gauss) |
|---|---|---|---|
| HA | 0.26 | 2.0032 | n.m. |
| HA-2,4-D | 0.27 | 2.0032 | n.m. |
| HA-MCPA | 0.33 | 2.0033 | n.m. |
| HA-Carbaryl | 0.12 | 2.0032 | n.m. |
| HA-Carbofuran | 0.16 | 2.0031 | n.m. |
| HA-Metolachlor | 0.19 | 2.0031 | n.m. |
| BC | 2.28 | 2.00265 | 4.3 |
| BC-2,4-D | 10.7 | 2.0026 | 3.9 |
| BC-MCPA | 11.7 | 2.00265 | 4.4 |
| BC-Carbaryl | 9.45 | 2.0027 | 4.1 |
| BC-Carbofuran | 10.1 | 2.0026 | 3.9 |
| BC-Metolachlor | 10.2 | 2.0027 | 4.1 |

n.m. –not measured

BC samples treated with pesticides revealed a substantial increase in FRs concentration (4-5 times) in case of all organic xenobiotics tested. Both ionic and nonionic polar substances seem to contribute equally to the enhancement of the aliphatic and PAH radicals formation in the studied BC samples.

These preeliminary data prove that: [i] HA and BC contain a radicals of different origin; [ii] indigenious radicals in HA are less prone to interaction with the studied pesticides than the radicals formed in a wheat-straw biochar during pyrolysis process; to fully explain and characterize the above mentioned interactions further studies need to be conducted; [iii] all of the studied pesticides classess enhanced the formation of aliphatic and PAH type of radicals in BC mixtures; [iv] before amending soils with BC its potential to generate potentially harmful organic radicals should be taken into consideration.

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# Humic acids as markers of soil organic matter quality in fire affected soils subjected to a mulching treatment

J. M. DE LA ROSA [(a)*], J. J. KEIZER [(b)], N. T. JIMÉNEZ-MORILLO [(c)], J. A. GONZÁLEZ-PÉREZ [(a)], G. ALMENDROS [(d)], F. J. GONZÁLEZ-VILA [(a)], D. VIEIRA [(b)], H. KNICKER [(a)]

(a) Instituto de Recursos Naturales y Agrobiología de Sevilla. Consejo Superior de Investigaciones Científicas. (IRNAS-CSIC), Reina Mercedes Av. 10. 41012 Seville, Spain.
(b) Centre for Environmental and Maritime Studies (CESAM), Campus Santiago. 3810-193 Aveiro, Portugal.
(c) Hercules Laboratory. University of Evora. Largo Marquês de Marialva, 8. 7000-089 Évora, Portugal.
(d) Museo Nacional de Ciencias Naturales. Consejo Superior de Investigaciones Cientíticas (MNCN-CSIC). Serrano, 115b. 28006 Madrid, Spain.
* E-mail: jmrosa@irnase.csic.es

**Keywords:** Wildfire, mulching, Py-GC-MS, Soil organic matter, humic acids

**Abstract.** Mulching is an effective treatment of post-fire soil erosion. However, little is known concerning its effects on soil organic matter (SOM) composition and properties. This study evaluated the long-term effects of forest residue mulch on the molecular composition of humic acids from a burnt eucalypt plantation in central Portugal. Mulching treatment increased the abundance of organic carbon and humics acids extracted five years after the wildfire. The molecular composition of soil samples indicated that mulching may help the recovery of the most labile fractions of fire affected soils. In addition, the composition of the HA extracted from both mulched and untreated fire affected plots was very similar.

## Introduction

In the last decades, wildfires have become the major environmental disturbance in Mediterranean countries [1]. One of the principal effects of wildfires is the loss of vegetation and litter cover. Consequently, wildfires typically enhance runoff generation as well as soil exposure to the direct impact of raindrops [2]. In addition, wildfires may cause a drastic alteration of soil organic matter (SOM) content and quality, soil properties and also carbon cycling and balance [3].

The most widely accepted measure to effectively mitigate post-fire soil erosion is mulching, i.e., the application of a cover of organic compounds on the soil surface [4, 5]. However, despite the fact that a significant part of the detrimental impacts of wildfires on the ecosystems is caused by qualitative and quantitative transformations of SOM [7], which is the soils' most functional fraction and a key attribute of soil quality, the knowledge of the effects of mulching on SOM composition in fire affected soils is still very scarce. Different types of organic matter are entering the soil during mulching with forest residues. The most labile organic matter is rapidly decomposed followed by condensation-polymerization of decomposition products to form microbially more recalcitrant substances, the accumulation of which can indicate a new equilibrium state. A precise characterization of SOM is crucial to provide recommendations on post-fire management practices aiming a durable soil recovery. This study provides the quantitative and qualitative composition of bulk soils and humic acids (HAs) from mulched and untreated soils of a eucalypt site affected by a fire.

## Materials and methods

### Area of study and sampling

The study area was located in the Sever do Vouga municipality (north-central Portugal), which was affected by a moderate to high severity wildfire that consumed 295 ha in July 2010. The burnt area consisted mainly of eucalyptus (*Eucalyptus globulus* Labill.) plantations, some maritime pine (*Pinus pinaster* Ait.) and a stand of cork oak (*Quercus suber* L.). At the end of August 2010, an abrupt and short (40 m) slope with southwest orientation was selected for installation of six slope-scale plots (approx. 100 $m^2$; see [5]). Three plots were treated with mulch at a rate of 13.6 Mg $ha^{-1}$, while the other three were left as control plots. In June 2015, five years after the wildfire, six samples of soils were taken from each plot at depths 0-2 cm, 2-5 cm and 5-15 cm.

### Extraction of Humic acids (HAs)

About 25 g of each sample were extracted with 200 mL of 0.1 M NaOH in polyethylene flasks in an $N_2$ atmosphere (repeated 5 times). The total humic extract was then precipitated by dropwise addition of 6 M HCl to precipitate the acid-insoluble HA fraction, which was redisolved in 0.1 M NaOH and centrifuged at 4.000 rpm for 20 min. The residue was discarded, and the brown supernatant was reprecipitated with 3 M HCl. Extracts were purified by dialysis and subsequently freeze-dried and weighed.

### Analysis of bulk soils and Humic acids.

### Determination of basic soil properties

Soil pH and conductivity were measured in triplicate by using a 2.5:1 water to soil ratio, whereas the water holding capacity (WHC) was determined by placing 15 g (d.w.) of each sample over a filter Whatman 2 into a funnel covered with a plastic film, satured with water and settled for 12 h. Elemental analysis (TC, TN) was carried out in bulk soils and HAs using a Thermo-Scientific Flash 2000 analyser.

### Analytical pyrolysis (Py-GC/MS)

Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) was performed using a double-shot

pyrolyser (Frontier Laboratories, model 2020i) attached to a GC/MS Agilent 6890N system. Samples were placed in small crucible capsules and introduced into a preheated micro-furnace at (500°C) for 1 min. The amounts of the different compounds identified by analytical pyrolysis were considered to be proportional to the peak areas of the total ion chromatographic trace.

### Solid state $^{13}$C NMR spectroscopy

The solid-state $^{13}$C NMR spectra were obtained in a Bruker Avance III HD 400 MHz instrument operating at a frequency of 100.64 MHz and using $ZrO^2$ rotors of 4 mm OD for $^{13}$C with Kel-F caps. The cross polarization (CP) technique was used during magic-angle spinning (MAS) of the rotor at 14 kHz for $^{13}$C. Contact time, pulse delay, line broadening and calibration of the $^{13}$C chemical shifts were performed according with [6].

## Results and Discussion

### Elemental (TC,TN) and basic analyses

The TC content of the mulched soils was significantly higher than that of the untreated fire affected soils. This difference was more evident at soil surface. The upper-most fraction also shows a decrease in soil pH (from 6.1 to 5.7) and a slight increase in the conductivity due to mulching addition. In contrast, N contents of bulk soils were not significantly altered due to mulching. The increase in the WHC of the 0-2 and 2-5 cm fractions of mulched plots is probably related with their increase in the TC contents. Mulching caused an increase in the amount of extracted HAs, this result was specially evident at the 0-2 cm depth fraction

**Table 1.** Elemental composition (TC, TN), pH, electrical conductivity and water holding capacity of soils. Elemental composition (C,N) and relative abundance of extracted HAs

| | Bulk soils | | | | | | Humic acids | | |
|---|---|---|---|---|---|---|---|---|---|
| Sample | Soil Depth (cm) | TC (g kg$^{-1}$) | TN (g kg$^{-1}$) | pH | Cond. (mS m$^{-1}$) | WHC (%) | Rel. Abund. wt (%) | TC (g kg$^{-1}$) | TN (g kg$^{-1}$) |
| Fire affected plots | 0–2 | 195±14 | 22±2 | 6.1±0.2 | 213±36 | 89 | 2.3 | 353±18 | 18±7 |
| | 2–5 | 121±11 | 20±2 | 7.3±0.1 | 572±14 | 43 | 2.2 | 280±40 | 16±5 |
| | 5–15 | 88±10 | 3±1 | 6.2±0.9 | 50±3 | 44 | 6.3 | 454±53 | 21±2 |
| Mulching conditioned plots | 0–2 | 305±14 | 24±3 | 5.7±0.2 | 254±14 | 97 | 4.2 | 295±21 | 14±2 |
| | 2–5 | 159±10 | 21±3 | 7.2±0.1 | 478±10 | 69 | 2.3 | 270±45 | 15±5 |
| | 5–15 | 93±10 | 4±1 | 5.1±0.1 | 93±5 | 47 | 5.4 | 336±52 | 18±4 |
| Mulching material | | 439±14 | 4±1 | 4.9±0.5 | 307±1 | 119 | | | |

### Analytical pyrolysis (Py-GC/MS)

Pyrochromatograms of bulk soils exhibited a greater presence of polysaccharidic compounds in mulched soils than in untreated soils, which suggested that mulching may help the recovery of the most labile fractions of fire affected soils. In addition, lignin compounds were also more abundant in the mulched soils.Analytical pyrolysis revealed a very similar molecular composition of HAs from mulched and untreateed plots. The most remarkable differences were the presence of lipid and peptidic homologues at the HAs isolated from mulched soils at 0-2 cm depth, a lower abundance of alkyl-homologues and a greater presence of lignins in HAs from mulched soils.

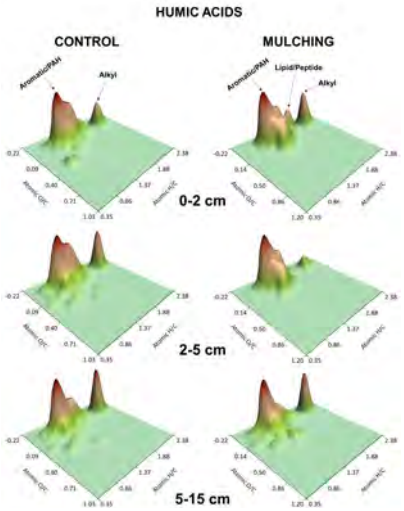



**Figure 1.** Surface density map displaying cumulative abundances of HA pyrolysis compounds of bulk soils defined by their H/C and O/C atomic ratios in a classical Van Krevelen's diagram [7].

### $^{13}$C NMR spectroscopy

$^{13}$C NMR spectra showed a greater abundance of O-alkyl-C and a lower presence of alkyl-C in the uppermost soil fraction of mulched plots in comparison with control ones. In contrast, mulching exhibited a limited effect on the $^{13}$C NMR spectra of HAs.

### Conclusions

The results of this study pointed out that, besides reducing runoff and erosion, mulching also contributed to the preservation of the organic carbon and HAs of a fire affected soil. Furthermore, mulching seemed not producing significant alterations on the molecular composition of the HA fraction, at least over the first 5 years after mulching.

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# Quantitative relationships of humic acids parameters and climate of local scales

M. DERGACHEVA [(a)*], N. BAZHINA [(a)], O. NEKRASOVA [(b)]

(a) Department of Biogeocenology, Institute of Soil Science and Agrochemistry SB RAS, 630090, Novosibirsk, Lavrent'ev Av., 8/2, Russia
(b) Institute of Natural Sciences and Mathematics, Ural Federal University,
620000 Ekaterinburg, Mira Av. 19, Russia
* E-mail: mid555@yandex.com

**Keywords**: humic acids composition, properties, local climatic parameters

**Abstract.** The possibilities of establishing quantitative relationships between the soil humic acid composition and properties and climatic indices of local sites of their position are examined using the example of the Western Tuva soils of different formation conditions. It was found that a definite combination of mean annual air temperatures and precipitation of local sites of the investigated soils corresponds to certain values of the ratio of the basic elements in humic acids (H:C).

## Introduction

An assessment of the humic substances system behavior in a changing environment under the natural and anthropogenic influence causes requires precise quantitative criteria characterizing the conditionality of certain humic acid parameters by the local conditions of their formation including the climate. It is the local combinations of temperature and humidity conditions that determine the specificity of humic acids composition and properties and their relationship with other components of humus in each individual case.

The purpose of this publication is to demonstrate one of the approaches to establishing the connection between the humic acids parameters and the climate indices of the local conditions of their formation on a quantitative basis.

## Objects and methods

Humic acids from the Western Tuva soils of different formation conditions were isolated by the traditional method by 0.1 N NaOH extraction after preliminary soil decalcination and subsequent precipitation with acid at pH = 2. Strict purification of preparations from ash elements was not carried out, because these procedures level the differences of humic acids caused by their natural state under environmental conditions [1].

Materials of the study of humic acids isolated from soils formed in different environment (from the tundra to the desert steppes) are discussed. Key areas with relatively homogeneous soil formation conditions (topography, dominant vegetation and soil type) were chosen.

A series of sections were made in these key areas. The mean annual temperature and precipitation were calculated for them based on derived regression equations reflecting the relationship between the climatic parameters and the elevation data taking into account the slope exposition and position relative to the wind rose [2].

The use of regression equations made it possible to calculate the quantitative characteristics of the climate for each soil, exposed by the individual sections at each key site. The calculation of the mean annual temperature and average annual precipitation was carried out using the regression equations, which for the Western Tuva have the following form:

average annual air temperature, °C = -2.1025-0.0021 H (r = 0.98);

average annual rainfall, mm = 125.335 + 0.1652 H (r = 0.97),

where H is the altitude above sea level.

Correction coefficients were introduced into these regression equations in the final calculations, taking into account the slope exposition and the position relative to the wind rose.

Since there are close correlations between the elemental composition and other quantitative characteristics of humic acids [3] only one of them is used in this investigation – the ratio of the basic structure-forming elements, hydrogen and carbon (H:C).

## Results and Discussion

The average statistical indicators of the humic acid composition of different soils (H:C) are given in Table 1.

**Table 1**. The average statistical values of the element ratio (H:C) of horizon A humic acids of some Werstern Tuva soils

| Key areas | Type of soils* | n | H:C |
|---|---|---|---|
| Mongun-Taiga | Lithic Leptosols Brunic | 24 | 1.25±0.06 |
| Sut-Khol | Lithic Leptosols Humic | 10 | 1.13±0.06 |
| Ak-Sug | Umbric Leptosols Dystric | 8 | 1.03±0.09 |
| Kara-Khol | Mollic Leptosols Eutric | 12 | 1.08±0.05 |
| Alash | | 10 | 0.92±0.03 |
| Hondergei | | 14 | 1.04±0.01 |
| Shanchy | Haplic Kastanozems Chromic | 4 | 1.28±0.09 |

*According to the WRB classification.

The data show that the humic acids of the Western Tuva soils have a wide range of values of this indicator, depending on the location of key areas in the region.

The quantitative characteristics of the main climatic indices for each studied soil were calculated using the registration equations that take into account the altitude of soil location above sea level, the exposure of the slope and the location relative to the wind rose. Part of the calculated climatic parameters for example is given in Table.2.

**Table 2**. Climatic parameters of soil formation for Western Tuva individual section (calculated)

| Section № | h a.s.l. | Average annual | |
|---|---|---|---|
| | | amount precipitation, mm | air temperature, °C |
| The key site Mongun-Taiga | | | |
| 1 | 2815 | 665 | -7.7 |
| 3 | 2690 | 640 | -7.5 |
| 6 | 2455 | 592 | -7.2 |
| 9 | 2352 | 571 | -6.9 |
| 1Ck | 2741 | 650 | -7.6 |
| 2CK | 2661 | 634 | -7.4 |
| 3CK | 2545 | 610 | -7.2 |
| The key site Kara-Khol | | | |
| 176 | 1476 | 301 | -5.2 |
| 186 | 1495 | 304 | -5.2 |
| The key site Alash | | | |
| 191 | 1460 | 250 | -3.1 |
| The key site Ak-Sug | | | |
| 221 | 1432 | 383 | -5.1 |
| 222 | 1329 | 363 | -4.9 |
| The key site Sut-Khol | | | |
| 207 | 1862 | 471 | -5.9 |
| 210 | 1815 | 462 | -5.8 |
| The key site Hondergei | | | |
| 78 | 1050 | 306 | -4.4 |
| 80 | 1020 | 300 | -4.3 |
| The key site Shanchy | | | |
| 1ш | 750 | 317 | -1.2 |

**Acknowledgment**

Part of analyzes used to justify a new approach for determining the quantitative relationships of the humic acid composition with the formation conditions was carried out by RFBR support (the research project No. 18-04-00714).

The average values of one of the most characteristic and time-stable parameters of the elemental composition of soil humic acids (H:C) was compared with calculated indicators of the main climatic characteristics (Fig. 1) that made possible to establish the patterns of combination of mean annual temperatures and average annual precipitation, which, under natural conditions, determine humic acids with certain parameters H:C (Figure 1).

Thus, knowing the value of the main structure-forming element ratio (H:C) in humic acids, it is possible to determine the annual average temperatures of air and precipitation (or other climate parameters), which is an important circumstance in assessing and predicting the possible state of the natural environment of local territories, including monitoring climate change.

Data on humic acids of modern soils and their quantitative connections to ecological formation conditions can be also used for reconstruction of paleoenvironment and ecological conditions of ancient man habitat as humic acids not only have got specific relations to environment, reflect and code information about ecological conditions of their formation in their composition and other features but well preserve the main characteristics in diagenesis [4].

Paleoenvironment reconstruction based on the quantitative parameter humic acids composition and their other features allows a step on a time scale to be reduced and objects of different preservation degree to be studying.

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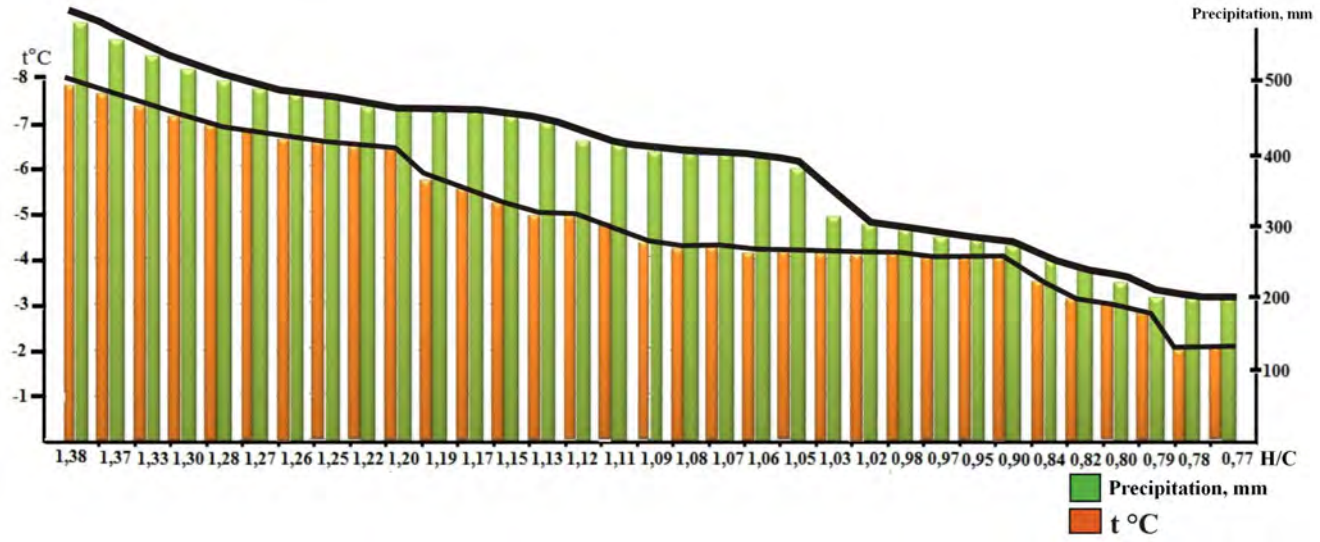



Fig. 1. The combination of average annual air temperatures and average annual precipitation, which determine under natural conditions humic acid formation with a certain ratio of the main structure-forming elements – H:C

# Structural features of humic acids extracted from layers of peat stratigraphic column from the West Siberian marsh according to IR-EXPERT Analytical system data

YU. DERYABINA [(a)], V. TIKHOVA [(a)*], T. KORNAKOVA [(a)], M. SARTAKOV [(b)], E. OSNITSKY [(b)]

(a) N. N. Vorozhtsov Institute of Organic Chemistry, Novosibirsk, 9 Lavrentiev Avenue, 63390 Russia
(b) Yugra State University, Khanty-Mansiysk, 16 Chekhov Str., Russia
* E-mail: tikhova@nioch.nsc.ru

**Keywords:** Humic acids, FTIR, peat, spectral databases

**Abstract.** IR-spectra of peat humic acids extracted from the 10 cm layers of 5 m length peat stratigraphic column were analyzed by IR EXPERT analytical system. The peat column was resulted from the Mukhrino marsh located in the Western Siberia Middle Taiga. Using IR EXPERT system allowed us to show changing of humic acids structures along the column depth, as well as in terms of their botanical and elemental composition.

## Introduction

IR spectroscopy is one of the basic and common methods used for more than half a century for the analysis of humic acids (HAs) extracted from various natural objects. The outline of IR spectrum is one of the diagnostic features of HAs [1]. Investigators of HAs use more and more complex and expensive methods though the possibilities of IR spectroscopy are far from being exhausted.

In the early 2000s, a new computer system for working with IR spectra of complex molecules was proposed in Novosibirsk [2]. It was called the "IR EXPERT", and was originally used only to work with the spectra of individual organic compounds. But later it was shown this system to be useful for the analysis of complex mixtures of natural compounds, for example - extractives of peat [3] and humic acids [4]. The application of this system to different types of HAs is of great interest.

This paper is devoted to the study of HAs isolated from layers (10 cm) of the peat stratigraphic column 5 m long.

## Materials and methods

The stratigraphic column was selected 30 kilometers to the south-west of Khanty-Mansiysk on the territory of the Mukhrino educational and experimental station (Yugra State University). This station is located on the left-bank terrace of the Irtysh River on a typical peatland (Mukhrino bog). The sampling point coordinates for the stratigraphic peat column: 60.89535°N; 68.639033°E.

Column was obtained using an Eijkelkamp Peat Sampler (Netherlands) and divided to samples from the surface up to a depth of 510 cm in increments of 10 cm. The sample of 0-10 cm was not examined because of a low yield of the humic acids; the samples of 60-70, 100-110, 170-190 and 200-210 cm corresponding to buried water layers were not examined either.

Humic acids were extracted according to standard Instorf procedures modified at the Department of General Chemistry of the Tyumen State Agricultural Academy [5], but without the hydrochloric acid demineralization which leads to a partial removal of aliphatic periphery.

The infrared spectra of HAs were registered in KBr (1:150) at room temperature in the range of 4000-400 $cm^{-1}$ with Bruker FTIR spectrometer Vector-22 using 32 scans, 2 $cm^{-1}$ resolution, and OPUS 3.0 software.

Humic acids were characterized in terms of their elemental and botanic composition [6].

The procedure of using the IR EXPERT system is performed in two steps:

1) The spectrum of a sample is compared with each spectrum in the database and the search report (SR) is formed from the structures whose IR spectra are the most similar to the spectrum of the considered compound.

2) IR EXPERT system analyzes the fragment compositions of the found (SR) structures and proposes a list of fragments that could be present in the sample structure. Fragments with high abundance in the studied structures and with a relatively low abundance among the structures of the whole database are collected [7].

## Results and Discussion

The method described above was used for fragmentation and comparison of the peat column HAs.

1) The closest spectral analogs were found for each HA spectrum by comparing full spectral curves with the Euclidian metric. As a result, 51 search reports (SR) were obtained; each consists of 10 structures from IR EXPERT Database. A close similarity of all SR was found. Three structures (71901, 74443, 36922) (Figure 1) are present in all SR with different degrees (coefficients) of similarity (from 0.2074 to 0.3800). The smaller the coefficient, the better the spectral similarity. And there are two more structures (28176, 71713) almost in all SR (in 39 among 51). All these compounds are definitely not equivalent to humic acids, but they contain virtually all their essential functional groups.

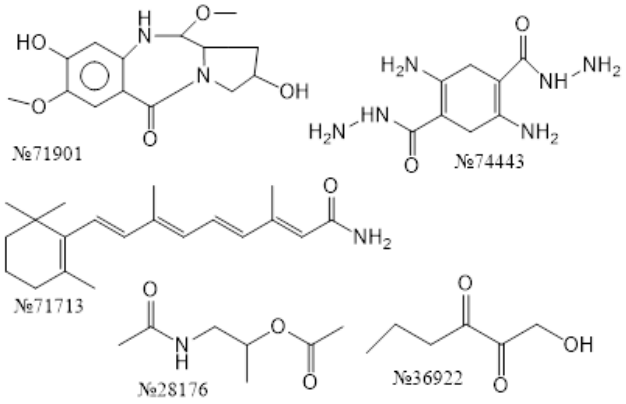



**Figure 1.** The most common structure of search answers.

The structure (№71901) is probably a fragment of melanoidins – irregular polymers with diverse structure, including heterocyclic and quinoid structures. Melanoidins are dark-colored humus-like compounds which are the products of microbiota and fungi vital (or destruction) activity, and can also be formed according to the Maillard reaction from monomeric fragments [8]. This structure is the closest spectral analog almost for all studied HAs, becides 2 HAs extracted from layers 20-30 cm and 30-40 cm. It contains carboxyl, hydroxyl, ether, amino, methyl groups, an aromatic ring and heterocyclic nitrogen. These groups are responsible for the main bands of the IR spectrum of HAs.

At a depth of more than 380 cm the structure of the HA becomes more diverse. It is due to the appearance of three more structures containing phenolic and carbohydrate fragments (Fig.2). Increase in oxidation of HAs is confirmed by elemental analysis data (Table 1).

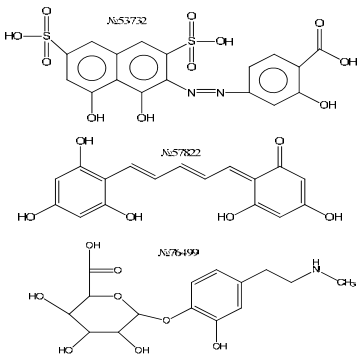



**Figure 2.** The structures that appear in the search reports for a depth of more than 380 cm.

It was unexpected that such characteristic structure of peat HAs as the pteridine skeleton was detected only in one layer 370-380 cm in this peat column.

All structures of 51 search reports were subjected to the decomposition procedure into their constituent fragments (from 3 to 10 vertex ones) followed by analysis of ten-vertex fragments for which the non-randomness factor of appearance in IR-EXPERT Database exceeds 0.9. It appears that the number of such fragments varies from minimum 152 (layer 250-260 cm) to maximum 3136 (layer 340-350 cm).

**Table 1**. Changes in the botanical and elemental composition of peat by the depth of the column

| Peat Type | Depth, cm | H/C | O/C |
|---|---|---|---|
| Scheuchzeria sphagnum peat sphagnum peat | 10 - 170 | 1.18 | 0.39 |
| Bog-depression sphagnum peat | | 1.16 | 0.38 |
| Scheuchzeria peat | 190 - 220 | 1.15 | 0.41 |
| Complex sphagnum peat | 220 - 330 | 1.12 | 0.40 |
| Sphagnum fuscum peat | | 1.08 | 0.41 |
| Scheuchzeria sphagnum peat | 300 - 380 | 1.14 | 0.43 |
| Grass-hypnum sapropel | 380 - 460 | 1.17 | 0.46 |
| Sapropel | 460 - 510 | 1.32 | 0.48 |

The use of IR EXPERT information-analytical system makes it possible

1) to identify important structures and structural fragments in HAs, that could not be reliably detected by other non-destructive methods of analysis;

2) to associate a spectrum with a combination of structural fragments rather than with the exact structure, that is very important for HAs;

3) to compare these fragment combinations to find similarities and differences for humic acids of different origin, age, climatic conditions, depth of occurrence, etc.

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# Soil organic matter and exchange cations composition of clayey soil from Southeastern part of the Sofia valley

E. DIMITROV [(a)*], M. KERCHEVA [(a)], T. SHISHKOV [(b)], E. FILCHEVA [(b)], R. SECHKOVA [(c)]

(a) Department of Soil Physics, Institute of Soil Science, Agrotechnology and Plant Protection "N. Poushkarov", 1080 Sofia, Bulgaria
(b) Department of Genesis, geography and classification of soils, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant protection, 1331 Sofia, Bulgaria
(c) Soil Chemistry Department, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant protection, 1080 Sofia, Bulgaria
* E-mail: e.dimitrov7@gmail.com

**Keywords:** organic carbon, humus type, exchange cations, soil texture

**Abstract.** Three clayey soils Haplic Fluvisol Eutric, Haplic Cambisol and Bathycalcic Vertisol from Southeastern part of the Sofia Valley and the Northwestern foot of the Lozen Mountain under different land use in one agro-climatic region have been selected to present characteristics of soil organic matter, exchange cations and soil texture in connection with soil formation. Organic carbon content in the surface horizons ranges from medium to high and in the depth sharply decreases except the third profile. The total sum of cation exchange capacity in the study profiles is very high due to textural classes.

## Introduction

The content of soil organic matter is connected with climate, topography, soil texture, type of clay, land cover and land use. The temperature and soil moisture regime is a decisive factor in the level of organic matter and soil texture in the soil to processes of structure formation. The type of clay minerals dominantly distributed in the soil often is related to a specific composition of soil adsorption system. Studying changes in the stock of organic carbon under the influence of land cover or land use requires information about natural variability. Arable land is characterized by lower organic carbon content due to soil aggregate degradation and better aeration of the surface layers, especially in dry and hot climate conditions. The results of this study could be used for more precise estimation of the current effects of different land use.

## Materials and methods

Soil samples were taken for analysis to determine extracted and no extracted fractions of organic matter, total organic carbon, exchange cations and soil texture. Soil organic carbon was determined by the modified method given by Turin [1, 2]. According to the method of Ganev and Arsova [3], cation composition was determined by the solutions of 1.0 N sodium acetate and 0.2 N potassium meliorate at pH 8.25. Soil fractions required for Soil Taxonomy texture classifications were determined with sodium pyrophosphate without preliminary removal of organic matter and carbonates. The data of organic matter and exchange cations composition and soil texture are from the soil profiles under different land use in one agro-climatic region.

Profile 1 is classified as Delluvial-meadow soil by Bulgarian classification [4]; Haplic Fluvisol Eutric by WRB [5]; Typic Udorthents by Soil Taxonomy [6]. Profile 2 is classified as Leached cinnamonic-meadow soil [4]; Haplic Cambisol [5] and Typic Eutrudepts [6]. Profile 3 is classified as Leached cinnamonic forest soil, like Smolnitsa [4], Bathycalcic Vertisol [5]; Typic Hapludert [6]. The three profiles are located at the Southeastern part of the Sofia Valley and the North-western foot of the Lozen Mountain near to the Iskar River.

## Results and Discussion

Data sets of organic carbon and organic matter fractions are listed in Table 1.

**Table 1.** Content and composition of soil organic matter in the studied soil

**a) Profile 1. Deluvial-meadow soil**

| Hor. | OC,% | $C^*_{ext}$,% | $C^*_{ha}$,% | $C^*_{fa}$,% | $C_{ha}:C_{fa}$ |
|---|---|---|---|---|---|
| $Ah_1$ | 2.71 | 0.81 | 0.39 | 0.42 | 0.93 |
| $Ah_2$ | 1.39 | 0.45 | 0.19 | 0.26 | 0.73 |
| $A_1$ | 0.82 | 0.27 | 0.13 | 0.14 | 0.93 |
| $A_2$ | 0.83 | 0.27 | 0.14 | 0.13 | 1.08 |

**b) Profile 2. Leached cinnamonic-meadow soil**

| Hor | OC,% | $C^*_{ext}$,% | $C^*_{h}$% | $C^*_{fa}$,% | $C_{ha}:C_{a}$ |
|---|---|---|---|---|---|
| $Ap_1$ | 1.77 | 0.44 | 0.19 | 0.25 | 0.76 |
| $Ap_2$ | 1.14 | 0.35 | 0.15 | 0.20 | 0.75 |
| AB | 0.89 | 0.31 | 0.20 | 0.11 | 1.82 |

**c) Profile 3. Leached cinnamonic forest soil, Smolnitsa like**

| Hor. | OC,% | $C^*_{ext}$,% | $C^*_{ha}$,% | $C^*_{fa}$,% | $C_{ha}:C_{fa}$ |
|---|---|---|---|---|---|
| $Ap_1$ | 1.50 | 0.55 | 0.34 | 0.21 | 1.62 |
| | 1.19 | 0.49 | 0.26 | 0.23 | 1.13 |
| $Ap_2$ | 1.10 | 0.43 | 0.27 | 0.16 | 1.74 |
| $A_1$ | 1.05 | 0.40 | 0.28 | 0.12 | 2.33 |
| | 1.03 | 0.41 | 0.23 | 0.18 | 1.28 |

* absolute %

The total carbon content of the Delluvial-meadow soil is high (2.7) in the surface horizon and the other two profiles of Leached cinnamonic-meadow soil and

Leached cinnamonic forest soil, like Smolnitsa is medium (1.7 and 1.5) respectively. In the depth, organic carbon sharply decreases except the Leached cinnamonic forest soil, Smolnitsa like where the decrease is more gradual. The extracted organic carbon with (0.1M $Na_4P_2O_7$+0.1M NaOH) in profile 3 is a part of a larger amount (37-41 %) compared to the other two profiles. In this profile the degree of humification is medium and humus type is fulvic-humic (1<Cha: Cfa<2.0) in the surface horizon and at depth about 30-40 cm change to humic (Cha: Cfa>2.0). In the surface horizons of the other two profiles dominate fulvic acids which determine the type of humus such as humic-fulvic which changes with a depth to fulvic-humic, according to the classification of Grishina and Orlov [7]. The explanation of these changes in the composition of the organic matter in depth is more intense condensation of humic acids under conditions of soil compaction.

The physicochemical characteristic is shown in Table 2.

**Table 2.** Cation exchange capacity CEC, $cmol^+$/kg and base saturation BS,%, pH in water, the total sum of exchange cations ($T_{8.2}$), CEC of soil strongly acidic ion exchanger ($T_{SA}$), CEC of slightly acidic ionexchanger ($T_A$)

**a) Profile 1. Deluvial-meadow soil**

| Horizon | pH | $CO_3$ % | $T_{8.2}$ | $T_{SA}$ | $T_A$ | Exch $H_{8.2}$ | BS, % |
|---|---|---|---|---|---|---|---|
| $Ah_1$ | 6.7 | - | 35.2 | 30.4 | 4.8 | 3.6 | 90 |
| $Ah_2$ | 6.9 | - | 34.8 | 30.8 | 4.0 | 2.8 | 92 |
| $A_1$ | 7.3 | - | 32.9 | 30.1 | 2.8 | 1.7 | 95 |
| $A_2$ | 7.2 | - | 32.1 | 29.0 | 3.1 | 2.0 | 94 |
| $AC_1$ | 6.9 | - | 33.1 | 28.9 | 4.2 | 3.3 | 90 |
| $AC_2$ | 7.0 | - | 34.7 | 31.3 | 3.6 | 2.6 | 93 |
| C | 7.1 | 0.56 | 33.9 | 30.6 | 3.3 | 2.4 | 93 |

**b) Profile 2. Leached cinnamonic-meadow soil**

| Horizon | pH | $CO_3$ % | $T_{8.2}$ | $T_{SA}$ | $T_A$ | Exch $H_{8.2}$ | BS, % |
|---|---|---|---|---|---|---|---|
| $Ap_1$ | 6.0 | - | 31.3 | 25.1 | 6.2 | 5.4 | 83 |
| $Ap_2$ | 5.8 | - | 30.7 | 24.9 | 5.8 | 6.1 | 80 |
| AB | 6.2 | - | 32.2 | 26.9 | 5.3 | 4.9 | 85 |
| $B_1$ | 6.6 | - | 32.3 | 27.3 | 5.0 | 4.0 | 88 |
| $B_2$ | 7.0 | 0.19 | 31.2 | 26.6 | 4.6 | 3.5 | 89 |
| $BC_k$ | 7.8 | 2.32 | 32.6 | - | - | 0.0 | 100 |
| $C_k$ | 8.0 | 4.89 | 34.4 | - | - | 0.0 | 100 |

**c) Profile 3. Leached cinnamonic forest soil, Smolnitsa like**

| Horizon | pH | $CO_3$ % | $T_{8.2}$ | $T_{SA}$ | $T_A$ | Exch $H_{8.2}$ | BS, % |
|---|---|---|---|---|---|---|---|
| $Ap_1$ | 6.1 | - | 35.5 | 28.7 | 6.8 | 5.5 | 85 |
| | 5.7 | - | 35.1 | 27.8 | 7.3 | 7.7 | 78 |
| $Ap_2$ | 5.9 | - | 34.2 | 27.5 | 6.7 | 6.0 | 82 |
| A1 | 6.5 | - | 34.2 | 28.4 | 5.8 | 4.6 | 86 |
| A2 | 6.7 | - | 34.4 | 29.8 | 4.6 | 3.6 | 90 |
| $Bi_1$ | 7.0 | 0.27 | 34.7 | 30.7 | 4.0 | 3.1 | 91 |
| $Bi_2$ | 7.0 | 0.42 | - | - | - | - | - |
| $Bk_3$ | 7.9 | 3.36 | - | - | - | - | - |
| $BC_k$ | 8.2 | 6.39 | - | - | - | - | - |

pH reaction is neutral in Profiles 2 and 3 deeper due to the appearance of carbonates about (5-6%) it is slightly alkaline. CEC in the study profiles is high up to 30-40 $cmol^+$/kg soil, at the obtained textural classes (Table 3). Base saturation is over 90% and could be assumed of dominated minerals from the smectite group.

**Table 3.** Textural classes according to USDA

**a) Profile 1. Deluvial-meadow soil**

| Horizon | Depth, cm | Textural class USDA |
|---|---|---|
| $Ah_1$ | 0-3 | Clay Loam |
| $Ah_2$ | 3-14 | Clay Loam |
| $A_1$ | 14-31 | Clay Loam |

**b) Profile 2. Leached cinnamonic-meadow soil**

| Horizon | Depth, cm | Textural class USDA |
|---|---|---|
| $Ap_1$ | 0-6 | Clay Loam |
| $Ap_2$ | 6-17 | Clay |
| AB | 17-36 | Clay |

**c) Profile 3. Leached cinnamonic forest soil, Smolnitsa like**

| Horizon | Depth, cm | Textural class USDA |
|---|---|---|
| $Ap_1$ | 0-18 | Clay |
| $Ap_2$ | 18-24 | Clay |
| $A_1$ | 24-50 | Clay |

## Conclusion

The data on soil organic matter content and composition presents potential storage of carbon stocks in the soils from valley and mountain. The humus type at the surface was determined as fulvic-humic (profile 3) which changes at the depth about 30-40 cm to humic type. Respectively, it is humic-fulvic in (profiles 1, 2) and is fulvic-humic deeper. The total sum of cation-exchange capacity is high due to textural classes.

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# Spectroscopic characterization of fulvic acids isolated from Czech soil and peat

V. ENEV [a,b]*, J. RYBÁRIK [a,b], L. DOSKOČIL [a,b], Š. SOVOVÁ [a,b], L. KUBÍKOVÁ [a,b], M. KLUČÁKOVÁ [a,b]

(a) Centre for Materials Research, Faculty of Chemistry, Brno University of Technology, Purkynova 118, 612 00 Brno, Czech Republic
(b) Institute of Physical and Applied Chemistry, Faculty of Chemistry, Brno University of Technology, Purkynova 118, 612 00 Brno, Czech Republic
* E-mail: enev@fch.vut.cz

**Keywords:** fulvic acids, spectroscopic methods, fluorophores, humification, protein-like

**Abstract.** Fulvic acids isolated from South-Bohemian peat and forest soil were characterized using spectral techniques and elemental analysis. Fluorophores of terrestrial fulvic acids lie within the α' and α regions with typical maxima centered in the range 240–280/400–500 nm and 300–380/400–500 nm ($\lambda_{ex}/\lambda_{em}$). Fulvic acids isolated from Humic Podzol were characterized also by two protein-like fluorophores. EEM spectra have the character of fingerprints for fulvic acids, respective humic materials. Distinctive $^{13}$C NMR signal of amino acids, typical for protein-like substance, was found in 45–106 ppm region.

## Introduction

Humic substances (HS) are a major component of natural organic matter (NOM) and are the dominant products of plant and animal tissue degradation by microbial activity. HS, the main organic constituents of lignite, soil, sediment and natural water are widely distributed over the Earth's surface, occurring in almost all terrestrial and aquatic environments. Humic substances are complex mixtures of high to low molecular weight species, so they are polydisperse systems with a specific distribution of molecular weights. They are operationally divided into three classes (fulvic acids, humic acids and humin) on the basis of their alkaline and acid solubility. Humic acids (HAs) are soluble at pH > 2, fulvic acids (FAs) are soluble under all pH conditions and humin (HU) is insoluble at all pHs [1]. The object of our study was to investigate the chemical properties and humification degree of terrestrial FAs.

## Materials and methods

Fulvic acids (FAs) were isolated from typical Czech forest soil Humic Podzol (locality Krkonoše, Czech Republic) and South-Bohemian peat (locality Třeboň, Czech Republic). Fulvic acids were extracted using a modified procedure recommended by the International Humic Substances Society.

Elemental composition of the FAs was determined using a CHNS/O analyzer Euro Vector EA 3000. Samples (~ 0.5–1.5 mg) were packed in tin capsules in the oven for combustion at 980°C. The calibration curves for C, H, N, S, O were obtained using a reference standard sample sulphanilamide. The % oxygen content was calculated by difference and the values obtained were corrected for ash and moisture content.

Steady-state fluorescence spectra were recorded in aqueous solutions of 10 mg·L$^{-1}$ FAs after overnight equilibration at room temperature, using FluoroLog luminescence spectrophotometer. The pH-values of the samples were adjusted to seven using a standard phosphate buffer. Total luminescence spectra (TLS) were obtained in the form of excitation/emission matrix (EEM) by scanning the wavelength emission over the range of 300–600 nm, also the excitation wavelength was in 5 nm steps from 240 to 550 nm. 1$^{st}$ and 2$^{nd}$ inner filter effects were corrected. The fluorescence intensity values of samples (in counts per second, CPS) were corrected using the method devised by Lakowicz [2].

$^{13}$C NMR spectra were recorded using a Bruker Avance 500 DRX NMR spectrometer. Samples were dissolved in 0.1 M NaOD in deuterium dioxide. The aromaticity ($f_a$), hydrophilicity and hydrophobicity ratio ($H_{fi}/H_{fo}$) and biological activity (*BiA*) of FAs were calculated from the $^{13}$C NMR spectra as a ratio of the integrated intensities.

## Results and Discussion

### Elemental composition

The elemental compositions are presented in Table 1 in atomic percent, because, in contrast to weight percent, it better reflects the proportion of hydrogen in the structure of fulvic acids. The elemental compositions of the FAs samples are comparable: C, 36.0–38.7 at.%; H, 37.4–40.0 at.%; N, 0.5–0.9 at.%; S, 0.0–0.2 at.% and O, 22.7–23.5 at.%. The H/C atomic ratio is considered as an indicator of aromaticity and, for our FAs, lay within range from 0.97 to 1.11. The aromaticity of fulvic acids from South-Bohemian peat was the higher of the FAs isolated from forest soil. The O/C atomic ratio reflects the amount of oxygen-containing groups (e.g. carboxylic acid, phenols, and ethers) in organic material. This ratio ranges from 0.59 to 0.65 in FAs. The higher value for the O/C ratio for FAs from soil can be attributed to the high ash content rather than be related to differences in the structure of FAs.

**Table 1.** Results of elemental analysis (atomic %), and calculated ratios

| sample | C (at.) | H (at.) | N (at.) | S (at.) | O (at.) | H/C | O/C |
|---|---|---|---|---|---|---|---|
| **FASBP** | 38.7 | 37.4 | 0.9 | 0.2 | 22.7 | 0.97 | 0.59 |
| **FAHP** | 36.0 | 40.0 | 0.5 | – | 23.5 | 1.11 | 0.65 |

### Steady-state fluorescence spectroscopy

The excitation-emission matrix spectra (EEM) of terrestrial FAs isolated from South-Bohemian peat and forest soil are shown in Figure 1. The fluorescence EEM spectra of terrestrial FAs were characterized by two unique fluorophores centered at an excitation/emission wavelength pair (EEWP) of 250/430–435 nm (fluorophore type A-fulvic-like respective sign α') and 300–305/425 nm (fluorophore type C-humic-like respective sign α). The characteristic C domain for humic substances originating from terrestrial origins with fluorescence maxima corresponding to excitation/emission wavelength pairs of 300–380/400–500 nm was also observed in our spectra [3]. Furthermore, fluorescence EEM spectrum of FAs isolated from forest soil was characterized by two fluorescence peaks located at EEWP of 280/320 nm (fluorophore type B-tyrosin-like respective sign γ) and 280/355 nm (fluorophore type T-tryptophan-like respective sign δ). The shorter emission wavelengths measured in the EEM spectra can be associated with simple aromatic structures (one- to three-ring) and low molecular weight components. In contrast, the longer emission wavelengths can indicate the presence of condensed aromatic rings and electron-withdrawing groups such as carbonyl-containing substituents, and hydroxyl and alkoxyl groups [4].

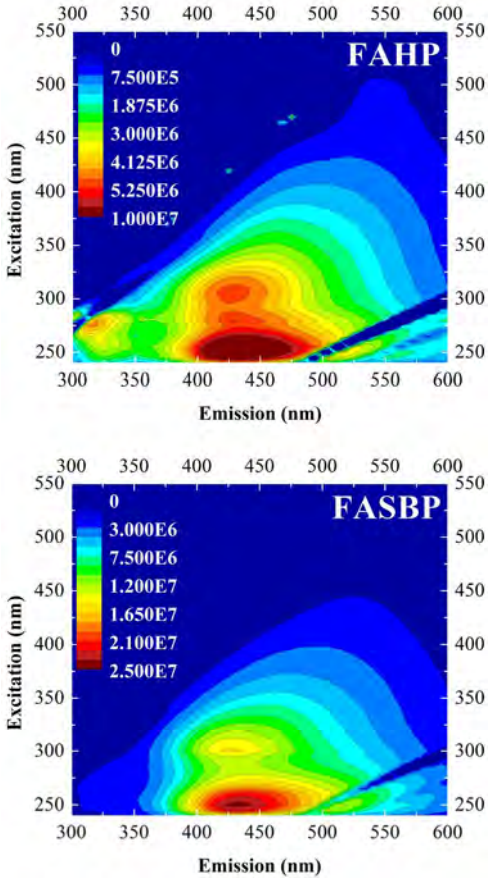



**Figure 1.** Excitation-emission spectra of FAs isolated from Humic Podzol (FAHP) and South-Bohemian peat (FASBP).

### $^{13}$C NMR spectroscopy

The quantitative $^{13}$C NMR spectra of the studied FAs showed typical resonance of humic substances: aliphatic carbon (0–45 ppm), saccharides and amino acids (45–106 ppm), aromatic and phenolic carbon (106–165 ppm) and carboxyl and carbonyl carbon (165–220 ppm) [5]. The typical resonance of FAs and values of $^{13}$C NMR indexes are presented in Table 2–3. The $^{13}$C NMR spectrum of FA isolated from forest soil contained in addition to the signals typical for FAs also many sharp signals of amino acids in the area of 45–106 ppm. The $^{13}$C NMR spectrum showed that FAs isolated from South-Bohemian peat contained the higher amount of aliphatic carbon ($sp^3$ hybridization) and carbonyl and/or carboxyl functional groups. The aromaticity ($f_a$) and *BiA* index of fulvic acids from South-Bohemian peat was the higher of the FAs isolated from forest soil. Whereas, FA isolated from forest soil was characterized higher value of $H_{fi}/H_{fo}$ ratio. The $H_{fi}/H_{fo}$ of FAs were calculated from the $^{13}$C NMR spectra as a ratio of the integrated intensities $I_{(45–106+165–220)}/I_{(0–45+106–165)}$.

**Table 2.** Average distribution of individual carbon types in the studied FAs from the $^{13}$C NMR spectra

| sample | 0–45 ppm | 45–106 ppm | 106–165 ppm | 165–220 ppm |
|---|---|---|---|---|
| **FASBP** | 24 | 20 | 30 | 26 |
| **FAHP** | 16 | 34 | 31 | 19 |

**Table 3.** $^{13}$C NMR ratios ($f_a$, *BiA* and $H_{fi}/H_{fo}$) of studied FAs

| sample | *fa* | *BiA* | *Hfi*/*Hfo* |
|---|---|---|---|
| **FASBP** | 0.41 | 1.27 | 0.85 |
| **FAHP** | 0.38 | 1.00 | 1.13 |

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# Chemometrics analysis of different fractions of soil organic matter

A. P. FERNANDES [(a)*], M. C. TAVARES [(a)], W. G. BOTERO [(a)], A. D. M. CAVAGIS [(b)], L. C. OLIVEIRA [(b)]

(a) Department of Chemistry, University of Alagoas, 57160000 Maceio, Brazil
(b) Department of Chemistry, University of São Carlos, 18052-780 Sorocaba, Brazil
* E-mail: fernandesdea@gmail.com

**Keywords:** extraction, humic substances, soil solution, simulated rainfall

**Abstract.** An investigation was made of the influence of the extraction on the characteristics of the organic matter fractions extracted from two samples of Brazilian soils. The extractions were carried out with NaOH, $NaNO_3$ and simulated rain. The ten fractions extracted were characterized by UV-vis spectroscopy, molecular fluorescence and acid-base titration. The parameters obtained from the characterizations indicated significant differences between the content and structural characteristics of the organic matter present in the fractions depending on the extractor used, underlining the importance of the development of methodologies and analytical procedures that minimize structural changes and are more representative of the active compounds in the environment.

## Introduction

Soil organic matter (SOM) is the main source of soil organic carbon, fundamental in the carbon balance, soil elements, environmental sustainability and climatic conditions with implications for biogeochemical processes in rivers, lakes and estuarine systems [1].

In order to better understand the behaviour of the SOM in the environment, including interaction with nutrients and contaminants, it is important to extract the material, with minimum changes in the original structures, reduced time, high yield and low contaminant content [2]. The International Humic Substances Society (IHSS) recommends the standardization of procedures for the collection, extraction and purification of humic and fulvic acids from different environmental matrices [3]. In order to minimize difficulties in comparing the characteristics of humic substances, some questions about the environmental representativeness, genesis and chemical structure of humic substances (HS), humic acids (HA) and fulvic acids (FA) extracted by NaOH. Thus, a variety of extractors such as $Na_4P_2O_7$, complexing agents, formic acid, acidic mixtures and organic solvents are used to understand the effect of the extractor on the characteristics of the fractions obtained [4].

Due to heterogeneity in the chemical composition of SOM, the structural characterization is very important for a better understanding of the activity and dynamics of these substances in the natural environment. In the study using different extractors, the chemical characterization presents itself as a mapping of the selectivity of the extractor to the structures preferentially extracted, becoming a differential when looking for a greater representativeness of the existing substances in the environment [4]. This work had the objective of extracting and characterizing different organic matter fractions of soils with different organic matter contents, using NaOH, $NaNO_3$ and simulated rainfall as extractors and chemometric analysis to better understand the relationships between the characteristics of each fraction.

## Materials and methods

**Soil samples.** Soil samples were collected between 0-20 cm depth in a semi-arid region (SS) with long periods of drought and in a region of humid climate (SU).

**Extraction HS, HA, FA.** Humic substances from semi-arid (SHS) and humid (SHU) soil, fulvic acid fractions (AFS, AFU) and humic acids (AHS, AHU) were extracted and purified following the procedure of IHSS [3].

**Extraction of the soil solution.** Soil solutions corresponding to the semi-arid and humid climate (SSS and SSU) were obtained using $NaNO_3$ 0.1 mol $L^{-1}$, ratio 1:10 soil: extractor, agitation for 4 h, followed by decantation for 2-16 h centrifugation to separate the supernatant and the soil residue [5].

**Extraction simulated rainfall.** A rainfall simulator was used with specifications cited by Oliveira et al., 2012 [6] equipped with a Veejet 80-150 sprinkler nozzle, internal diameter of 12.7 mm, 2.7 m above the surface of the soil, operating at a pressure constant of 41 kPa, during 30 min with an average intensity of 96.66 mm $h^{-1}$.

**Characterization of the extractions.** The different fractions of the SOM were characterized by the $E_{465}/E_{665}$, $E_{250}/E_{365}$, $E_{270}/E_{407}$ ratios, molecular fluorescence spectroscopy ($A_4/A_1$, $A_{465}$), determination of carboxylic and phenolic groups by potentiometric titration ($Q_1$ and $Q_2$).

**Chemometrics analysis.** In order to evaluate the similarity between the different fractions of the SOM, a chemometrics analysis was performed with the MINITAB 12.

## Results and Discussion

From the hierarchical cluster analysis (HCA) it is possible to observe the formation of clusters in a hierarchical structure in which the samples are placed in groups and subgroups depending on the similarity between them. The cluster in Figure 1 shows the groups formed by the different fractions of SOM from the similarity of their chemical characteristics.

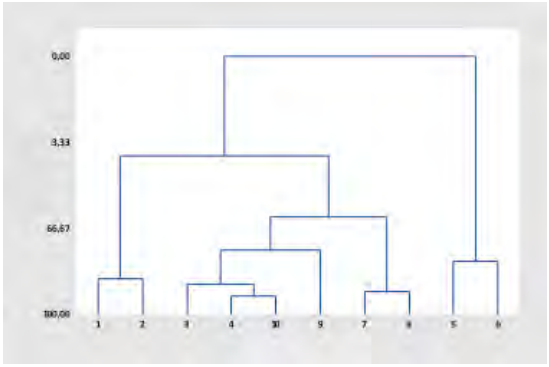



**Figure 1.** Cluster for SOM fractions corresponding to parameters $E_{465}/E_{665}$, $E_{207}/E_{470}$, $E_{250}/E_{365}$, $A_4/A_1$, $A_{465}$, $Q_1$ and $Q_2$.

In Figure 1 it is possible to observe the formation of a group that relates the characteristics of SHS and SHU with the similarity of 86.16%. These fractions were the result of the same type of extraction and were not submitted to purification treatment, which justifies this similarity. The second group, the result of three groupings, relates AFS, AFU, SLS, SLU, SSS and SSU with 62.24% similarity. The third group relates to the AHS and AHU fractions, which are fractional samples from the respective HS and that were submitted to the purification procedure, leading to such different characteristics.

It can also be observed that the HS group and the group of the other fractions with the exception of the HA present a relation of 38.54%, whereas this new group does not present similarity with the HA group. This may be justified by the fact that HA was purified using an HCl/HF treatment that can lead to structural alterations of the parent compounds.

The principal component analysis (PCA) is shown in Figure 2, where the first component (PC1) represents 79.1% of the total data variance and is produced mainly by the parameters of $E_{270}/E_{407}$ and $E_{250}/E_{365}$ positively in PC2 and negatively in PC 1. The second component (PC2) explains 12.9% of the total data variance. The HS group is influenced by parameter $E_{465}/E_{665}$, whereas the parameters $Q_1$, $Q_2$ and $A_4/A_1$ have a greater influence on the formation of the HA group. Already the group of the other fractions is influenced by the parameters $E_{270}/E_{407}$ and $E_{250}/E_{365}$ while $A_{465}$ has no influence on the distribution of the groups.

The chemometrics analysis allowed the observation of the similarity of the fractions and the formation of three groups based on the parameters obtained in the characterization. The group of humic acids, presenting higher aromatic condensation and molecular size, a higher degree of humification, and higher content of carboxylic and phenolic groups.

The AFS, AFU, SSS, SSU, SLS, SLU fractions show lower composition in condensed aromatic structures, lower molecular size, the higher contribution of lignin derivatives, less humid and lower content of phenolic and carboxylic groups in relation to humic acids. Humic substances (SHS and SHU) therefore have intermediate characteristics.

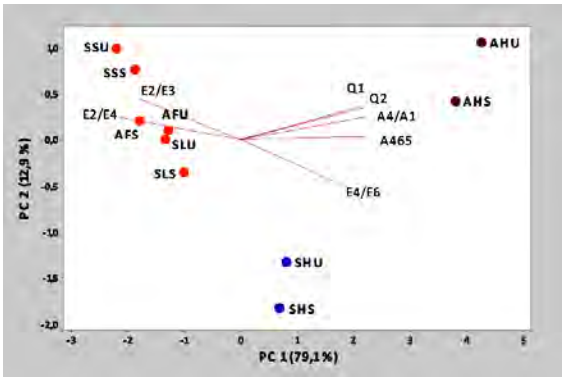



**Figure 2.** Principal componente analysis for SOM fractions corresponding to parameters $E_{465}/E_{665}$, $E_{207}/E_{470}$, $E_{250}/E_{365}$, $A_4/A_1$, $A_{465}$, $Q_1$ and $Q_2$.

From the studies carried out, it can be inferred that the extraction procedure influences the characteristics of the extracted organic material. This comparison is important from the environmental point of view since it allows to obtain more realistic parameters regarding the SOM active fraction and its influence on the bioavailability of contaminants to the environment, for risk assessments and molecular modelling.

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# Organic matter content and quality of Bulgarian soils

E. FILCHEVA [(a)*]

(a) Department of Genezis, Geography and Classifications of Soils, ISSAPP "N. Poushkarov,"1080 Sofia, Bulgaria
* E-mail: filcheva@itp.bg

**Keywords:** soil, humic substances, humic acids, fulvic acids, heavy metals

**Abstract.** Based on the ratio $Ch_1/Cf_1$ soils in Bulgaria are divided into five groups: a. izohumic saturated (A) b. izohumic unsaturated (B) c. texture differentiated (C), hydromorphic (D) and e. shallow and poorly developed (E). The difference is statistically proven with significance level of p 0.005 -0.01. The main characteristics of these groups are described in the paper.

## Introduction

The quantity and quality of soil organic matter have a positive influence on many soil properties, mainly on the soil physical properties and the processes of structure formation. Humus substances interact with mineral colloids and form organo-mineral compounds and associates with complex nature and structure. An important role of soil organic matter is to bind heavy metals and some organic contaminants in polluted soils [1, 2, 3].

Humus substances play an important role in soil processes, transport, accumulation and mobility of metal ions [1, 2, 3]. The stability of the complexes formed depends on the metal ion and the maturity of the humus substances. It was found that humic acids bind more strongly to metal ions than fulvic acids [4]. The composition of the SOM and especially the prevalence of humic acids over the fulvic acids give some sense to suggest formation of more stable complexes with metal ions – contaminants.

## Materials and methods

Soil organic carbon content was determined by modified Turin's method, soil organic matter composition was determined by the method of Kononova-Belchikova [1, 2, 3].

Information on the composition of soil organic matter of 354 profiles (19 soil types) of publications of different authors was generalized. Based on the ratio Ch/Cf in the surface, soils in Bulgaria are divided into five main groups. The variation (minimum and maximum) of the main components of soil organic composition (Ch, Cf, Ch/Cf, Cres.,) for the soils in the each group define characteristics of the main groups (A, B, C, D and E). The generalized data were published earlier [3].

## Results and Discussion

A key indicator in the soil grouping is the ratio between the organic carbon content of humic and fulvicacids in topsoil ($Cx_1/Cf_1$). The difference is statistically proven with significance level of p 0.005 - 0.01. When the above mentioned criteria ($Cx_1/Cf_1$) is not sufficient, additional values for Cres., Cx and Cf are used as the differences, which are statistically proven. Based on the above, soils in Bulgaria are divided into five main groups as follows: a. izohumic saturated (A) b. izohumic unsaturated (B) c. texture differentiated (C), the hydromorphic (D) and e. shallow and poorly developed (E) [2]. Groups of izohumic saturated soils and texture differentiated soils are subdivided into subgroups.

The main soil groups are characterized as follows:

Group of izohumic soils saturated (A), with humic type of humus soils that have developed under Xerothermic conditions: Calcic Chernozems, Haplic Chernozems; Luvic Chernozems (Ch/Cf <2> 1.5) and Haplic Chernozems, Vertisols, Rendzic Leptosols (FAO, 2006) with ratio Ch/Cf> 2.

Group of izohumic soils unsaturated (B) combines Mountain-meadow soils (Umbrisols, FAO, 2006) and Brown forest soils (Cambisols, FAO, 2006). The humus horizon is Umbric type and the carbon content ratio in humic and fulvic acids in surface horizon (Ch/Cf) is <1.

Group of texture differentiated soils (C): Albic Luvisols, Planosols and Haplic Luvisols on the basis of differences in the hydrothermal system is divided into two subgroups.

Group of Hydromorphic soils (D) combines Mountains (Peat, Histosols, FAO, 2006) and lowland (Marsh meadow, Gleysols, FAO, 2006) soil types and is formed solely on the basis of hydromorphic conditions of soil formation.

Litosols and Regosols which significantly differ in terms of humus formation and conditions are combined into one group (E). The process of humus formation is: a. Litosols - advanced in Litosols, but it is limited in depth of close lying parent materials and b. Regosols - no depth limitation, but the process is in its initial stage.

The established regularities in the change of the ratio between the amount of humic and fulvic acids are as function of the soil formation factors [5] and confirmed by the summarized results of a earlier survey [1, 2]. This allows the ratio Ch/Cf to be used as a diagnostic feature in soil classification, which is confirmed by the other authors [5, 6].

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**Supplementary material**

Data on the composition of soil organic matter (Cf, Cf, Ch/Cf, Cres. of 354 profiles (19 soil types) for the first two depths of the profiles were statistically treated and generalized.

**Group of Izohumic saturated soils (A)**

The total carbon content, the amount of humic and fulvic acids, their ratio and unextracted organic carbon (Cres.) in izohumic saturated soils are within the following limits (data based on the statistical generalized profiles of the virgin variants).

**Table 1.** The main characteristics of the group of Izohumic saturated soils (A):

| Horizons | C,% | Ch,% | Cf,% | Ch/Cf | Cres.,% |
|---|---|---|---|---|---|
| First depth | 1.86-5.57 | 0.34-1.68 | 0.24-0.82 | 1.39-2.73 | 1.29-3.07 |
| Second depth | 1.03-2.93 | 0.22-1.07 | 0.13-0.67 | 0.88-2.07 | 0.55-1.34 |

In the group of **Izohumic unsaturated soils (B)** entered the Mountain meadow (Umbrisols, FAO, 2006) and Brown forest soils (Cambisols, FAO, 2006). The conditions of humus formation in these soils are some what similar, hummus type is - Moder: Climatic conditions which form the Mountain meadow soils (Umbrisols, FAO, 2006) are characterized with low intensity of mineralization of plant residues of subalpine and alpine herbaceous vegetation. Under these acidic conditions creates where the transformation of organic matter leads to formation of Umbrik horizon in the highest parts of the Alpine passes into “Moder” type.

**Table 2.** The main characteristics of the group of Izohumic unsaturated soils (B)

| Horizon | C,% | Ch,% | Cf,% | Ch/Cf | Cres.,% |
|---|---|---|---|---|---|
| First depth | 6.87-10.17 | 1.44-3.11 | 1.46-2.61 | 0.99-1.19 | 3.85-4.60 |
| Second depth | 3.07-8.26 | 0.60-1.74 | 1.0-2.03 | 0.60-0.86 | 1.28-2.44 |

**Group of Texture differentiated soils (C)**

Pseudopodzolic (Light gray-podzolic soils, Cinnamonic-podzolic and Yellow podzolic) soils occupy about 0.33 million ha, 3% of the total area of the country. The conditions of humus formation and humus accumulation in Pseudopodzolic soils are unfavorable. The type of organic matter in soils is fulvic across soil profile (Ch/Cf <0.5). The content of humic acids is low and sharply decreased along the profile depth. Morphological findings in the darker bottom of the profile is associated with some increase in organic carbon content, probably due to the movement of mobile humic acids in this horizon and their bounding with calcium to form low mobile compounds. Consequently, low pH and periodic surface waterlogging, the degree of humification in these soils, especially in the area of waterlogging is low, except at the top of eluvial horizon, and in the dark part (lower profile). Humic acids in the surface horizon are represented by "free" and bounded with mobile $R_2O_3$. The degree of condensation of aromatic nuclei is low ($E_4$: $E_6$ 5-7).

**Table 3.** The main characteristics of the group of Texture differentiated soils (C)

| Horizons | C,% | Ch,% | Cf,% | Ch/Cf | Cres.,% |
|---|---|---|---|---|---|
| First depth | 1.57-3.70 | 0.14-0.58 | 0.26-0.61 | 0.66-1.2 | 1.02-2.51 |
| Second depth | 0.48-1.02 | 0.13-0.14 | 0.07-0.27 | 0.07-0.50 | 0.48-0.7 |

**Group of Hydromorphic soils**

Organic carbon content is high to very high, the type of organic matter in this group according to the classification of Grishina and Orlov [6] is fulvic-humic to humic. Peat lands are subdivided to mountains and lowlands. Key features of SOM for soil hydromorphic group of soils (data are based on generalized statistical profiles [2]. The type of SOM throughout the depth of the profile is humic (Ch/Cf about 2.5) and only in the lower part humic-fulvic or fulvic. The degree of humification in these soils is very low. Humic acids are bounded with calcium and have a high optical density due to the carbonate soil forming materials.

**Table 4.** The main characteristics of the group of Hydromorphic soils (D)

| Horizons | C,% | Ch,% | Cf,% | Ch/Cf | Cres.,% |
|---|---|---|---|---|---|
| First depth | 6.34-23.43 | 1.32-7.58 | 1.08-3.91 | 0.96-3.38 | 3.94-13.33 |
| Second depth | 3.6-33.09 | 0.79-5.02 | 0.55-2.92 | 1.44-2.54 | 1.98-26.09 |

# Quantitative and qualitative characterisation of humic products with spectral parameters

E. FILCHEVA [(a)*], M. HRISTOVA [(a)], P. NIKOLOVA [(a)], T. POPOVA [(b)]
K. CHAKALOV [(b)], V. SAVOV [(c)]

(a) N. Poushkarov Institute of Soil Science, Agrotechnology and Plant Protection,
7, Shosse Bankya Str., 1080 Sofia, Bulgaria
(b) Balkan Plant Science Ltd., Bld, Tsar Osvoboditel 25A, Bulgaria
(c) Biology Faculty "St. Kl. Ohridski" Sofia University, 8, Blvd. Dragan Tsankov", Sofia, Bulgaria
*E-mail: filcheva@itp.bg

**Keywords:** Humic products; Fulvic acids; Humic acids; Optical characteristics; Ratio $E_{465}/E_{665}$; $E_{300}$; $E_{850}$

**Abstract.** In recent years the application of different humic products for the treatment of soils and plants has considerably increased. The characteristics of humic products, i.e. content and composition of organic carbon and the maturity, provide valuable information, essential for their adequate application. Such information is of crucial importance for the manufacturers, business consultants and users involved in the production, distribution and implementation of humic products. This study presents a correlation between the quantitative indicators of commercial humic products and their spectral characteristics via measurements of UV (300 nm); visible (445 and 665 nm); near-IR (850 nm) spectra.

## Introduction

Humic products play an essential role in soil. Rapid and easy estimation studies are useful to assess the properties of humic products. Such studies consist of spectrophotometric measurements of the extinction of the HAs at 465 nm ($E_4$) and 665 nm ($E_6$) and of the determination of the $E_4/E_6$ ratio which can be used to assess the maturity of HAs [1].

The evaluation of HAs in the $E_4/E_6$ ratio is frequently being used to assess the degree of condensation and the aromaticity of the carbon structure, the carbon content and the molecular weight of humic products [2, 3]. Spectroscopy in the UV and NIR ranges is a useful approach for monitoring the composting process, but the projections of near-infrared spectroscopy (NIRS) yielded better results than the precision of UV calibration [4]. Other researchers have shown a weak correlation between the HAs/FAs ratio with the UV index [5].

In the present study, as in our previous ones, we are dealing with humic products of different origins and manner of production - from leonardite, lignite biotransformed with *Trichoderma* sp. (Plantagra), plant materials after pyrolysis and composting. The basis of our research is represented by standard quantitative methods for determining the content of organic matter as well as HAs and FAs amounts within organic matter. Our previous studies proved that there is a good correlation between the composition of the humic products as a mixture of humic acids (HAs) and fulvic acids (FAs) and their optical characteristics of spectral measurements at wavelengths of 300, 445, 665 and 850 nm [6].

This study presents the correlation between the quantitative indicators of commercial humic products and their spectral characteristics by measurements in the visible area at 300 nm, 445 and 665 nm and in the near-infrared area at 850 nm.

## Materials and methods

This study describes 14 humic commercial products (liquid and solid) used as soil improvers and leaf feeders. For all humic products, we determined the concentration of organic carbon via the modified Turin's method [6].

The precipitation of humic acids from the starting solution determines the composition of the humic products in terms of humic acids (HAs) and fulvic acids (FAs). The specific extinction can be assessed via measuring the absorption of HAs spectra at the following wavelengths: 300, 465, 665 and 850 nm. The degree of aromaticity and condensation of humic products determines the optical density of the HAs via the $E_4/E_6$ ratio.

Extinctions of the studied preparations were measured by spectrophotometer WPA Light wave II. The apparatus has a measuring range of 200 to 1100 nm.

## Results and Discussion

Table 1 shows the types of humic products, the content of total organic carbon, the content of carbon in HAs and FAs, the ratio HAs/FAs, the ratio $E_4/E_6$, $E_{300}$ and $E_{850}$. The investigated products are divided into two groups by the ratio HAs/FAs. The first group include HAs/FAs ratios below 1, indicating that FAs dominate (2L, 6L, 7L, 14L).

The second group includes ratios above 1, indicating that HAs dominate (1S, 3L, 4S, 5S, 6L, 7L, 8L, 9S, 10L, 11S, 12S, 13S, 14L). The content of total organic carbon widely varied from 5.50 to 375 g/l in both groups. The content of carbon in HAs, as a percentage of the total carbon in fulvic type products, accounted for 1.29 to 16.00%, while in the humic types, it represented 51.43 to 91.92%. The ratio $E_4/E_6$ had a minimum value of 2.97 (13S) and a maximum value of 6.35 (6L) (Table 1).

**Table 1.** The main parameters of humic products

| Type of humic product | TOC g/l | HAs / FAs | $E_4/E_6$ | $E_{300}$ $cm^{-1}$ | $E_{850}$ $cm^{-1}$ |
|---|---|---|---|---|---|
| 1S Agros | 147 | 1.37 | 4.50 | 0.73 | 0.03 |
| 2L Biohumas | 101 | 0.01 | 6.35 | 2.34 | 0.04 |
| 3L Bioprantagra | 246 | 4.23 | 4.14 | 2.34 | 0.03 |
| 4S Potassium humates | 247 | 8.15 | 4.16 | 0.64 | 0.02 |
| 5S Humates sodium | 357 | 1.46 | 3.43 | 0.63 | 0.02 |
| 6L Humic products | 5.00 | 0.19 | 5.72 | 2.42 | 0.04 |
| 7L Humic acid BB | 76.6 | 0.05 | 5.65 | 2.15 | 0.05 |
| 8L HAs | 7.00 | 1.06 | 4.23 | 0.74 | 0.02 |
| 9S HAs (Leonardite) | 280 | 5.09 | 4.37 | 2.25 | 0.05 |
| 10L HAs | 6.20 | 6.75 | 3.99 | 1.90 | 0.03 |
| 11S Humic products | 303 | 2.29 | 3.98 | 1.40 | 0.04 |
| 12S Humintech | 297 | 11.3 | 3.58 | 2.50 | 0.08 |
| 13S Lumbri 21 | 140 | 8.33 | 2.97 | 2.50 | 0.07 |
| 14L Lumbri bio | 185 | 0.01 | 5.45 | 2.48 | 0.05 |

Absorption at 465 nm reflects the organic material at the beginning of humification, while absorption at 665 nm indicates a high degree of condensation of aromatic constituents (4).There is a direct relationship between the groups with prevailing HAs or FAs in the humic products and the $E_4/E_6$ ratio. When FAs were dominant, the $E_4/E_6$ ratio ranged from 5.45 to 6.35, i.e. for the humic products Limbi bio (14L), with a high quality, and Biohumas (2L), with the lowest degree of condensation. The $E_4/E_6$ ratio ranged from 2.95 to 4.50 when HAs were dominant. The highest degree of condensation was found in the preparation of Lumbi 21 (13S), while the humic product Agros (1S) showed the lowest degree of condensation. Results published by other authors also prove that $E_4/E_6$ ratios of HAs of around 4.04 indicate high quality. In the case of FAs domination, the $E_4/E_6$ ratio reaches values of up to 6.17; when HAs dominate, the value is 4.34 [7].

For example, we introduce a wavelength of 300 nm established as the maximum wavelength [6]. Our research is in agreement with other studies which reported that spectra of HAs and FAs are similar, but the extinction values decreased with increasing wavelengths [7]. The maximum extinction ($E_{300}$) values at 300 nm covered an extensive range of 0.63 (5S) to 2.50 (12S) in the second group (HAs). In the other group (FAs), the $E_{300}$ values did not vary significantly and ranged from 2.1 (7L) to 2.5 (14L).

The values of the minimum extinction at 850 nm ($E_{850}$) showed a similar variance. Based on these results, $E_{300}$ and $E_{850}$ are more stable indicators for humic products when FAs dominate. Our study found no significant correlation between $E_{300}$ and $E_{850}$ ($R^2 = 0.5717$).

## Conclusions

The presented optical characteristics for humic products show that there is a direct relationship, especially between HAs/FAs and $E_4/E_6$ ratios.

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# Variability of soil carbon and nitrogen content and quality of humic substances in Norway spruce stands at two contrasting altitudes

E. GÖMÖRYOVÁ [(a)*], G. BARANČÍKOVÁ [(b)], E. TOBIAŠOVÁ [(c)], D. GÖMÖRY [(a)]

(a) Faculty of Forestry, Technical University in Zvolen, 960 53 Zvolen, Slovakia
(b) National Agricultural and Food Centre – Soil Science and Conservation Research Institute, 82713 Bratislava, Slovakia
(c) Department of Soil Science, Slovak Univeristy of Agriculture in Nitra, 94976 Nitra, Slovakia
* E-mail: gomoryova@tuzvo.sk

**Keywords:** forest soils, heterogeneity, soil organic matter, spruce stands

**Abstract.** Variability of C and N concentration and humic substances (HS) quality were evaluated at two plots located at the altitudes of 484 and 1340 m a.s.l. in Poľana Mts. (Slovakia). The data showed that C and N content exhibited higher variability at the lower altitude than at the higher altitude. Also C and N content differed significantly during the vegetation period with a higher amount in the summer. On the other hand, HS quality did not differ distinctly within the plots; however, differences were found between the sites at different altitudes.

## Introduction

Soil properties can vary both vertically and horizontally, from short distances (micro-scale) to distances of tens to hundreds of meters, from the field level to the regional or landscape level [1]. Vertical gradients of soil organic matter (SOM) content are well documented, recently the focus has been on horizontal patterns. Forest soils exhibit generally much higher horizontal heterogeneity than agricultural sites [2]. On the small scale level (forest stand) the variability results from direct and/or indirect effects of trees via different quantity and quality of above- and belowground litter input, differences in microclimate, as well as spatial distribution of water and nutrient uptakes by trees.

Spatial variability of SOM is known to influence ecosystem processes at various scales [3]. The aim of the study is to evaluate the small scale spatial variability of SOM quantity and quality in two spruce stands located at contrasting (lower and higher) altitudes. We hypothesize that changes in the precipitation amount and air temperature with the altitude will be reflected not only in the amount and quality of SOM, but also in their variability, as the effect of trees can be enhanced or inhibited through different mezoclimate conditions. Studying the influence of climatic and site specific factors on SOM along an altitudinal gradient is important for understanding the response of SOM to global warming. Moreover, the results may help to improve sampling designs.

## Materials and methods

The study was performed in the Poľana Mts. (Slovakia) at two localities with Norway spruce stands (*Picea abies* L.) situated at the altitude of 484 m a.s.l. (Iviny) and 1340 m a.s.l. (Poľana). The soil type at the plots is representied by Gleyic Cambisol (Iviny) and Andic Cambisol (Poľana), both formed from andesite slope depositions. Soil samples were taken from the depth of 0–10 cm (A-horizon) in May, July and October 2012. Regular grid with 10 m distance of sampling points was established. Totally 61 samples were taken from each plot at each sampling date.

In air dried soil samples, total C and N concentration was determined using Vario Macro CNS Analyzer. Three or two samples at Iviny and Poľana, respectively, with contrasting C concentrations were used for the isolation of humic acids (HA). HAs were isolated according to the modified method established by the IHSS. In HAs elemental analysis, optical parameter $E^{1\%}_{6}$ and carboxylic groups were determined. Elemental analysis of HA was performed using a CHN analyzer Perkin Elmer CHN 2400. Carboxylic groups were determined according to Schnitzer et al. [4]. Varian Carry 50 was used for the determination of optical characteristics $E^{1\%}_{6}$.

## Results and Discussion

The soils differed significantly in the C and N concentration, while the soils at the higher altitude exhibited approx. three times higher C and N content than those on the lower location. This outcome is not unexpected: (i) it was shown that SOM in allophanic soils is intensively accumulated due to the effect of allophane and the formation of Al-organic complexes [5, 6]; (ii) with increasing elevation increased organic C and N is usually observed [7].

**Table 1.** C and N concentration (%) and C/N ratio: means and coefficients of variation (CV) at localities at different altitudes and at different times of sampling

| | C | | N | | C/N | |
|---|---|---|---|---|---|---|
| | mean | CV | mean | CV | mean | CV |
| **Iviny, 484 m a.s.l.** | | | | | | |
| May | 5.0 a | 51.6 | 0.3b | 47.4 | 14.8a | 9.6 |
| July | 6.2 b | 41.2 | 0.4a | 32.7 | 14.2b | 9.2 |
| Oct | 4.2 a | 60.2 | 0.3b | 47.7 | 13.6c | 11.9 |
| **Poľana, 1340 m a.s.l.** | | | | | | |
| May | 17.2a | 28.7 | 1.1a | 16.2 | 14.9a | 12.6 |
| July | 16.1a | 20.3 | 1.1a | 11.7 | 14.5a | 11.7 |
| Oct | 16.1a | 23.2 | 1.1a | 13.1 | 14.3a | 12.2 |

Means with the same capital letters do not differ significantly; based on Duncan's pairwise tests.

Poľana Mts. represent a volcanic range where humid conditions in higher locations and the presence of volcanic glass lead to the occurence of amorphous allophane which significantly influences other soil properties including SOM accumulation in contrast to lower sites, where soils can often dry off due to higher temperatures and lower precipitations, while allophane changes to a crystallic form and loses its ability to affect SOM accumulation. Surprisingly, the overall C/N ratio did not differ much between localities.

The soils differed in the temporal course of their measured characterstics during the vegetation season (Table 1). While C, N and also C/N ratio did not differ significantly in Andic Cambisol between sampling dates, C and N content in Gleyic Cambisol was significantly higher in summer in comparison to samples from spring and autumn. C/N ratio differed significantly between all sampling dates and decreased from spring to autumn.

The lower locality was characterized by a higher variability of C and N content, but a lower variability of C/N ratio. The variability of C and N content was 2–3 times higher compared to the higher altitude, especially CV of C content (41–60 %). The variability changed during the vegetation season at both localities. The lowest CV regarding the C and N content was found in summer, the highest in October at the lower altitude and in May at the higher altitude. The variability of C/N ratio did not change considerably during the course of vegetation period, while in comparison to C and N content, its variability was substantially lower at the locality Iviny (3–4 times).

The indicators of SOM quality, evaluated on the basis of chemical composition of HA, indicate their small variability within the plots (Table 2) despite of the fact that analyses were performed though on limited number of samples but differing distinctly in the C and N content. However, the differences between plots were obvious and indicated a higher stability of HS at the higher altitude. Fernandez-Getino et al [8] showed that among environmental factors, vegetation type, the effect of geological substrate and soil taxonomical differences had the most distinct impact on HA characteristics. Climatic factors have also distinct effect on characteristics of HA and SOM as shown by Wang et al. [7]. The change in SOM pools in bamboo plantations and in the rate of humification with elevation was primarily affected by changes in climatic conditions along the elevation gradient. However, when the composition of SOM characterized by NMR spectroscopy and photometric analysis was considered, site-specific factors such as residual SOM from the previous forest and intensive cultivation history could also have an important effect on the HA composition and humification of SOM. The results in our study also indicate that the mesoclimate plays a significant role in the quality of HS and affects it more strongly than the within-plot variation in microclimate associated with the effect of trees.

**Table 2.** Characteristics of HA quality at localities at different altitudes

| Locality | H/C | O/H | E1%6 | COOH |
|---|---|---|---|---|
| Poľana 13 | 0.85 | 0.53 | 14.1 | 4.32 |
| Poľana 18 | 0.81 | 0.53 | 15.6 | 4.81 |
| Iviny 19 | 1.00 | 0.44 | 13.3 | 3.71 |
| Iviny 28 | 0.94 | 0.46 | 13.3 | 3.42 |
| Iviny 46 | 1.01 | 0.42 | 13.2 | 3.42 |

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# Isolation of humic and fulvic acids from the Suwannee river: A report on the 2016 IHSS-sponsored expedition

R. M. HOZALSKI [(a)*], P. BLOOM [(b)], M. PERDUE [(c)]

(a) Department of Civil, Environmental, and Geo- Engineering, University of Minnesota, 55455 Minneapolis, MN, USA
(b) Department of Soil, Water, & Climate, University of Minnesota, 55108 St. Paul, MN, USA
(c) Retired
* E-mail: hozalski@umn.edu

**Keywords:** DAX resin, natural organic matter

**Abstract.** An IHSS-sponsored sampling crew was at the Suwannee River field site for about 4 weeks from mid-May to mid-June of 2016. Approximately 18,700 liters of river water with an average dissolved organic carbon concentration of 49.1 mg/L (total mass of 918 g as C) was processed through DAX resin columns. After desalting and freeze drying, the final yields of humic acids (HAs) and fulvic acids (FAs) were 241 and 937 g, respectively for a total yield of 620 g as C. This resulted in a final C recovery of 67.5%, with the greatest mass losses resulting from the freeze drying process. These Surwannee River humic materials are a valuable resource for the research community as evidenced by the high rate of publications and citations concerning their usage.

## Introduction

The Okefenokee National Wildlife Refuge (ONWR) in southeastern Georgia and northeastern Florida, USA is a remarkable, nearly pristine blackwater ecosystem. The thriving ecosystem includes the headwaters of the famous Suwannee River, where pH is often below 3.5 and the concentration of dissolved organic matter is more than an order of magnitude greater than in the world-average river. It is mainly because of the relatively minimal human impact and very high concentration of dissolved organic matter that the headwaters of the Suwannee River in the ONWR was selected nearly forty years ago as the location from which samples of natural organic matter would be isolated for the standard and reference collection of the International Humic Substances Society (IHSS). Over the years, ten standard and reference samples have been collected there and distributed to hundreds of scientists throughout the world for their research and for interlaboratory comparisons of research methodologies. This paper concerns the latest expedition (Figure 1) on behalf of the IHSS to obtain humic acids (HA) and fulvic acids (FA) to replenish the supplies in the standard and reference collection of the society. We will also discuss the impact that these Suwannee River materials have had on the science and engineering fields.

## Materials and methods

**Resin preparation.** DAX-8 resin was purchased from Sigma-Aldrich Corporation and was cleaned and pre-conditioned using sequential solvent washes in a soxhlet apparatus according to the method of Thurman and Malcolm (1981).[2] Although XAD-8 was used in the original method, DAX-8 and XAD-8 perform comparably in the isolation of humic substances from water (Peuravuori et al.[1] 2001). The cleaned resin was stored in methanol.

**Preparation of resin columns.** At the field lab, the resin slurry was placed into ~9 liter glass columns and rinsed with 10 bed volumes of reverse osmosis (RO) water to remove any residual $CH_3OH$. The resin in the columns was then rinsed three times sequentially with 0.1 M NaOH and 0.1 M HCl.



**Figure 1.** Photo from the 2016 expedition showing some of the crew members and the Suwannee River in the background. From left to right: Jeyson Man, Ryan Earls, Mike Perdue, Daniel Chang, and Ray Hozalski.

**Collection of humic susbtances.** River water was pumped from the Suwannee River and filtered sequentially through a 1 micron polypropylene filter and a 0.45 micron GE Flotrex filter directly into 25-liter polyethylene carboys. The filtered samples were transferred back to the field lab for further processing. In total, we collected and processed 18,700 liters of river water.

At the field lab, each 25-liter carboy of filtered water was acidified to pH 2 with HCl. The acidified water was pumped at a flow rate of 1 liter/min through each of three parallel columns of DAX resin. After 275 liters (about 30 bed volumes) of water was processed on each column, the adsorbed humic substances on each column were collected by back-eluting with 15 liters of 0.1 M

NaOH at a flow rate of 0.35 liter/min. After elution of the humic susbtances, the columns were re-acidified by back-elution with 10 liters of 0.1 M HCl at the same flow rate. The concentrated humic substance solutions were acidified to pH 4 with HCl and stored in a refrigerator.

**Separation of HAs and FAs fractions.** The concentrated solutions were diluted to a DOC of approximately 1000 pm DOC then acidified to pH 1 with HCl and allow to stand for 48 hours under refrigeration allowing the humic acid to precipitate. Then, the HAs were separated from the supernatant solution by centrifugation. The FAs solution was decanted off into a container and the humic acids was re-suspended in a minimum volume of water and transferred to a storage bottle. The HA was diluted to approximately 500 ppm DOC and the pH was increased to 4 to dissolve the HAs. Both the HAs and FAs solutions were stored in a refrigerator.

**Desalting and freeze drying.** The pH 1 FAs concentrates were loaded onto a column of DAX-8 resin. The adsorbed fulvic acids was rinsed by pumping 2-3 bed volumes of water through the column to reduce the Cl- and then the column was back-eluted with 0.1 M NaOH. The alkaline eluent was then passed directly through a $H^+$-saturated cation exchange resin to remove cations (e.g., $Na^+$, $Ca^{2+}$) from the sample. The humic acids solutions were then adjusted to pH 2 with HCl and treated like the fulvic acid. Finally, the $H^+$-saturated humic and fulvic acids were freeze-dried at a commercial freeze-drying facility.

### Results and Discussion

The sampling crew was at the Suwannee River field site for about 4 weeks from mid-May to mid-June of 2016. Information on the Suwannee River Sill sampling site can be found in Yin and Brook (1992).[3] Approximately 18,700 liters of water was processed with an average DOC concentration of 49.1 mg/L for a total mass of 918 g as C. We returned to the laboratory at the University of Minnesota with 839 g as C of concentrated humic susbtances for an intermediate recovery of 91% of the orignal C in the river water After desalting, the yield was 772 g as C (84%). The final yields of freeze dried humic acids and fulvic acids were 241 and 937 g, respectively for a total of 620 g as C. This results in a final C recovery of 67.5%, with the greatest mass losses resulting from the freeze drying process.

### History and Impact

The first IHSS samples were isolated in 1982-1983 and introduced to the scientific community at the first meeting of IHSS in mid-1983. Two plots were generated using Web of Science on March 8, 2018 (Figures 1 and 2). The query spanned the years 1980-2018 and included the following search terms: “Suwannee” AND (“fulvic” OR “humic” OR “natural organic matter” OR “NOM”). Figure 1 shows the number of papers that have been published per year by scientists from all over the world. The rate of publication has grown steadily since the first papers appeared in 1987. Figure 2 shows the impact of the published papers through the number of citations per year of those papers by other scientists. The exponential growth of citations since 1988 has now reached a rate of over 4000 citations per year.

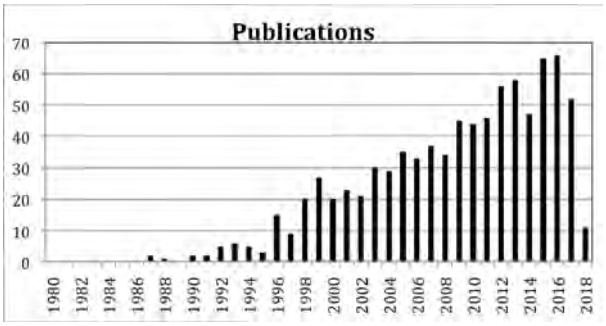



**Figure 2.** Number of published papers per year dealing with fulvic acids, humic acids, and natural organic matter from the headwaters of the Suwannee River in the Okefenokee National Wildlife Refuge. From 1980 to March 8, 2018.

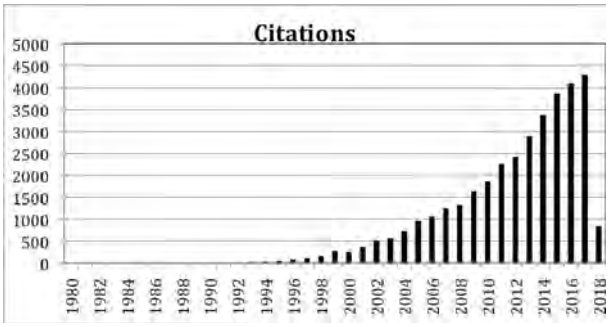



**Figure 3.** Number of citations of published papers per year dealing with fulvic acids, humic acids, and natural organic matter from the headwaters of the Suwannee River in the Okefenokee National Wildlife Refuge. From 1980 to March 8, 2018.

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# Variability of soil chemical properties in the lands of Chavdar village, Bulgaria

B. HRISTOV [(a)*], N. DINEV [(b)], I. NIKOVA [(b)], M. HRISTOVA [(a)]

(a) Department of Genesis, geography and classification of soils, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant protection, 1080 Sofia, Bulgaria
(b) Soil Chemistry Department, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant protection, 1080 Sofia, Bulgaria
* E-mail: bisseru@gmail.com

**Keywords:** soil chemical properties, soil monitoring, agrochemistry, soil variability

**Abstract.** The present study is related to monitoring areas polluted from mining industry over lands with intensive agriculture in the area of Chavdar village. The study area of the survey is localized in the north part of village Chavdar, the watershed of Topolnitsa River in the east, close to the tailing pond. It provides information about chemical and agrochemical properties, variability and correlation between different soil parameters. In that area could be found processes of soil degradation induced by human activities like soil erosion, contamination, soil sealing, domestic waste and other materials on the soil surface. The results show that there is a significant variation of chemical, agrochemical and physicochemical properties.

## Introduction

Lands of Chvadar village are parts of the Zlatitsa-Pirdop field. They were formed through various geological periods. The soil is from shallow to deep and has different physicochemical characteristics and fertility [1]. The lands of the region are polluted from mining industry for copper and gold for centuries [2]. The soils, with the exception of meadow varieties, can generally be defined as low to a medium content of humus and nutritional elements. Much of lands are weak developed, acid, fragmented, heavy, loamy by texture, poor physical and physicochemical properties and difficult to cultivation [3].

The aim of the study is to clarify chemical and agrochemical properties which provide information about soil variability and correlation between different soil parameters.

## Materials and Methods

Figure 1 shows study area - the village of Chavdar with mining tailing ponds in the east (Zlatiza-Pirdop filed; Google Earth).




**Figure 1.** The study area - a village of Chavdar.

The area is characterized with the following soil's type: Deluvial (Colluvial) meadow soils, Alluvial soil, Brown forest soils. The soils types in the fields are Deluvial (Colluvial) meadow soil (medium thick); Undeveloped cinnamon forest (shallow) soils, Alluvial-meadow and Alluvial soils [4]. These soils correlate mainly with Cambisols, Luvisols, Fluvisols and Phaeozems in WRB classification [5].

They are spread around the village of Chavdar, Sofia region, with the elevation of about 520 meters. The parent materials are colluvial gneisses and schist deposits, the vegetation is composed mainly of meadow grasses associations.

The fixed located points of GIS oriented was sampled and detected for following main chemical and physicochemical properties:

- Soil organic carbon % (SOC) and humic acids [6,7];
- The cation exchange capacity in $cmol^+/kg$ (CEC) via a method of Ganev and Arsova [8];
- The mobile forms of phosphorus and potassium in mg/100g via acetate-lactate method of Ivanov [9];
- pH is measured in water [10];
- The mobile forms of ammonia and nitratenitrogen in mg/kg by Bremner and Keeney [11];
- Microsoft Excel 2010 is used for statistics.

## Results and Discussion

The soil chemical properties in the surface horizons 0-20 cm show high variability (Table 1).

The soil results show that pH varies from 5.0 to 7.0 (Table 1) and the mean value is 5.9, which is consistent with other studies in the area which show that the soil reaction is low acidic values pH approximately 5-6 [2]. The soil surface is heterogeneous, there are spots with pH more than 7 and spots with low pH about 5. The reason of alkalic reaction of soils is using the calcium or magnesium meliorations (liming materials). Such practices were used to eliminate high acidity and to blockade free heavy metals ions mainly copper [12]. There are also soils in the region which are rich in calcium, because of carbonated parent rock and river Topolnitsa brings base cations form the surrounding area [3]. Table 2 shows that soil pH correlate basically with exchangeable basic cations such as $Ca^{2+}$, $Mg^{2+}$ and it has a negative correlation with $Al^{3+}$ and total acidity ($H^{8.2+}$).

**Table 1.** Descriptive statistics of main chemical and physicochemical properties

| | pH(H2O) | Org.C.% | Humic Acids % | CEC | ex. H+ | ex. Al+ | ex. Ca+ | ex.Mg+ | B.S.% | Σ NH4+NO3 | P2O5 | K2O |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 5.94 | 1.61 | 0.41 | 27.25 | 7.29 | 0.75 | 16.90 | 3.10 | 72.70 | 25.64 | 3.50 | 22.49 |
| Standard Error | 0.17 | 0.11 | 0.03 | 1.27 | 0.72 | 0.16 | 1.40 | 0.12 | 2.89 | 1.63 | 0.96 | 1.59 |
| Median | 5.80 | 1.47 | 0.38 | 27.40 | 7.80 | 0.40 | 16.00 | 3.20 | 71.40 | 23.60 | 1.80 | 21.70 |
| Mode | 5.00 | 1.54 | 0.38 | 24.90 | 5.50 | 0.00 | 16.00 | 3.20 | 55.00 | 23.00 | 0.20 | 21.70 |
| Standard Devia | 0.89 | 0.61 | 0.15 | 6.86 | 3.85 | 0.84 | 7.52 | 0.66 | 15.58 | 8.76 | 5.15 | 8.59 |
| Sample Varianc | 0.80 | 0.37 | 0.02 | 47.10 | 14.84 | 0.70 | 56.55 | 0.44 | 242.71 | 76.78 | 26.57 | 73.76 |
| Range | 2.40 | 3.00 | 0.79 | 37.40 | 12.60 | 2.30 | 38.70 | 2.50 | 49.00 | 38.10 | 21.40 | 27.70 |
| Minimum | 5.00 | 0.73 | 0.22 | 18.90 | 0.00 | 0.00 | 9.50 | 2.00 | 51.00 | 8.60 | 0.20 | 12.10 |
| Maximum | 7.40 | 3.73 | 1.01 | 56.30 | 12.60 | 2.30 | 48.20 | 4.50 | 100.00 | 46.70 | 21.60 | 39.80 |
| Count | 29.00 | 29.00 | 29.00 | 29.00 | 29.00 | 29.00 | 29.00 | 29.00 | 29.00 | 29.00 | 29.00 | 29.00 |

**Table 2.** Correlation matrix between main chemical and physicochemical properties.

| | *pH(H2O)* | *OC%* | *Humic Acids%* | *CEC* | *ex. H+* | *ex. Al+* | *ex. Ca+* | *ex.Mg+* | *B.S.%* | *ΣNH4+NO3* | *P2O5* | *K2O* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH(H2O) | 1 | | | | | | | | | | | |
| OC% | 0.20 | 1 | | | | | | | | | | |
| Humic Acids | 0.05 | 0.85 | 1 | | | | | | | | | |
| CEC | 0.18 | 0.49 | 0.58 | 1 | | | | | | | | |
| ex. H+ | -0.94 | -0.13 | 0.00 | 0.00 | 1 | | | | | | | |
| ex. Al+ | -0.86 | -0.12 | 0.06 | -0.30 | 0.74 | 1 | | | | | | |
| ex. Ca+ | 0.61 | 0.52 | 0.53 | 0.87 | -0.49 | -0.60 | 1 | | | | | |
| ex.Mg+ | 0.55 | 0.08 | 0.04 | 0.47 | -0.36 | -0.63 | 0.53 | 1 | | | | |
| B.S.% | 0.97 | 0.20 | 0.04 | 0.19 | -0.96 | -0.85 | 0.63 | 0.51 | 1 | | | |
| ΣNH4+NO3 | 0.05 | -0.15 | -0.16 | -0.03 | -0.03 | 0.01 | -0.04 | 0.16 | 0.02 | 1 | | |
| P2O5 | 0.50 | 0.02 | 0.11 | -0.10 | -0.53 | -0.31 | 0.16 | 0.23 | 0.49 | -0.21 | 1 | |
| K2O | 0.35 | 0.00 | -0.02 | -0.09 | -0.44 | -0.33 | 0.16 | -0.14 | 0.40 | -0.20 | 0.31 | 1 |

The content of SOC has significant variation from 0.73 % to 3.73 % in the studied survey area (Table 1). Usually, in cultivation fields, it is about 1.5 % which is something typical for arable lands in Bulgaria.

The high content of organic carbon has in the area of marshes close to Topolnitza river which is situated in the south-east part of the survey filed (point 79, Figure 1). The type of humus in the region is predominantly humic-fulvic with ration Ch/Cf between 1 – 0.5 [3]. In Table 2 is shown that SOC correlates with humic acids, CEC and exchangeable $Ca^{2+}$, a fact proven by other scientists [13].

The amount of "mineral" nitrogen, the sum of ammonium and nitrate nitrogen is from moderate to low and ranging between 46.7 to 8.6 mg/kg, despite high amounts of nitrogen fertilizers that are used in the agricultural lands.

The amount of available phosphorus oxide in studied soils is low (Table 2). The values are ranging from 0.2 to 21.00 mg/100g and the median and mode are respectively 1.8 and 0.2. Therefore, the amounts less than 5 mg/100g of soil, the plants will suffer a severe shortage of this element.

The potassium supply in studied soils is in better than mineral nitrogen and phosphorus. The main reason for that is the fact that soils in Bulgaria naturally have good reserves of potassium. Under the influence of surrounding mountains in the region and surface waters, potassium-containing sediments are weathering and realize good quantities of potassium oxide in lowland fields.

**Conclusion**

Intensive agriculture and pollution in the region in last decades drastically influenced soil properties and decreased soil fertility. There is a significant variation of physicochemical and agrochemical properties. The soils with high copper-contamination and acid pH are meliorated with calcium and magnesium-rich materials. Nowadays following soil's parameters as pH, CEC and base saturation show favourable values. However, the amount of SOM, available forms of nitrogen and phosphorus is low. That's why such soil monitoring is recommended as an operational model, supporting the planning of scientific researchers, adequate land use activities and good practice.

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# The role of organic matter to retention uranium in soils and sediments

M. HRISTOVA [(a)]*, R. LAZAROVA[(b)], D. STANEVA [(b)], I. YORDANOVA [(b)]

(a) Department of Genesis, geography and classification of soils, N Poushkarov ISSPPA, Sofia, Bulgaria
(b) Isotope Laboratory, N Poushkarov ISSPPA, Sofia, Bulgaria
* E-mail: marihristova@mail.bg

**Keywords:** TOC, radionuclides, uranium, soils, sediments

**Abstract.** The human population is continuously exposed to ionizing radiation from several natural sources, among which are radioactive nuclides. Terrestrial radioactivity is mostly due to the naturally occuring radionuclides in the $^{238}$U and $^{232}$Th series and $^{40}$K. The soils are important matrixes for assessing of radiological contamination of the environment. The aim of the paper was to survey the correlation between the content of total organic carbon and $U_{238}$ in a set of samples composed of soils and sediments with different origin. The experiment set of soils and sediments are samples from Interlaboratory tests lead by Wageningen Evaluating programs for Analytical Laboratories (WEPAL). The investigation gives conclusion the prime role of organic matter to retention U and the secondary role from clay minerals according to the correlation matrix.

## Introduction

The human population is continuously exposed to ionizing radiation from natural sources such as radioactive nuclides that are present in the Earth's crust since its formation. The primary sources of human exposure to external radiation are mainly the radionuclides in the $^{238}$U and $^{232}$Th series and $^{40}$K [1, 2]. The soils are important matrixes for assessing the radiological exposure of the public and biota and verifying radiological contamination of the environment [3].

Uranium is one of the radioelements whose mobility in soil may vary strongly depending on soil type and physicochemical properties [4]. While naturally occurring U is widespread in the Earth's crust at concentrations of ~1 to 3 ppm, higher concentrations can be found, including within organic matter-rich sediments [5, 6, 7].

The aim of the paper was to survey the correlation between the contents of organic carbon and uranium 238 in a set of soils and sediments samples with different origins.

## Materials and methods

**Soils and sediments sample set.** The experiment was carried out with a set of soils and sediments, which are samples of Interlaboratory tests lead by WEPAL. Table 1 shows that we have investigated 9 samples, most of them originate in Netherlands.

**Table 1** Samples identity (ID), type and origin

| ID | Type | Origin |
|---|---|---|
| 860 | Soil | Netherlands |
| 861 | Calcareous clay | Logrono /Spain |
| 876 | Clay | Bologna/Italy |
| 881 | Clay | Markness/ Netherlands |
| 909 | Marshland | Zurich/ Netherlands |
| 910 | Clay soil | Maren-Kessel/ Netherlands |
| 919 | Sandy soil | Halle/ Netherlands |
| 989 | River clay | Wageningen/ Netherlands |
| 992 | Sandy clay soil | Den Helder/Netherlands |

The following parameters extracted by the Final Report of WEPAL testes as the median absolute deviation (MAD) are given for all soils and sediments: Organic carbon via the modified Turin's method [8, 9]; pH is measured in water [10]; Content of clay, silt and sand; Cation-exchange capacity (CEC). Specific mass activity of $^{238}$ U in all samples was determined by gamma-spectrometry via standard procedures [11]. The $^{238}$U concentration was derived from the weighted mean of the photopeaks of $^{234}$Th (63.5 and 92.6 keV) [12].

The correlation matrix of the soil parameters was made using the Excel/Microsoft office by MAD of $pH_{H2O}$, TOC, $U_{238}$, clay, silt and sand and Cation-exchange capacity (CEC). MAD is a robust measure of the variability of a univariate sample of quantitative data.

## Results and Discussion

Figure 1 is presented data from the determination of pH.

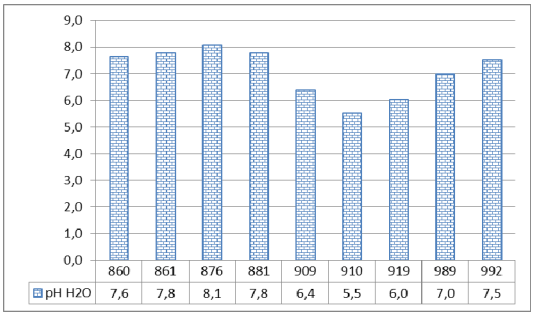



**Figure 1** Determination of pH in $H_2O$.

They show that most of the samples have neutral pH reaction. The most acid sample is ID 910, clay soil and the most alkalic sample is clay from Italy with ID 876. The experimental set ranges from moderately acidic to slightly alkaline [13].

Figure 2 is presented data from TOC and content of $^{238}$U in Bq.kg$^{-1}$.

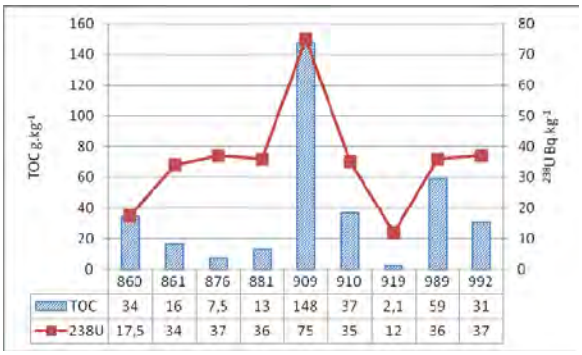



**Figure 2** Determination of TOC and $^{238}U$.

The values of TOC strongly vary in the sample set. The mean value with the standard deviation is 42.2 ± 39.3 $g.kg^{-1}$. The sample with the lowest value for TOC is ID 876 clay sediment and the highest value is ID 909 Marshland. The experimental set ranges from very low content to very high content of organic carbon. [14]. The content of $^{238}U$ in the set varies between 12 and 75 $Bq.kg^{-1}$. In the whole of Europe, the mean content of $^{238}U$ in the topsoil layer varies in the range of 16 to 110 $Bq.kg^{1}$ according to the data in UNSCEAR [2].

Table 2 shows correlation matrix between main parameters. This study confirms the correlation between the amount of U and the TOC as other investigations [1, 3, 5].

Table 2. Correlation matrix between parameters

| | **TOC** | $U_{238}$ | Clay | Silt | Sand | CEC |
|---|---|---|---|---|---|---|
| **TOC** | 1.00 | | | | | |
| **U238** | **0.84** | 1.00 | | | | |
| **Clay** | 0.51 | 0.58 | 1.00 | | | |
| **Silt** | 0.32 | 0.65 | 0.73 | 1.00 | | |
| **Sand** | -0.30 | -0.64 | -0.72 | -1.00 | 1.00 | |
| **CEC** | 0.91 | 0.87 | 0.72 | 0.44 | -0.42 | 1.00 |

Surveys of some authors said that behaviour of U in terrestrial ecosystems is closely related to environmental redox conditions. In an oxidant environment, uranium has +VI valence ($UO_2^{2}$+ uranyl ion), but in anaerobic conditions, it may be reduced to the +IV state in $U(OH)_4$ and $U(OH)_3$ [15,16]. In our research the sample 909 Marshland that contains the highest U and TOC confirms the precipitation of U in that reductive conditions. As a conclusion according to the correlation matrix we may note the primary role of organic matter to retention U and the secondary role of clay minerals.

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# Micromorphology of toplayer in Bulgarian Anthrosols

R. ILIEVA [(a)*], M. ZHIYANSKI [(b)], B. BANUSHEV [(c)]

(a) University of Forestry, 10 "St. Kl. Ohridski" Blvd., 1797 Sofia, Bulgaria
(b) Forest Research Institute – BAS, 132 "St. Kl. Ohridski"Blvd., Sofia, Bulgaria
(c) University of Mining and Geology "St. Ivan Rrilski", 1700 Sofia, Bulgaria
*** E-mail:** rossitz@abv.bg

**Keywords:** SOM, UGI, Micromorphology, Anthrosols

Considering the intensive urban and suburban sprawls the anthropogenic impact on soils increases. This creates urban soils, which are important for ensuring supply of different ecosystem services and need of detail investigations of their properties and functions.

In the present paper the microscopic observation of thin sections derived from the toplayer of Anthrosols was performed for a first time differentiating the types of urban green infrastructure (UGI) in Sofia city.

Micromorphological indicators that give important information about the state of the soil organic matter (SOM) were traced. Micromorphological analyses showed that the soil organic matter is represented by plant residues in a different phase of transformation, charcoal plant tissues, excrements of living activity of the soil biota and a little fragmented humus plasma. Among the commonest coarse constituents there are: quartz grains, which often present wavy extinction; polycrystalline quartz grains, which were included in the category "quartzite"; feldspar grains, which are varied and include microcline and plagioclase.

The results obtained could be used as a fundamental basis for further analyses directed toward better classification of urbanized soils and in indicating the conditions of urban green infrastructure.

# Comparison of size and molecular mass distributions of humic acids originating from different source matrices

M. KALINA [(a)*], M. DROSSOS [(b)], D. SIRUCEK [(a)], S. SOVOVA [(a)], A. PICCOLO [(b)], M. PEKAR [(a)]

(a) Materials Research Centre, Faculty of Chemistry, Brno University of Technology, 612 00 Brno, Czech Republic
(b) The Interdepartmental Research Centre on Nuclear Magnetic Resonance for the Environment, Agroo-food and New Materials (CERMANU), University of Naples Federico II, 800 55 Portici, Italy
* E-mail: kalina-m@fch.vut.cz

**Keywords:** humic acids, molecular mass, polydispersity, size exclusion chromatography, static light scattering

**Abstract.** This work was focused on comparison basic characterists of humic acids isolated from different source matrices and a commercial sample of lignohumate by means of the determination of their elemental composition, particle size, zeta potential and molecular mass distribution. The obtained resuls showed comparable characteristics for individual humic acids obtained from different sources and significant difference in the case of commertial lignohumate. The data are in good agreement with the supramolecular model of humic structure.

## Introduction

A determination of absolute molecular mass of humic substances and their separate fractions as well as the detailed description of humic acids (HAs) conformation in aqueous solutions represent the central problems of humic related research, despite the fact, that these basic characteristicts of humic substances determine other important properties such as solubility, mobility and reactivity in natural systems [1]. These scientific difficulties and disunity is connected with extremely high polydispersity and heterogeneity of humic matter. Historically, the definition of HAs structural models was connected with technological advances in available analytical instrumentations. One of the first generally accepted models of HAs structure, was a macromolecular model, which was assuming molecular mass of HAs in the range of several hunder thousands of Daltons [2]. Mainly due to the introduction of size exclusion chromatography (SEC) and nuclear magnetic resonance (NMR) into humic-related research, the supramolecular model of humic structure was adopted [1].

## Materials and methods

Humic acids used in the experimental part of the work were isolated by the standard procedure recommended by International Humis Substances Socienty (IHSS) [3] from a luvic chernozem soil (Ap horizon, Praha-Ruzyne, Czech Republic) (SHA), South-Moravian lignite (Mikulcice, Czech Republic) (LHA), commercial compost (Central composting plan, Brno) (CHA) and commercial peat sample (Agro-profi, Czech Republic) (PHA). Moreover, a sample of IHSS leonardite HA standard (1S104H) (IHA) and a sample of commercial Lignohumate A (Amagro company, Czech Republic) (LH) were also utilized.

All the studied solid samples of HAs were characterized by basic physico-chemical methods. Ash and organic matter contents in all studied samples were determined by standard thermogravimetric procedures. Elemental compositions were determined using a CHNS/O analyzer Euro Vector EA 3000.

Prior to subsequent colloidal and molecular mass characterizations, the solutions of individual HAs/LH in phospate buffer (pH ≈ 7, concentration of HAs/LH = 0.6 g/dm$^3$) were prepared.

Dynamic and electrophoretic light scattering were used to characterize aqueous solutions of individual HAs from the view of their particle size distributions and zeta potential. Both these measurements were performed usign Zetasizer Nano ZS instrument (Malvern Instruments).

The main part of this contribution was dealing with the comparison of two size-exclustion chromatographic techniques, which can be used for determination of the molecular mass of HAs. Firstly, the classical HPSEC/UV-VIS/dRI instrumentation (combined instrument from Perkin Elmer, Norwalk, USA and Fison Instrumentsm USA) based on Biosep S2000 SEC column (600 x 75 mm, Phenomenex, Torrance, USA) [1] was used. In this method, the molecular mass was determined relatively to the column calibration obtained by set of polystyrene sulfonate standards (1.53, 5.18, 16.0, 34.7, 57.5 and 505.1 kDa). The second used technique was based on the combination of SEC chromatography (Biosep S2000 column, 600 x 75 mm, Phenomenex, Torrance USA) with multiangle light scattering (MALS) and differential refractometry (dRI). SEC/MALS/dRI sytem (combined instrumentation from Agilent Technologies, Santa Clara, USA and Wyatt Technology, Santa Barbara, USA) used in the work represented the absolute technique for determination of molecular mass, with no needs for column calibration with standards.

## Results and Discussion

### Elemental and Thermografimetric analysis

Thermogravimetric analysis on individual isolated HAs proved high purity of obtained HAs (content of ash up to 2.0 wt.%). The low concent of ash was also detected in the sample of IHSS leonardite standard 2.4 wt.%). On the other hand, a commercial lignohumate (LH) contained 33.4 wt. % of ash, indicating the persistence of inorganic residues from its commercial production. The

summarization of the data from the elemental analysis of all studied HAs and LH are listed in Table 1. These data are presented in atomic percents and represent compositions recalculated on dry, moisture-free samples.

**Table 1.** Results from elemental analysis (atomic %) and caltulated H/C and O/C atomic ratios

| sample | C | H | N | S | O | H/C | O/C |
|---|---|---|---|---|---|---|---|
| **SHA** | 40.1 | 40.0 | 2.7 | 0.2 | 17.0 | 1.00 | 0.42 |
| **LHA** | 43.9 | 40.2 | 0.7 | 0.2 | 15.0 | 0.92 | 0.34 |
| **CHA** | 37.7 | 43.2 | 3.6 | 0.0 | 15.5 | 1.15 | 0.41 |
| **PHA** | 36.7 | 46.8 | 1.0 | 0.0 | 15.5 | 1.28 | 0.42 |
| **IHA** | 48.1 | 35.2 | 0.8 | 0.2 | 15.7 | 0.73 | 0.33 |
| **LH** | 38.9 | 43.1 | 3.6 | 0.8 | 13.6 | 1.11 | 0.35 |

The obtained elemental compositions represents comparable values to the data, wich can be found for HAs originating from different sources in literature [1]. The ratio H/C indicates highest aromaticity for PHA, CHA and LH sample. On the other side O/C value indicate the highest content of oxygen-containig groups for SHA, CHA and PHA.

### Dynamic and electrophoretic light scattering

Subsequently the prepared aqueous dispersions of HAs/LH in phosfate buffer were characterized using dynamic and electrophoretic light scattering. The summary of measured *Z*-averaged values of hydrodynamic particle sizes as well as the values of determined zeta potential and conductivities of individual samples is shown in Table 2. The data from ELS and conductivity measurements are indicating no significant difference in collodal behavior between the studied samples of HAs. The only difference was found in measured distributions of particle sizes of individual samples. In particular LH exhibited particle size distributions shifted to the lower particle sizes.

**Table 2.** Summary of determined *Z*-average values of individual HAs hydrodynamic particle sizes and their zeta potential ($\zeta$) and measured conductivities ($\kappa$)

| sample | *Z*-*d*H (nm) | $\zeta$ (mV) | $\kappa$ (mS/cm) |
|---|---|---|---|
| **SHA** | 154 ±15 | –28.4 ± 1.1 | 6.6 ± 0.5 |
| **LHA** | 262 ± 24 | –31.3 ± 1.7 | 6.9 ± 0.2 |
| **CHA** | 189 ± 26 | –29.1 ± 2.2 | 7.0 ± 0.3 |
| **PHA** | 203 ± 31 | –24.0 ± 1.5 | 6.2 ± 0.1 |
| **IHA** | 222 ±21 | –27.2 ± 1.1 | 5.8 ± 0.6 |
| **LH** | 50 ± 16 | –28.3 ± 1.3 | 7.6 ± 0.2 |

### Size exclustion chromatography (SEC)

Molecular mass of individual studied samples of HAs and LH was determined by means of HPSEC/UV-VIS/dRI using an Origin 8.0 software relatively taking into account the column calibaration made by the series of polystyrenesulfonate standards [2]. The summary of calculated molecular masses can be found in Figure 1. Basicaly all the obtained chromatograms consisted of two separate areas, first one, more sharp, can be attributed to the presence of supramolecular agregates in analysed samples. The second one, broader, can be assigned to the presence un-associated humic molecules, which are beeing released from humic supramolecular structure during SEC separation.

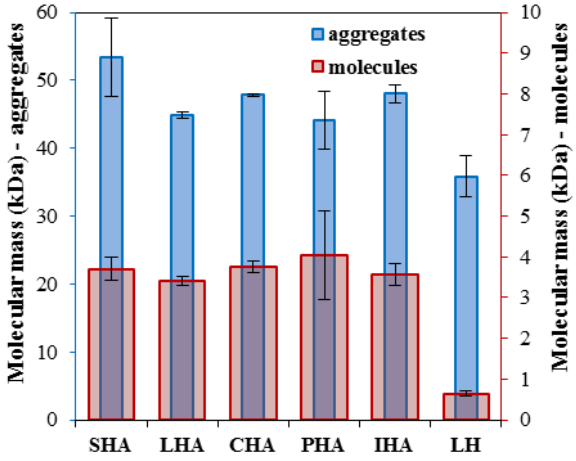



**Figure 1.** Molecular mass of individual studied HAs and LH determined by HPSEC/UV-VIS/dRI.

Similarly to DLS analysis, the chromatographic data confirmed the comparable behavior of individual studied HAs almost regardless the source matrix. On the other hand, LH showed much lower determined molar masses of both supramolecular aggregates and non-associated molecules. All the obtained results are in good correlation to the data published for humic acids from diffferent source matrices in literature [2].

Subsequently the individual samples were also analysed using the method of SEC/MALLS/dRI, which in comparison to the generally used HPSEC/UV-VIS/dRI is an absolute method for molecular mass determination. The obtained results confirmed the dominant presence of HAs mass in the form of the supramolecular aggregates. It also confirmed the significantly smaller mass for LH sample in comparison with HAs.

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# Thermal properties of clay soils with different humus content from Sofia field

M. KERCHEVA [(a)], K. DONEVA[(a)*], E. DIMITROV [(a)], ST. STOINOV [(a)], T. SHISHKOV[(b)]

(a) Department of Soil Physics, Institute of Soil Science, Agrotechnology and Plant Protection "N. Poushkarov", 1331 Sofia, Bulgaria
(b) Genesis, Geography and Classification of Soils, Institute of Soil Science, Agrotechnology and Plant Protection "N. Poushkarov", 1331 Sofia, Bulgaria
* E-mail: caeruleus2001@yahoo.com

**Keywords:** clay soils, soil thermal properties, soil structure, humus characteristics

**Abstract.** Thermal characteristics of clay soils with different humus content from the Sofia field were estimated by applying the model of De Vries at soil water contents at different suctions. The influence of humus content on water retention characteristics were quantified by statistical relationships. The influence of water content on the apparent thermal diffusivity of the surface humic horizon was estimated by applying the Harmonic method on longterm soil moisture and temperature field data measured in one of the studied sites.

## Introduction

Pedoclimate expressed by soil temperature and water regimes influence humus accumulation and composition. These regimes are driven by climate conditions and realized under specific soil properties. Soil thermal properties are the most complex soil characteristics as they depend not only on static (or slow changing) parameters such as soil composition and structure, but on the higly variable soil water content and to less extent on soil temperature. There are few publications which estimated thermal characteristics of clay soils from the Sofia field obtained by different methods [1-4]. The cited publications do not investigate the dynamic of the apparent thermal diffusivity under variety of soil moisture conditions observed in the field.

The current study estimates the thermal characteristics of clay soils with different humus content from the Sofia field by applying the model of De Vries at soil water contents at different suctions. The influence of water content on the apparent thermal diffusivity of the surface humic horizon was estimated by applying the Harmonic method on longterm soil moisture and temperature field data measured in one of the studied sites.

## Materials and methods

Three soil profiles from the Sofia field with almost homogeneous with depth fine texture were selected in this study: (1) non-cultivated Vertisols from, Bozhurishte [5]; (2) non-cultivated Deluvial-meadow soil and (3) cultivated Vertisol (Bathycalcic) from Gorni Lozen [6]. The soil texture was classified as Heavy clay (1) and Clay (2 and 3) according to USDA (Soil Survey Division Staff, 1993).

**Table 1.** Content of clay (<0.002 mm) and humus in topsoil and subsoil soil layers

| Profile | clay, % | | humus, % | |
|---|---|---|---|---|
| | 0-20 cm | 20-100 cm | 0-20 cm | 20-100 cm |
| 1 | 60-64 | 62-65 | 4.4 - 3.4 | 3.1 - 1.4 |
| 2 | 45-48 | 43-47 | 5.2 - 1.6 | 1.3 - 1.2 |
| 3 | 47-50 | 53-57 | 2.6 - 1.9 | 1.7 - 1.0 |

Water contents at saturation and suctions pF 2.5 and 4.2 of these profiles are plotted on Fig. 1 based on the data published in [5] and [6]. Soil water saturation is estimated by soil total porosity.

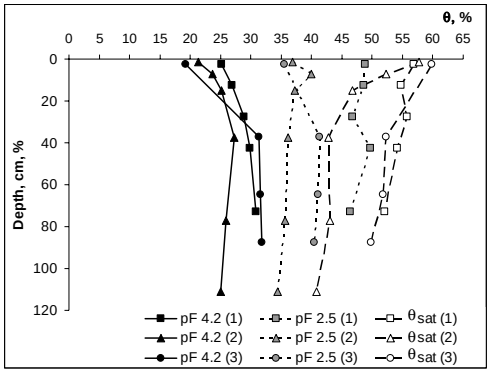



**Figure 1.** Water content (θ, %vol.) at saturation (θsat), at suctions pF 2.5 (field capacity) and pF 4.2 (wilting point) along the depth of profiles 1, 2 and 3.

Soil thermal conductivity (λ) was estimated by De Vries model (Van Wijk and De Vries, 1963; Ochsner, 2001) as described in [2, 7]. Thermal conductivity of solids (λs) is among the important model parameters. An estimate of λs=2.0 $W.m^{-1}.K^{-1}$ was found to fit with the data measured on samples from Profile 1 [1, 2] as well as with the data measured with KD2-Pro device of air-dried and water saturated samples of Profile 2 [7]. The heat capacity of soil was calculated as the weighted sum of the heat capacities of the soil constituents (Table 2).

**Table 2.** Thermal conductivity and volumetric capacity of main soil constituents used in calculations

| | water | air | mineral | humus |
|---|---|---|---|---|
| $\lambda$, $W.m^{-1}.K^{-1}$ | 0.596 | 0.025 | 2.0 | 0.25 |
| $C_v \times 10^{-6}$, $J.m^{-3}.K^{-1}$ | 4.18 | 0.0015 | 2.0 | 2.51 |

Thermal diffusivity ($a$) of soil is expressed as

$$a = \lambda / Cv \quad (1)$$

The apparent thermal diffusivity ($a$) was calculated by the Harmonic method (Horton et al., 1983; Gao et al.,

2009). The term "apparent" means that both processes - conduction and convection - are performed in heat transfer in moist soil and in the presence of a temperature gradient. The procedure as described in [7] was applied on each of the selected 128 daily records of diurnal soil temperature measurements at 2 and 10 cm at clear sky days during April-October when soil moisture content was also measured. The records were selected from the archive (1985-1990) of the bioclimatic station Gorni Lozen at the Institute "N. Poushkarov".

**Results and Discussion**

The humus content in the studied profiles varied from low (1-2%) in the subsoils of soil Profiles 2 and 3 to high in surface layers of non-cultivated Profiles 1 and 2 (4.4-5.2%) (Table 1). The Vertisol in Bozhurishte has the deepest humus horizons with more than 2% humus content till 90 cm soil depth.

Humus content as well as thermal properties are linked with soil structure. The positive effect of humus content on soil structure is well manifested by the relationships between volume of water retained at saturation ($\theta_{sat}$, %) (Eq. 2) and at field capacity ($\theta_{pF2.5}$, %) (Eq. 3) with clay and humus contents:

$$\theta_{sat} = 31.6 + 0.25\times clay + 2.64\times humus, \quad R^2=79.9\%, SEE=2.4\% \qquad (2)$$

$$\theta_{pF2.5}= 9.1 + 0.56\times clay + 0.94\times humus, \quad R^2=91.6\%, SEE=1.6\% \qquad (3)$$

The volume of water unavailable to plant (wilting point, pF 4.2) decreased with increasing of humus content (Eq. 3):

$$\theta_{pF4.2}= 16.3 + 0.29\times clay - 1.88\times humus, \quad R^2=86.4\%, SEE=1.4\% \qquad (4)$$

The simulated thermal conductivity (λ) and volumetric heat capacity (*Cv*) increased from dry to water saturated conditions correspondingly 7 and 3.5 times in average for the whole Profile 1 (Table 3). The changes of these properties in the other two profiles are of the same extent in humic horizon (7 and 3-4 times, correspondingly for λ and *Cv*), but they are less in subsoil (5 and 2.4 times for λ and *Cv*). In all cases the estimated thermal ptoperties of the studied clay soils have higher values for subsoils. Exceptions are the higher values of *Cv* at saturation for topsoils of Profiles 2 and 3. Thermal diffusisvity in all cases increases with θ till field capacity and then it decreases. A part of the relationship between *a* and θ simuleate by De Vries model is presnted on Fig. 2 for humic horizon of Profile 1. As it is shown by other studies (e.g. [7]) the simulated values can be fitted with laboratory measurements.

In case of the apparent diffusivity the scaterring is great although the cases were selected to be at days with clear sky conditions (Fig. 2). Medians of daily values of *a* vary from $2.7\times10^{-7}$ to $5.7\times10^{-7}$ $m^2.s^{-1}$. They are situated above the simulated values with De Vries model but follow its course. The annual temperature wave method of Marinova [3] produce the highest value $7.1\times10^{-7}$ $m^2.s^{-1}$.

**Table 3.** Thermal conductivity ($\lambda$ $W.m^{-1}.K^{-1}$), volumetric heat capacity (*Cv*, $J.m^{-3}.K^{-1}$) and thermal diffusivity (*a*, $m^2.s^{-1}$) of **top**soil and **sub**soil of profiles (Pr.) for dry, saturated and moist soil at pF4.2 and pF 2.5 suctions

| Pr. | | $\lambda$, | | $Cv\times10^{-6}$ | | $a\times10^{7}$ | |
|---|---|---|---|---|---|---|---|
| | | top | sub | top | sub | top | sub |
| 1 | θ=0 | 0.15 | 0.16 | 0.9 | 0.9 | 1.68 | 1.76 |
| | $\theta_{pF4.2}$ | 0.60 | 0.74 | 2.0 | 2.2 | 2.99 | 3.38 |
| | $\theta_{pF2.5}$ | 0.98 | 1.02 | 2.9 | 2.9 | 3.32 | 3.50 |
| | $\theta_{sat}$ | 1.05 | 1.09 | 3.2 | 3.2 | 3.23 | 3.41 |
| 2 | θ=0 | 0.18 | 0.23 | 1.0 | 1.2 | 1.80 | 2.03 |
| | $\theta_{pF4.2}$ | 0.63 | 0.96 | 2.0 | 2.3 | 3.20 | 4.25 |
| | $\theta_{pF2.5}$ | 0.97 | 1.16 | 2.6 | 2.7 | 3.79 | 4.37 |
| | $\theta_{sat}$ | 1.11 | 1.24 | 3.2 | 3.0 | 3.53 | 4.20 |
| 3 | θ=0 | 0.14 | 0.18 | 0.8 | 1.0 | 1.69 | 1.80 |
| | $\theta_{pF4.2}$ | 0.36 | 0.84 | 1.6 | 2.3 | 2.20 | 3.65 |
| | $\theta_{pF2.5}$ | 0.80 | 1.02 | 2.3 | 2.7 | 3.46 | 3.77 |
| | $\theta_{sat}$ | 1.02 | 1.12 | 3.3 | 3.1 | 3.06 | 3.59 |

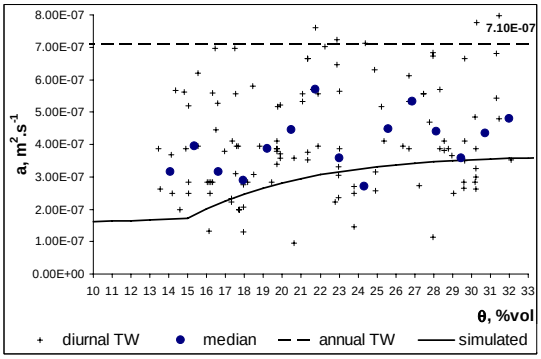



**Figure 2.** Apparent thermal diffusivity (*a*, $m^2.s^{-1}$) of humic horizon of Deluvial-meadow soil at different water content determined by diurnal and annual temperature wave (TW) methods, and model of De Vries (with $\lambda s=2$ $m^2.s^{-1}$).

The obtained information is a base for further improvement of the estimations using direct measurements and accounting the effect of swelling-shrinkage of these soils.

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# Peat humic substances: composition and influencing factors of their formation

M. KLAVINS [(a)], O. PURMALIS [(a)*]

(a) Department of Environmental Sciences, University of Latvia, Raina blvd.19, Riga, Latvia
* E-mail: oskars.purmalis@lu.lv

**Keywords:** peat humic substances, humification

**Abstract.** Humic substances (HS) isolated from three different ombrothrophic bog profiles in Latvia with different degree of humification, age, origin, botanical composition are studied. Multi-parameter characterization of macromolecular properties of peat HS were performed in order to analyze structural changes and properties of HS during decay and humification processes. Characterization of HS was used by various methods including fluorescence spectroscopy, FTIR, NMR and UV spectrometry as well as analysis of the elemental composition, functional group content and hydrophobicity. The results show the structural changes of macromolecular properties of HS during humification affecting their properties, behavior in the environment as well as complexing properties in contact with environmental pollutants. Formation of HS in bogs is greatly influenced by botanical composition of peat; also important is the presence of microbial biota and their metabolites with such transformation processes of organic matter as humification and coalification. Degradation of organic material, enzymatic reactions and synthesis of stable compounds are ongoing simultaneously. Results have proven that relatively aliphatic material during transformation become aromatic with increasing amount of carbon and nitrogen.

## Introduction

Peat is a light brown to black organic material formed under waterlogged conditions from partial decomposition of mosses and other bryophytes, sedges, grasses, shrubs, or trees [1]. Total area of bogs on Earth is approximately $4 \times 10^6$ km$^2$ which is around 3% of the terrestrial area, and around 87% of those are in boreal and subarctic regions. HS represent a significant part (which may exceed 50%) of the peat mass volume thus representing a significant portion of importance in biogeochemical cycle of the organic material [2]. The formation is linked to humification from plant tissues and biomolecules in the presence of microorganisms: abiotic, biotic and pyrolytic material degradation [3].

HS were isolated from three different ombrothrophic bog profiles in Latvia with different degree of humification, age, origin, botanical composition are studied. Multi-parameter characterization of macromolecular properties of peat HS were performed in order to analyze structural changes and properties of HS during decay and humification processes. HS from full peat profiles were choosed for studies, because in such stable and an anaerobic environment as bogs are, transformation processes of organic matter differ a lot from other sources. Studies of full peat profiles also allow to track changes in the structure of organic matter during humification process. Previous studies of peat HS confirm their aliphatic structure and polysaccharides as a major design element, but are not fully explaining the increase of aromaticity during humification, as well as the influence of vegetation. Studies of structure and properties of peat HS besides creating better understanding of transformation of organic material itself are also important for applied manufacturing and industrial extraction needs in order to obtain products with desirable characteristics.

During humification it is observed decrease of methoxyl groups and amount of polysaccharides in structure of humic substances [4], but quiet often increases the aromaticity, amount of the lipids and furans as degradation product of polysaccharides. Humification process also decreases number of carboxylgroups and aldehydes, as well as reduces the amount of oxygen in lignin and aromatic compounds [5] - it is affected by both the type of vegetation and its diversity as well as an environmental conditions.

## Materials and methods

**Sampling.** Full peat profiles (from the surface to the underlying sediment) were obtained by using peat corer (*Eijkelkamp*) and distinct layers were cut considering different decomposition degree and botanical composition of sediments. From corresponding peat samples HA were extracted by using procedures recommended by International Humic Substances Society (IHSS).

**Methods.** Characterization of HS was used by various methods including fluorescence spectroscopy, FTIR, NMR and UV spectrometry as well as analysis of the elemental composition, functional group content and hydrophobicity.

## Results and Discussion

An important role in humification plays cellulose and hemicellulose because they are forming most of peat-forming vegetation, as well as lignin and its transformation products which can be associated with vascular plants [2]. From these compounds during humification as transformation products are phenol and polyphenol compounds, providing a significant parts of the structure of HS, and they can transform (decarboxylation and demethylation) into quinones. Whereas transformation products of lignin are not only phenolic compounds, but also aromatic amines and other simpler compounds that can form complexes with carbohydrates and proteins as well as to take part in the esterification reaction [2].

Peat-forming carbohydrates are commonly considered to be labile and rapidly destroyable during

peat formation process, whereas our results show that the ring carbon atoms of carbohydrates do not change significantly in the structure of HAs in the main body of the bog during humification process. At the same time, a comparison of carbohydrate amount in peat-forming mosses with carbohydrate content in the HAs isolated from upper peat layers shows significant differences (Table1), which means that only a part of carbohydrate structures so abundant in mosses are incorporated into the structure of peat HAs. On the other hand, *Sphagnum* carbohydrates in the structure of peat HAs are refractory and are not much degraded during humification process. One of the major structural features common for humic substances is their acidity – presence of carboxylic groups. Our results show that C of carboxyls and esters (resonance at 160-190 ppm) indeed belongs to major structural groups, and changes the amount of these structures within the bog profile are not very significant. Again, if compared with the presence of carboxyl functional groups in the composition of peat forming mosses, major differences can be found, as their concentration is low in the structure of the samples of living peat-forming biota. It means that carboxyl groups appear at the first steps of living organic matter degradation in the upper peat layers (acrotelm).

**Table 1.** Integrated areas of the main signal regions of the CP MAS $^{13}C$ NMR spectra of peat-forming bryophytes and Eipurs Bog peat (%)

| Mosses and peat | 212-190 ppm | 190-160 ppm | 160-140 ppm | 140-110 ppm | 110-90 ppm | 90-50 ppm | 50-0 ppm |
|---|---|---|---|---|---|---|---|
| *Sphagnum magellani-cum* | 0.1 | 4.0 | 0.6 | 0.5 | 15.0 | 70.0 | 9.9 |
| *Sphagnum girgensoh-nii* | 0.8 | 4.7 | 0.9 | 5.0 | 14.0 | 65.2 | 9.3 |
| *Pleurozium schreberi* | 0.5 | 5.2 | 0.6 | 3.4 | 13.2 | 65.9 | 11.2 |
| Eipurs 0-25 cm | 0.5 | 3.8 | 1.1 | 1.8 | 14.0 | 65.2 | 13.5 |
| Eipurs 230-240 cm | 0.6 | 4.0 | 2.4 | 4.5 | 10.6 | 52.2 | 25.7 |
| Eipurs 456-462 cm | 2.3 | 8.4 | 4.9 | 7.6 | 6.3 | 34.1 | 36.4 |

**Conclusions**

Variability of functional group content and structural units of peat HA in bog profiles depends on the peat-forming vegetation, microbial degradation and humification processes. Although the botanical composition plays an important role in forming the structure of humic substances, however, the dominant driving force is humification process, which includes presence of microbial transformation of organic material. First stage of transformation processes in bogs is taking place in aerobic environment; the duration and intensity differs in studied bogs. Aerobic is transforming to anaerobic degradation in water-saturated part of the bog, where oxidation replaces reducing conditions, due to the different ongoing reactions and microbial activity.

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# Spatial distribution of soil organic carbon after patchy applications of farmyard manure and its changes over time

V. KOUTEV [(a)*], M. VENELINOV [(a)], M. NENOV [(b)], M. HIMMELBAUER [(c)]

(a) University of Forestry, Agronomy Faculty, 10 Kliment Ohridsky Blvd., Sofia 1756, Bulgaria
(b) Institute of Soil Science, Agrotechnologies and Plant Protection "Nikola Poushkarov", Sofia, Bulgaria
(c) Institute of Hydraulics and Rural Water Management, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Wien, Austria
* E-mail: koutev@yahoo.com

**Keywords:** Farmyard manure application, soil organic carbon, spatial distribution and temporal changes, kriging maps

**Abstract.** Field studies on spatial distributions of soil organic carbon (SOC) after patchy application on alluvial soils of matured cow farmyard manure were carried out. A sampling network was created using GPS equipment, samples were collected at three soil depths. The data were analyzed using geostatistics generating kriging maps of SOC distributions. The results showed that shortly after manure applications, the SOC of the fertilized spots reached 2.12 % in the top soil (30 cm) and sharply dropped underneath. Two years later, the SOC contents 1.47-1.69 % were quite similar along depth suggesting a significant leaching downwards, bellow 90 cm. Kriging method permitted high precision results between the sampling points and provided a base for improvement of manure application management. Significant leaching of SOC down soil profile was observed only on sandy alluvial soil.

## Introduction

Application of dairy manure to agricultural fields increases crop yields along with improvement of the soil characteristics such as water-holding capacity and organic matter (fertility) of the soil. Continuous use of manure or compost over years, however, can lead to accumulation of residual nitrogen (N), phosphorus (P) and other nutrients in the soil. These accumulations can be then transferred in runoff to lakes or leached to the groundwater and thus to have negative effects on the environment. For instance, the accumulation of P can cause eutrophication of drainage water; $N\text{-}NO_3$ can provoke degradation of drinking water; microbial pollution of soil and air can occur [1, 2]. Hence, the long-lasting enrichments of soils with nutrients can pose environmental threats long after application has ceased [3, 4].

Livestock farms often have fields where manure was not applied uniformly, resulting in soil areas with distinguished very high and very low levels of P and K contents. This can be a problem both for crop yields in low manured areas and excessively applications of nutrients in high fertilized areas. Grid soil sampling technique can be used to guide the conventional manure application in order to optimize its economic value against commercial fertilizers by producing more uniform yields. In addition, it provides maps of soil test values, which can support proper design and planning of manure applications.

The objective of this study was to evaluate spatial distributions (horizontal and vertical) of soil organic carbon after improper patchy applications of manure in vegetable farms on fluvisols and their changes over time using grid soil sampling approach and subsequent geostatistical analyses of the data.

## Materials and methods

Field studies were carried out in vegetable farm using drip irrigation on tomato field on sandy alluvial soil (fluvisol) in 2012, 2014 and 2017 and in mixt farm on a field with cabbage on loamy alluvial soil (fluvisol) in 2016 (Table 1).

**Table 1**. Soil physical and chemical characteristics

| Alluvial soils | Soil texture, mm | | C org % | pH |
|---|---|---|---|---|
| | <0.001 | <0.01 | % | KCl |
| Sandy soil | 4.9 | 9.7 | 0.51 | 5.4 |
| Loamy soil | 11.2 | 21.8 | 1.18 | 4.7 |

In late 2011, the farmers from first farm applied 20 tons matured cow farmyard manure to a part of the field of about one-half hectare. The manure was stored at the end of the field and the spreading was made by harrow. Most of it remained within 1/3-2/5 of the field (close to the manure piles). Therefore, this part of the field obtained manure rate of at least 120 tons per ha. During the growth periods, drip irrigation was applied over the entire field. The situation in second farm was similar.

A network of 15 sampling points was created in the first farm using Garmin GPS equipment. At each point, samples were collected at three soil depths of 0-30 cm, 30-60 cm and 60-90 cm for soil organic carbon (SOC) content determined by the Tyurin method [5]. Next, the data were analyzed using geostatistical methods generating kriging maps of soil organic carbon distributions using GS+ software.

## Results and Discussion

The results of the measured organic carbon contents in the top soil are presented on the kriging map in Figure 1. The non-uniform (patchy) manure application resulted in highly heterogeneous SOC distributions across the tomatoes field. The highest contents were observed from zero to about 40-45 meters field length. Behind this length, the SOC values changed insignificantly.

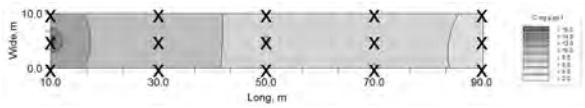
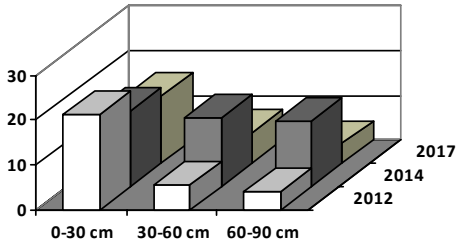



**Figure 1.** Kriging map of SOC spatial distributions ($g\,kg^{-1}$) in the top soil across the tomatoes field after the patchy manure application.

The soil organic carbon in the fertilized part of the field in the end of 2012, was quite high in the top 30 cm, i.e. 2.12%, but only 0.55% between 30-60 cm and 0.42 % at 60-90 cm depth (Figure 2a). At the poorly (nearly non-) fertilized part, the SOC contents were lower, but more homogeneously distributed down the profile *i.e.* 0.67 % at 0-30 cm, 0.55 % at 30-60 cm and 0.38 % at 60-90 cm soil depths (Figure 2b). Two years later in 2014, the SOC values in the fertilized field part were 1.69 %, 1.55 % and 1.47 % suggesting a significant redistribution down the soil profile. Hence, three years after the manure application the SOC increased even at deeper soil layers most probably due to the easy water movement trough the light textured sandy soils (> 80 % sand). In 2017, the rates of organic matter mineralization and leaching declined, especially in the subsoil. The results of the SOC contents were 1.52 % in 0-30 cm, but only 0.68 % and 0.54 % at the two sampling depths underneath (Figure 2a).

a)




b)

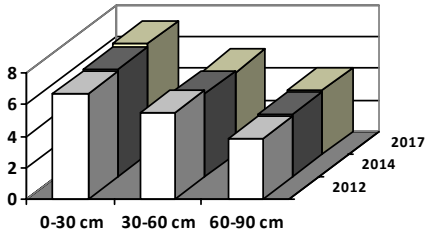



**Figure 2.** Distributions of SOC (g.kg-1) down the soil profile in the a) fertilized and b) non-fertilized parts of the tomato field.

On the non-fertilized part of the field, the SOC contents remained almost unchanged, *i.e.* 0.68%, 0.53% and 0.42% at the three sampled depths, respectively (Figure 2b).

In second farm, at the cabbage field on loamy alluvial soils we assessed only the result of the massive FYM application before 5 years. Comparing soil with and without manure application permitted us to obtain enough information about SOC leaching. The results showed that the manure organic carbon moved to less extend down the profile. In the most fertilized part, the SOC content was 1.67 % at 0-30 cm, 0.80 % at 30-60 cm and 0.68 % at 60-90 cm soil depths, while in the non-fertilized one the SOC values were 0.94 %, 0.68 % and 0.71 %, respectively.

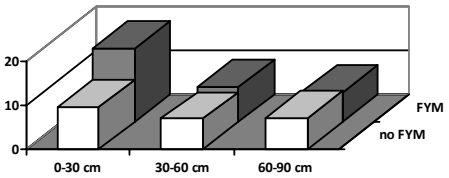



**Figure 3.** Distributions of SOC ($g.kg^{-1}$) down the soil profile in the fertilized (FYM) and non-fertilized (no FYM) parts of the cabbage field.

In our study, the grid soil sampling and the generated kriging maps of the SOC distributions over the vegetable fields provided a better view on the processes occurred shortly after the patchy manure application and in the following years. It is a base for further improvements of rates and patterns for farmyard manure applications. Significant leaching of SOC down soil profile was observed only on sandy alluvial soil.

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# The role of lignin phenols in organic-mineral interactions in soils

I. V. KOVALEV [(a)*], N. O. KOVALEVA [(a)]

(a) Lomonosov Moscow State University, Soil Science Faculty, Moscow, Russia
* E-mail: kovalevmsu@mail.ru; natalia_kovaleva@mail.ru

**Key words**: lignin phenols, particle-size fractions of soils, Fe-Mn-concretions

**Abstract.** The authors give a quantitative characterization of lignin phenols in soils and in particle-size fractions of grey forest soils (Russian Plane) and soils of the North Caucasus. Most of lignin phenols oxidation products (50 %) are associated with the coarse sand (>250 μm) fraction. However, in silty fractions (< 2 μm, 2-20 μm) there is a maximum of oxidation level and degree of transformation of the side chains of the lignin. The possible influence of soil mineralogical composition on the specific surface area of soil aggregates and on possible adsorption of lignin phenols is shown. Fe-Mn-concretions of all sizes contain the lignin of higher plants.

## Introduction

The complex aromatic structure and hydrophobic properties of lignin, as well as its high biochemical stability determine the unique role of this biopolymer in the processes of humification and organic-mineral interactions. However, this type of information is extremely limited. The purpose of the study is to identify patterns of intake, composition and transformation of lignin and its derivatives in different particle-size fractions and Fe-Mn concretions in soils of different zones.

## Materials and Metods

The chosen sites study included: light-gray forest soils (Grey Phaeozems Albic; Eutric podzoluvisols), light-gray forest gleyic and gley soils (Greyic Phaeozems Albic; Gleyic Greyzems) and gray soils of the southern taiga (Moscow district), as well as soils of vertical zones of Northern Caucasus

Lignin phenols were isolated using Amelung's version [1] of the Ertel–Hedges procedure [2]. The determination of lignin in the soils included the alkaline oxidation of the sample with copper oxide at170°C under pressure in a nitrogen atmosphere, the precipitation of HAs, and the preconcentration of phenolic products on compact disposable C18 columns under pressure. Lignin preparations were isolated by the evaporation of ethyl acetate on a rotary evaporator. The phenol components of lignin were derivatized into trimethylsilyl ethers and separated on a gas liquid chromatograph-mass spectrometer (Hewlett-Packard, Palo Alto, CA, USA) with a flame ionization detector and a capillary column. Individual reaction products (vanillin, syringic aldehyde (al), syringic acid (ac), p-coumaric acid, and ferulic acid) were identified by comparing the retention times and peaks with those of the known components and amounts used as external standards. The reproducibility of results was 95%. The alkaline oxidation of vascular pant tissues and their remains by copper oxide in the soil yields 11 phenols [2], which can be grouped in accordance with their chemical nature into three structural families: vanillin (guaiacyl) (V), syringyl (S), and cinnamyl (C). Thus, the sum of oxidation products (VSC) reflects the total content of lignin in the sample. Determination of carbon, nitrogen, sulfur is performed on element CNS analyzer (VARIO EL, Elementar GmbH, Hanau). Particle-size separation was made by ultrasonic method (Branson, 450W). The mineralogical composition of soil was investigated by x-ray diffraction (D5000 SIEMENS).

## Results and Discussion

Quantile distribution of lignin (VSC), the degree of oxidation (ac/al)v), the degree of lignin transformation in relation to the original plant tissue (T, %) in different particle-size fractions of soils of the Northern Caucasus and in gray soils of the Moscow region showed that with decreasing size fraction (< 2 μm, 2-20 μm), the amount of lignin in is decreased 10 times in comparison with the large fractions (20-250 μm, and >250 μm) (Fig. 1).

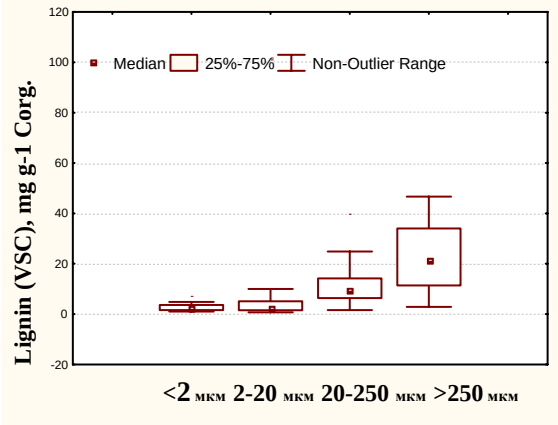



**Figure 1.** Statistical distribution of lignin (VSC), mg/g Corg.) in particle-size fractions of soils studied.

Most of lignin phenols oxidation products (50 %) are associated with the coarse sand (>250 μm) fraction. However, in silty fractions there is a maximum of oxidation level (ac/al)v and degree of transformation of the side chains of the biopolymer (T,%) therefore, low values of S/V-relations, which are due to selective loss of syringyl aromatic structures, and demethylation of methoxyl groups, (check this sentence). Thus, in small fractions (<2 μm, 2-20 μm), maximum destruction of lignin phenols is observed. In the finer fractions the highest content of biophilic elements: carbon, nitrogen,

sulfur, due to microbial resynthesis is also observed. In the large fractions poorly decomposed residues enriched in lignin, are accumulated, whereas the fine fractions of soils are dominated by products of microbial activity. The higher degree of lignin phenols oxidation level on the surface of the aggregate than inside of it, leads to a higher ratio of acid/aldehyde (Ac/Al). These facts are in agreement with that the destruction of lignin occurs in the aerobic conditions. In the reductive zones, lignin weakly decomposes and its relative accumulation occurs, as in Ap horizon of Gleysoils [3].

Adsorption of lignin phenols on the mineral surface is facilitated by a high specific surface area of the mineral phase and its positive charge. Biopolymer molecules are encrusted with clay minerals and become inaccessible to microorganisms. Probably, this is the reason for the high correlation of the content of lignin oxidation products with the value of the specific surface area. The correlation coefficient (P = 0.95) between the values of the degree of oxidation level and the specific surface area ($m^2/g$) in light gray soils of the Kolomna Opole is about 0.97. The high values of the specific surface area are due to the presence of clay minerals: groups of illites, groups of vermiculite and mixed-layer minerals (Illite-montmorillonite), groups of kaolinite. It can be assumed that oxidized monomers of lignin are fixed by clay minerals.

The "ideal" matrix for adsorption of lignin phenols and enzymes are amorphous hydroxides of Fe and Al. They are widely distributed in the soil in the free form or in the form of Fe-Mn nodules [7]. Possessing a large specific surface, the lignin compounds become the center of further concretions formation. Indeed, it was shown that nodules of all sizes (>100 samples) contain the lignin of higher plants [3, 4]. In concretions from light grey soils the content of lignin oxidation products is 10 times less than in fine-grained soils, however, the content of acids exceeds that of aldehydes. The degree of lignin transformation in relation to the original plant tissues (T, %) in ferro-manganese nodules increases by 4 times (Table. 1). As a result, the molecules of humic acids in Fe-Mn-nodules have twice the aroma in comparison with humic acids of soils. However, the number of carboxyl structures in humic acids of concretions is two times higher than in the humus of soils. Perhaps these fragments of the molecules increase the share of fulvic acids in the group composition of humus of concretions, according to the group and fractional composition of humus [6].

**Table 1.** The lignin content (VSC) in soils and Fe-Mn-nodules, n=5.

| Gleysoils Mollic | Lignin (VSC), mg g-1 Corg | Vanillic acids/vanillin (Ac/Al)v | Syringic acids/ syringaldehyde (Ac/Al)s | T, % |
|---|---|---|---|---|
| Ap, 0-15 cm | 11.9 | 0.2 | 0.4 | 5.8 |
| Fe–Mn Nodules (1-5mm) | 1.3 | 0.6 | 1.4 | 20.8 |

Therefore, the oxidized monomers (and possibly oligomers) of lignin, as well as lignin phenols, become a part of humic acids [4, 5, 6]. The processes of interaction of lignin phenols with fine soil fractions play a key role in the stabilization of biopolymer and the formation of stable aggregates..

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# Transformation of humic acids by two domain laccase of *Streptomyces anulatus* at alkaline pH

A. LISOV [a,b,c], L. TRUBITSINA [b], A. ZAVARZINA [a,d], Z. LISOVA [a,b], I. TRUBITSIN [a,b], A. LEONTIEVSKY [b, c]

(a) Faculty of Soil Science, Moscow State University, 119234 Moscow, Russia
(b) G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Russia
(c) Pushchino State Institute of Life Sciences, Pushchino, Russia
(d) A. A. Borissyak Paleontological Institute, Russian Academy of Sciences, Moscow, Russia
* E-mail: zavarzina@mail.ru

**Keywords**: humic acids, laccase, polymerization, oxidative coupling, alkaline soils

**Abstract**. We describe here the properties of two domain laccase (2D laccase) of *Streptomyces anulatus* (SaSL) and the role of the enzyme in humic acids (HA) transformation at alkaline pH values. We have demonstrated for the first time the ability of 2D bacterial laccase to transform HAs. Exclusively polymerization reactions took place during interaction of SaSL with initial HAs, as well as with their high-molecular and low-molecular fractions. Our results expands the knowledge on humification processes under alkaline conditions and show importance of enzyme-catalyzed oxidative coupling in HA molecular organization.

## Introduction

Humic substances (HS) are widespread in the environment, being the major component of stable organic matter in soils, sediments, and waters. Despite their biospheric significance, HS structure and formation processes has not been defined yet. Initially HS were considered as macromolecules with random coil conformations in solution while more recent studies describe HS as supramolecular associations of small molecules held together by weak dispersive forces [3]. Interactions with phenoloxidase laccase (EC 1.10.3.2) seem to be promising approach to reveal molecular organization of HS. The enzyme catalyzes oxidation of phenolic substrates by $O_2$ to phenoxy radicals and quinones, which may subsequently couple with one another. Degradation reactions can also occur if initial substrate is polymeric [5]. Laccases are widespread in soils being produced by fungi and bacteria [1]. Fungal laccase is typically a monomeric protein containing three cupredoxin domains and catalyzes substrates oxidation at acidic or less often at neutral pH values [5]. Purified fungal laccase can effectively depolymerize HS from soils [9] or can catalyze polymerization of monomeric phenols into humic-like polymers [7]. Along with 3D laccases bacteria contain two-domain laccase form. Unlike 3D laccase, 2D laccase is a homotrimer, the enzyme is thermostable, resistant to inhibitors, high salt concentrations and catalyzes oxidation of phenolic compounds at alkaline pH values [6]. The role of 2D laccases in HS transformation is unknown, although alkaline conditions in soils are not unusual. They can arise locally on the contact with carbonates or due to activity of soil microbiota excreting alkaline substances [4]. Sodic soils with alkaline pH are widespread in arid and semiarid regions. Alkaline conditions can arise from improper soil management practices. HA transformation at alkaline pH values in presence of 2D laccase has not been studied so far. Therefore, the aim of this work was to study the interaction of 2D laccase with HA and to elucidate its role in humification.

## Materials and Methods

### Microorganism, the protein expression, purification and characterization.

Strain *S. anulatus* VKM Ac-728 was obtained from All-Russian collection of microorganisms (http://www.vkm.ru/index.htm). All procedures for bacteria cultivation, 2D laccase's gene cloning and the protein expression were identical to those previously described [6]. The purification conditions and protein characterization were similar to those described earlier [6].

### Humic acids preparations

Humic acids from sod-podzolic soil (HAS), chernozem (HAC) and peat (HAP, Merck preparation) were used. Extraction, purification, fractionation and characterization procedures were as described earlier [8, 10].

### Reactions of 2D laccase with HA

The reaction was carried out in 50 mM Tris-HCl buffer pH 8.8 and 30°C during 48 h. Concentration of each HA was 1 mg/ml, quantity of 2D laccase – 5 U/ml. The laccase activity was determined by the rate of ABTS oxidation. The reaction mixture contained 1 mM ABTS in 20 mM Na-acetic buffer, pH 4.5, and the enzyme preparation. Molecular weight distributions of HA were studied by size-exclusion chromatography on Sephadex G-75 column 2 × 35 cm. The eluent used was 25 mM Tris-HCl buffer pH 8.2 with 50 mM NaCl, 0.1% SDS and 0.01% $NaN_3$, the flow rate was 0.3 ml/min. Void volume ($V_0$) was determined as an elution volume of blue dextran 2000, total void volume (Vt) was determined with 0.5 M NaCl (detection by a conductometer).

**Results and Discussion**

The molecular weight of SaSL according to SDS-electrophoresis, was 40 kDa, while molecular weight of the native protein was 114 kDa, which indicates that SaSL is a homotrimer. SaSL has a high thermal stability. The enzyme was stable at 70 and 80$^{o}$C and remained active for an hour at 90$^{o}$C. The temperature optimum was 85$^{o}$C. The enzyme catalyzed the oxidation of an electron donor ABTS at acid pH values with an optimum of 3.5 and catalyzed the oxidation of phenolic compound 2,6-dimethoxyphenol at alkaline pH values with an optimum of 9.0. The enzyme catalyzed the oxidation of electron donors at acid pH more efficiently than the oxidation of phenolic compounds at alkaline pH. The properties of SaSL were similar to those previously described for 2D laccases [6].

During interaction of 2D laccase with all HA studied, an increase in the high molecular weight (HMW) fraction, eluting in $V_0$, occurred. The amount of low-molecular (LMW) fraction correspondingly decreased, indicative of polymerization reactions. No polymerization reaction was detected in the controls without SaSL. To confirm the predominance of polymerization, the reactions of the enzyme with LMW and HMW fractions of HAS and HAP were studied. Again, exclusively polymerization reactions occurred irrespective of HA initial molecular weight, although only slight increase in Vo occurred in case of HMW fraction. Reactions of HA with 2D laccase are distinct from those reported for fungal laccases which can depolymerize HA in vitro [9]. The increase in HA's molecular weight during its oxidation at alkali pH was expected. Under alkaline conditions, monomeric phenolic compounds can polymerize even without a catalyst [2]. However, our results show that HA oxidation and subsequent polymerization does not occur without a catalyst even at alkaline pH.

**Conclusions**

We have demonstrated for the first time the ability of 2D bacterial laccase to transform HAs at alkaline pH. Exclusively polymerization reactions took place, which did not occur without a catalyst. This expands our knowledge on humification processes under alkaline conditions, which are unfavorable for 3D fungal laccases. Our results do not support exclusively supramolecular organization of HS. Initial HAs contain true polymeric material eluted in a void volume of the column. Substantial increase in its amount in presence of laccase occured only in unfractionated preparation in expense of LMW components (co-polymerization reactions), while pure HMW fraction exhibited only slight further polymerization even in presence of catalyst.

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# Preliminary investigation into the stimulation of plant growth promoting bacteria (PGPB) by soil amendment with a commercial humic acid

K. LITTLE [(a)], H. M. GAN [(b)], M. ROSE [(c)], W. R. JACKSON [(a)], T. CAVAGNARO [(d)], A. PATTI [(a)]

(a) School of Chemistry, Monash University, Clayton, Victoria, 3800, Australia
(b) School of Life and Environmental Sciences, Deakin University, Geelong, Victoria, 3216, Australia
(c) NSW Department of Primary Industries, Wollongbar Primary Industries Institute, Wollongbar, New South Wales, 2477, Australia.
(d) School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Glen Osmond, South Australia, 5064, Australia.
* E-mail: karen.little@monash.edu, tony.patti@monash.edu

**Keywords:** humic, DNA sequencing, plant growth promoting bacteria, rhizobium

**Abstract** The stimulation of, or soil inoculation with plant growth promoting bacteria (PGPB) is recognized as a potential means of decreasing fertilizer inputs, the benefits of which are to decrease greenhouse gas emissions, nitrate run off and soil acidification. Rhizobium form symbiotic relationships with leguminous plants and supply nitrogenous compounds by fixing atmospheric nitrogen. In an incubation study, application of a commercial humic acid product was shown to increase the abundance of bacteria of the *Proteobacteria* phylum, specifically the *Rhizobiales* order and therein the *Beijerinckiaceae*, *Bradyrhizobiaceae*, *Hyphomicrobium* and *Rhizobiaceae* families. The results of this preliminary study may give some insight into the growth promoting effects of commercial humic acids.

## Introduction

Commercial humate products are marketed with general claims of stimulation of the microbial community and the promotion of 'beneficial' soil microbes. There is little published information about the direct impacts of humic substances on soil microbial communities [1]. Of the few studies available, some show that humic substances increase the growth of some microbial groups [2], whilst other studies show minimal [3] or even negative [4] impacts.

Agricultural production relies on the provision of plant essential nutrients such as N, P and K however overuse can cause detrimental environmental impacts including emissions of nitrous oxide. A growing awareness of inoculation with PGPB represents an important strategy for sustainable management and reduction of negative environmental impacts. Within the broad range of PGPB, the *Proteobacteria* phylum is the most represented, with a number of bacteria classified to this phylum capable of forming symbiotic relationships with leguminous plants. The bacteria fix atmospheric $N_2$ and convert it to ammonium ions, thereby supplying a source of N other than that from inorganic fertilizer. Some of these bacteria are also capable of producing phytohormones and solubilizing inorganic phosphate, thereby promoting plant growth in a number of ways.

Advances in high-throughput 16S rDNA amplicon sequencing technologies provide a considerable amount of taxonomic information and have changed our understanding of microbial diversity in the environment. Here we present the results of an incubation study in which we sought to determine if the addition of a commercial lignite-derived humic acid product would have an effect on the composition of the soil microbial community, in particular PGPB.

## Materials and methods

The soil used for this incubation study was pH 5.8, low in nutrients and organic matter (1.9%) and of a sandy texture. A basal fertilizer application of 100 kg/ha N, 40 kg/ha P and 60 kg/ha K was applied. The lignite-derived amendment was a commercially available, soluble, potassium humate in granular form (SHG). To 250 mL microcosms, SHG was thoroughly mixed into the soil at equivalent rates of 0, 20 and 300 kg/ha. Five replicates were prepared of each treatment. During the six week incubation period at 25°C, the moisture content was maintained at 60% field capacity.

Soil respiration was measured at 0.8, 1, 4, 7, 14, 21, 28 and 42 days. At six weeks incubation, soil genomic DNA was extracted using the PowerSoil DNA isolation kit. PCR was performed, targeting the V3-V4 region of 16S rDNA, followed by sequencing using the Illumina MiSeq. The sequence data was overlapped and trimmed using PandaSeq. The output was clustered at 97% identity cutoff and chimera-filtered using the Greengene OTU database. Taxonomy assignment and abundance estimations were performed using QIIME. Sequences have been submitted to the European nucleotide archive (ENA) with accession numbers ERS663195 to 663209.

## Results and Discussion

The addition of SHG increased soil CO2 emissions, indicating stimulation and increased respiration of the soil microbial community. Sequences belonging to 25 bacterial phyla were identified. The dominant phyla were *Actinobacteria* (28–35%), *Proteobacteria (*21-31%), *Chloroflexi* (6-11%), *Planctomycetes* (6-8%) and *Acidobacteria* (8- 10%), phyla that are commonly dominant in soil [5]. Phyla representing less than 1% of the microbial community, including unclassified sequences were classified as Other (2-3%). Of particular

interest was the significant increase in the abundance of *Proteobacteria* ($p$=0.02) between the unamended soil and the 300 kg/ha application rate.

Analysis of the families of the *Proteobacteria* phylum by ANOSIM indicated a significant difference in the composition of the soil microbial community between the unamended control and the soil amended with 300 kg/ha of SHG. To determine which families contributed to this difference, SIMPER analysis (output table not shown) indicated that the *Xanthomonadace* family (15%) was the largest contributor to dissimilarity, with a significant increase ($p$=0.03) in abundance with increasing application rate of SHG. Within the family *Xanthomonadace*, *Lysobacter* was of particular interest. Species classified to this genus have demonstrated antagonistic activity against a number of plant pathogens across a range of crop types [6].

Also of particular interest within the *Proteobacteria* phylum was a significant increase in the *Rhizobiales* order and therein the *Bradyrhizobiaceae, Beijerinckiaceae Hyphomicrobium* and *Rhizobiaceae* families, with increasing SHG application. The shift in microbial community composition with SHG amendment was further demonstrated by abundance data clustering according to application rate.

Soil pH is a well-recognized moderator of microbial activity. An increase in soil pH was detected with increasing application rate of SHG (data not shown) however analysis by Spearman's rank order correlation indicated that pH was not significantly correlated with the increase in abundance of these four families (*Bradyrhizobiaceae* $p$=0.05, *Beijerinckiaceae* $p$=0.37, *Rhizobiaceae* $p$=0.94 *Hyphomicrobium* $p$=0.34). Further classification of the sequencing data identified the genera *Bradyrhizobium* of the *Bradyrhizobiaceae* family and *Rhizobium* of the *Rhizobiaceae* family, species of which are well documented nitrogen-fixing, symbiotic bacteria [7].

The application of SHG to this soil type increased the abundance of bacterial families of the *Rhizobiales* order, members of which can form symbiotic relationships with leguminous plants and promote growth through the provision of N and phytohormone production. Further investigation is required using a range of humic products, in a broader range of soil types and in the presence of plants. Further identification of the bacteria to the operational taxonomic unit level (OTU) and microbial functional assays would be required to extend on the knowledge gained here.

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# Properties of fulvic acids extracted by two different methods

D. ŁOMIŃSKA-PŁATEK [(a)*], A. M. ANIELAK [(b)]

(a) Department of Environmental Engineering, Institute of Water Supply and Environmental Protection, Cracow University of Technology, 24 Warszawska, 31-155 Cracow, Poland
(b) Department of Environmental Engineering, Institute of Water Supply and Environmental Protection, Cracow University of Technology, 24 Warszawska, 31-155 Cracow, Poland
* E-mail: dominika.lominska@pk.edu.pl

**Keywords:** fulvic acids, waste water, extraction of fulvic acids, humic substances

**Abstract.** FA were extracted from the wastewater treatment plant by using two different methods. The first one with using ammonia water ($NH_4OH$) and the second with process of ion exchange in a hydrophobic ion exchanger by using aqueos sodium hydroxide (NaOH). The main aim was to balance and assess the amount of FA in the primary and secondary effluent isolated by using different methods. The studies have shown that concentration of FA in the primary effluent was bigger than in the secondary effluent in both methods.

## Introduction

Humic substances (HS) are a major form of the natural organic matter (NOM). HS have different compositions and their properties depend on the source of occurrence and method of extraction [1]. Approximately 50% of the earth's carbon is in the form of humic and fulvic acids. Due to the heterogeneity of structure, size and associate in solutions as their concentration increases, characteristic of HS can be a problematic [2].

Fulvic acids (FA) belong to dissolved humic substances. FA are soluble in water under all pH. They are light yellow to yellow-brown substances. There are many different methods of extraction of FA. According to the International Humic Substances Society (IHSS) the most suitable method is undefined. Kuwatsuka et al. [3] compared two methods of preparation of humic and fulvic acids, IHSS Method and NAGOYA Method. According to author despite the fact that there is a lot of number of preparation methods of humus it is dificult to compare the data which are obtained by reserachers. It can be the reason why the inconsistency of the preparation methods has been an obstacle to the development of studies on humus.

In the presented work the authors examined the content of FA in the primary and secondary effluent. The main aim of the research was to compare FA isolated by using two different methods.

## Materials and methods

The samples were collected directly from the primary and secondary effluent at the wastewater treatment plant several times and the results are averaged. The selected wastewater treatment plant treat mainly wastewater from individual users and industrial plants. The WWTP comprises mechanical and biological wastewater trains, sludge processing and biogas trains. The plant works as a three stage Bardenpho system and operates five identical biological reactors.

### Extraction of FA with $NH_4OH$

Samples were acidified with HCl to pH < 2 in order to prevent development of biological life in the samples. After 24 hours wastewater were filtred on a medium thickness filter with glass ball <0.5 cm diameter and then they were passed throug the ion exchange column with a 1 L/hr flow. Next 0.5 mol / L $NH_4OH$ was directed into the ionite to desorb adsorbed fulvic acids.

Received effluent was concentrated in a vacuum evaporator at 85°C and under vacuum of 350 mbar. After evaporation on water bath at 80°C, the sample was dried in order to obtain dry residue of FA.

### Extraction of FA with aqueous NaOH

Samples were acidified with HCl to pH < 2 in order to prevent development of biological life in the samples. After 24 hours wastewater were filtred on a medium thickness filter with glass ball <0.5 cm diameter and then they were passed through the ion exchange column with a 1L /hr flow. NaOH solution was directed into the ionite to desorb adsorbed fulvic acids. Next the permeate was directed to the cation exchanger. Received effluent was concentrated in a vacuum evaporator at 85°C and under vacuum of 350 mbar. After evaporation on water bath at 80°C, the sample was dried in order to obtain dry residue of FA.

## Results and Discussion

The concentration of the FA solution was determined in a vacuum evaporator and was specified by the determination of dry residue.

**Table 1.** Comparison of the properties of FA extracted from the different samples

| Samples | Fulvic acids [$g/m^3$] | Ash [%] | ω [-] | Literature |
|---|---|---|---|---|
| Primary effluent $NH_4OH$ | 14.8 | 11.55 | 3.8 | [4] |
| Primary effluent NaOH | 17 | 77.73 | 1.7 | - |
| Secondary effluent $NH_4OH$ | 5.2 | 9.74 | 2.3 | [5] |
| Secondary effluent NaOH | 4.45 | 37.44 | 2.0 | - |

The biggest concentration of FA for both methods had primary effluent 14.8 [g/m$^3$] ($NH_4OH$) and 17 [g/m$^3$] (NaOH). Concentration of FA in the secondary effluent was 5.2 [g/m$^3$] ($NH_4OH$) and 4.45 [g/m$^3$] (NaOH). Based on Żdanow formula [6,7] calculated the degree of oxidation (ω) of the extracted FA. The degree of inside oxidation was higher in all samples for $NH_4OH$ method. For primary effluent it was 3.8 ($NH_4OH$) and 1.7 (NaOH). In FA extracted from the secondary effluent the ω was 2.3 ($NH_4OH$) and 2.0 (NaOH). The high degree of oxidation means the high level of humification [6,7].

The high content of ash in the FA from the primary effluent extracted by NaOH method can testify to the high content of inorganic pollutants. The mass reduction for temperature from 30°C to 200°C and it is quite similar for both samples. The mass remained constant at a temperature of approx. 600°C for the NaOH method and 650°C for the $NH_4OH$ method.

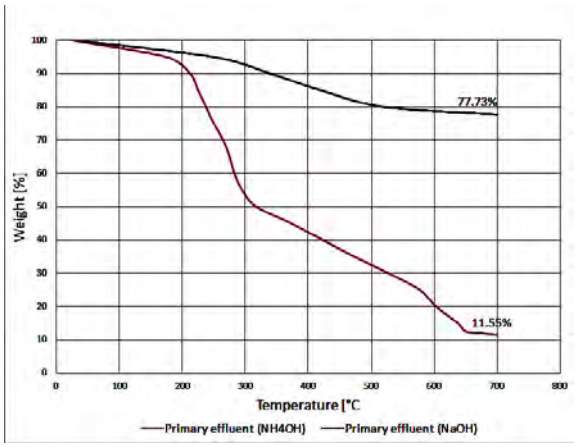



**Figure 1.** Mass reduction in wastewater samples after primary treatment as a function of temperature.

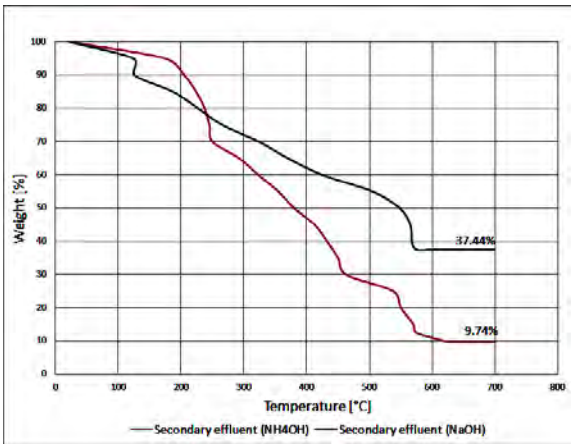



**Figure 2.** Mass reduction in wastewater samples after secondary treatment as a function of temperature.

The mass reduction for temperature from 30°C to 120°C is quite similar for both samples. The mass remained constant at a temperature of approx. 580°C for both samples.

**Table 2.** Elemental composition of FA extracted by different methods

| Elements | Primary effluent $NH_4OH$ | Primary effluent NaOH | Secondary effluent $NH_4OH$ | Secondary effluent NaOH |
|---|---|---|---|---|
| Elements | Content [%] | | | |
| C | 33.24 | 45.31 | 43.51 | 46.02 |
| H | 4.6 | 3.86 | 5.37 | 5.13 |
| N | 10.03 | 3.05 | 9.44 | 1.57 |
| S | 1.87 | 11 | 2.7 | 7.2 |
| O | 50.26 | 36.78 | 38.98 | 40.08 |
| Literature | [4] | [-] | [5] | - |

The results of the elemental composition were converted to ashfree mass. A study of the elemental composition provided information regarding the structure of isolated acids, the origin of their organic matter as well as the degree of aromatization and condensation. The content of C and H in all samples was similar and it is characteristic for FA. It is worth noticed that the content of N was different due to the methodology. Method with using $NH_4OH$ generates more N in comparison to NaOH method.

## Conclusions

The main aim was to balance and assess the amount of FA in the primary and secondary effluent with two different methods. The studies have shown that concentration of FA in the primary effluent was higher than in the secondary effluent in both methods. As a result of using the NaOH method in the secondary effluent, more inorganic impurities were removed. Based on the research, it can be stated that selected WWTP discharges less FA compared to the input pollution load no matter what different method was used.

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# Soil organic matter management in a tropical low carbon Agriculture: Data review and current challenges

L. MARTIN-NETO [(a)], D. M. B. P. MILORI [(a)], A. M. TADINI [(a)], A. BERNARDI [(b)], P. VILLAS-BOAS [(a)]

(a) Embrapa Instrumentação, 1356-970 São Carlos, SP, Brazil
(b)Embrapa Pecuária Sudeste, 13560-970, São Carlos, SP, Brazil
* E-mail: ladislau.martin@embrapa.br

**Keywords:** soil carbon sequestration, spectroscopic analysis, no-till, crop-livestock-forest integrated systems

**Abstract.** Soil organic matter management in tropical environments is a challeging issue, considering erosion process and high temperature and humidity with fast carbon compounds mineralization. However using soil conservative practices, as no-tillage in croplands and more recently in crop-livestock-forest integrated systems, Brazil reached important achievements. From long-term field experiments data combined with spectroscopy tools analysis, utilizing soil humic substances and whole soil samples, several results demonstrated viability to sustain a tropical low carbon agriculture.

## Introduction

Land use change in Brazil is a highly relevant topic, considering several aspects as: deforestation, soil degradation, global climate change, related with greenhouse gas emissions and carbon sequestration, and other aspects Numbers are huge, considering country size (850 million of ha) and currently 30% of total land is under agricultural use, with 60 million of hectares under croplands and 200 million of ha are pastureland. More than 60% of total land area of Brazil (around 500 million of ha) is under native vegetation, mainly in the Amazonian rainforest. Besides this Brazilian government launched, in 2010, a voluntary Low Carbon Agriculture Plan stimulating some main practices to reinforce sustainable development, at the farms level, they are: no-tillage, biological nitrogen fixation, crop-livestock-forest integrated systems, pastureland recovery, planted forest and animal production waste management [1]. In this context a paramount topic is soil organic matter (SOM) dynamics in these tropical and sub-tropical environments, generally under high temperatures and humidity, with fast soil carbon turnover rate. Also normally in Brazilian tropical soils the SOM content is low and the main soil clay particle is kaolinite type, with low cation exchange capacity, and consequently all agricultural sustainable practice must include an adequate SOM management. In this abstract will be presented some field and laboratory results, majority them published internationally, demonstrating feasibility that adequate SOM management can help to render an economically viable agriculture and simultaneously generate soil carbon sequestration conditions, sustaining a low carbon emission agriculture in the tropics.

## Materials and methods

**Field Sustainable Practices.** Field long-term experiments with croplands under no-till and crop-livestock-forest integrated systems in tropical and sub-tropical areas, compared with reference areas, will be presented.

**SOM Dynamcs and Characterization**. Data on SOM content, obtained with CHN Analyser and by Laser-induced breakdow spectroscopy (LIBS), permitted evaluate soil carbon dynamics. SOM characterization was performed in whole soil samples, physical and chemical soil fractions, including soil humic substances, using spectroscopic analysis, as LIBS, laser-induced fluorescence (LIF), $^{13}C$ NMR, EPR, UV-Vis fluorescence and other.

## Results and Discussion

### SOM dynamics

Long-term experiments conducted in experimental fields and private farms were analysed. Bayer et al., 2006 [2] identified from data of several croplands sites under no-tillage a capacity of accumulus reaching to 500 kg C/ha.year in sub-tropical soils (south of Brazil) and 350 kg C/ha.year in tropical areas (savannah areas mainly), compared with conventionally cultivated sites (Figure 1). Other sites with crop-pastureland(livestock)-forest integrated systems have demonstrated important soil carbon accumulus, mainly in tropical areas of Brazil, with value above 1.0 ton C/ha.year [3].

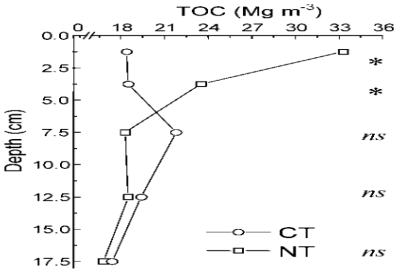



**Figure 1.** Data showing total organic carbon (TOC) content, in conventional tillage (CT) and no-tillage (NT) croplands sites, in tropical region of Brazil [2].

Many others experiments are being conducted in different regions of Brazil and will be basis to Monitoring/Reporting/Verification (MRV) procedure, coordianated by a Multiinstitucional Low Carbon Agriculture Laboratory, under responsibility of Embrapa, as part of Brazilian Government commitment with IPCC. The voluntary goal of avoided agricultural greenhouse gases (GHG) can reach to 162.9 millions of tons of $CO_2$eq until 2020 [1].

### SOM Characterization

Besides quantitative data the SOM characterization is very relevant to identify mechanisms of soil carbon accumulation helping in the adequate management practices. So several studies were and are in progress with analysis of whole soil samples, physical and chemical soil fractions (including soil humic substances) using different spectroscopic tools.

In the past our research group in Embrapa performed SOM characterization basically with soil humic substances extractions in analysis with $^{13}C$ NMR, EPR, UV-Vis light and fluorescence spectroscopies. Relevant information were obtained including an initially unexpected reduction in the humification degree of soil humic substances in croplands areas under no-till with SOM increase, as can be seen in Figure 2 [4]. This kind of result was explained considering that newly incorporated humic substances, especially in sites under no-till, are less transformed than indigenous soil humic materials reducing humification degree.

In more recent years a relevant effort was made to identify and validate new spectroscopic tools to perform SOM analysis with whole soil samples and our group reached to two excelent options based in laser spectroscopies, laser-induced fluorescence (LIF) and laser-induced breakdwon spectroscopy (LIBS) [5, 6, 7]. Measurements of SOM characteristics using LIF, with use of SOM humification index **(HLIF),** in whole soil samples demonstrated a reduction of humification degree of SOM in areas under no-till comparatively to conventional tillage areas, Figure 3 [4]. These results indicated that observation made using soil humic substances (**Figure 2**) was consistent with results obtained using an innovative procedure to work with whole soils samples to SOM characterization, with similar results generated (**Figure 3**).

### Final Consideration

The combination of long-term field experiments and use of more versatile spectroscopic tools, mainly based in laser spectroscopies, to SOM analysis will be relevant to MRV procedure and to sustain the Brazilian Low Carbon Agriculture Plan, generating a valuable reference policy also to other countries, mainly in tropical regions.

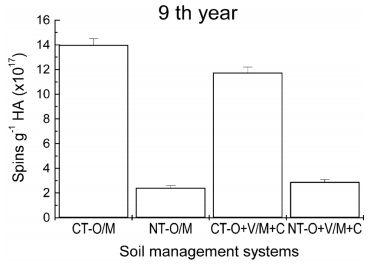



**Figure 2.** Quantification by EPR of semiquinone-type free radical in soil humic acids (HA), extracted from soils under conventional tillage (CT) and not-ill (NT) with different crops sequences (oat-O, maize-M, vigna-V, caupee-C), after 9 years of management [4].

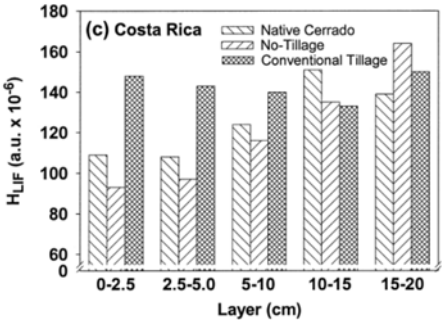



**Figure 3**. SOM humification index ($H_{LIF}$), obtained by laser induced fluorescence (LIF), with whole soil samples, from farm field, in Costa Rica city, Brazil, under different managements [5].

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# Fluorescence properties of humic substances from Podzols affected by alkali fly-ash blown out from the dumping site of the power plant

L. MIELNIK [(a)*], J. WEBER [(b)], M. PODLASIŃSKI [(c)], A. KOCOWICZ [(b)]

(a) Department of Physics and Agrophysics, West Pomeranian University of Technology in Szczecin, 71-459 Szczecin, Poland
(b) Institute of Soil Science and Environmental Protection, Wroclaw University of Environmental and Life Sciences, 50-375 Wrocław, Poland
(c) Department of Soil Science, Grassland and Environmental Chemistry, West Pomeranian University of Technology in Szczecin, 71-459 Szczecin, Poland
* E-mail: lilla.mielnik@zut.edu.pl; jerzyweber@gmail.com

**Keywords:** fluorescence spectroscopy, EEM spectra, fluorescence index, humic and fulvic acids

**Abstract.** The changes in humic substances structures extracted from soils affected by alkaline fly-ash blown out from the dumping site of Belchatow power plant were studied using the fluorescence spectroscopy. Humic and fulvic acids were extracted from ectohumus horizons of Podzols located 50 m and 4 km from the dumping site. The alkalization caused by the influence of fly-ash dumping site contribute to creation of humic substances with a more complex structure containing the higher molecular weight fractions, indicated by lower intensity of the fluorescence.

## Introduction

Combustion of lignite in power plants generates a huge amounts of alkaline residues stored in dumping sites, which contribute to the alkalization of surrounding soils. Previous research revealed differences in morphology of affected Podzols, in which Bhs illuvial horizons have been transformed into Bs horizons [1]. These processes have to be initiated in ectohumus horizons, following changes in humic substances properties.

Fluorescence spectroscopy is one of the methods used for characterization of properties of humic substances [2]. At present, three-dimensional excitation-emission matrix (3D-EEM) is a prevalent method used to characterise humic substances and to distinguish their different origins [3]. The aim of this paper was to characterize changes in fluorescence spectroscopic properties of humic substances undergoing in ectohumus horizons of Podzols affected by deposition of the alkali fly-ash.

## Materials and methods

The samples were collected from ectohumus horizons in direct vicinity of the dumping site (50 m), as well as in control area (4 km away). Samples were collected in five replications. The FA and HA were extracted with standard IHSS method [4].

Fluorescence emission and excitation/emission matrix (EEM) spectra were recorded with a Hitachi F 7000 Fluorescence Spectrophotometer. The recording were performed using a non-fluorescent quartz cuvette with path length of 1 cm. The spectral recording were performed at room temperature for the constant concentration of 10 mg $C_{(HA/FA)}$ $dm^{-3}$ in 0.05 mol $dm^{-3}$ $NaHCO_3$ solution. Each FA and HA sample was pre-filtered through a syringe filter with 0.45 µm pore size.

From fluorescence spectroscopic data the following indexes have been calculated: (a) the humification index (HIX), defined as the ratio between the area in the upper quarter (Σ435–480 nm) and the area in the lower quarter (Σ300–345 nm) of the fluorescence emission spectra at excitation wavelength 254 nm [5]; (b) biological index (BIX) calculated from emission spectra at $\lambda_{ex}$=310 nm, as divided intensity of 380 nm by 430 nm [6], (c) fluorescence index ($f_{450}/f_{500}$) from emission spectra excited by $\lambda_{ex}$=370 nm, as ratio of the intensity at 450 nm and 500 nm, [7]; and (d) humification index A465 proposed by Milori [8], which is equal to the area below fuorescence curve at $\lambda_{ex}$=465 nm.

## Results and Discussion

Fluorescence emission spectra of studied FA and HA showed a similar shapes for the excitation wavelengths used (Fig. 1). These similarities indicate that analyzed substances represent biopolimers of similar basic structures. However, the differences can be observed in the intensity of the fluorescence.

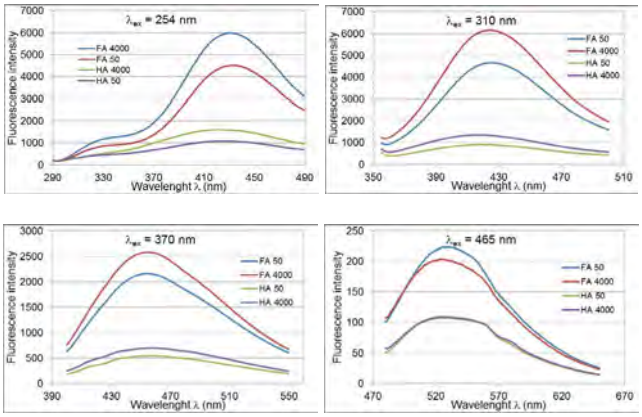



**Figure 1**. The emission fluorescence spectra obtained with different waivelength excitation $\lambda_{ex}$.

The highest intensity of the fluorescence showed FA, while HA indicated the lowest values. The higher fluorescence emission resulted from less complex

molecular structure of FA, in comparison with HA. These kind of structures usually are characterized by less intensive intramolecular fluorescence quenching. [2, 9].

Obtained results pointed out to the influence of the dumping site on the humic substances properties of the surrounding soils. FA and HA isolated from soils located 50 m from the dumping site indicated lower intensity of the fluorescence, compared to FA and HA collected from soils located at a distance of 4 km. This indicates that alkalization caused by influence of the dumping site contributes to creation of humic substances fractions with a higher molecular weight structures, containing a larger number of electron withdrawing substituents, that affect the reduction of the efficiency of fluorescence [9, 10].

The humification index (HIX) is used to assess the degree of maturity of soils of various origins. Humic substances collected close to dumping site and in control sites did not indicate clear differences in HIX (Table 1). FA showed higher HIX values than HA. This is probably related to the fact that the degree of coupling in unsaturated aliphatic chains of FA molecules increases, while the HA molecules are characterized by a more "rigid" structure, made up of condensed aromatic or heterocyclic rings [5, 6, 8, 10].

**Table 1.** Calculated fluorescence indexes: HIX, BIX, $f_{450}/f_{500}$, $A_{465}$ of FA and HA

| Sample | HIX | BIX | $f_{450}/f_{500}$ | $A_{465}$ |
|---|---|---|---|---|
| FA 50 | 12.5 | 0.4 | 1.4 | 68 |
| FA 4 000 | 11.7 | 0.4 | 1.5 | 62 |
| HA 50 | 5.8 | 0.7 | 1.3 | 39 |
| HA 4 000 | 6.9 | 0.7 | 1.3 | 37 |

BIX values indicate differences between the FA and HA fractions. Higher BIX values are obtained for the high molecular weight fraction of HA, lower – for the less compound structure characterized by FA fraction, whereas the impact of the dumping site on BIX values was not observed.

The fluorescence index ($f_{450}/f_{500}$), which strongly correlates with the complexity of the structure and the aromaticity, is used to differentiate organic matter sources. For soil organic matter, this coefficient is about 1.3 [4, 7]. Higher values of index $f_{450}/f_{500}$ are observed for FA, lower to HA. There are also slight differences for FA depending on the distance from the dumping site. Higher values of $f_{450}/f_{500}$ of FA located in control site suggest their relatively lower aromaticity.

The $A_{465}$ humification index, according to Milori [8], can provide information on the degree of internal transformation of HA. FA indicated distinctly higher $A_{465}$ values, when compared with HA.

The EEM contours exhibited four characteristic peaks at λex/em of 220–240/300–350 (peak A), 22-2700/400–450 (peak B); 300-340/400–450 (peak C); and 270-300/300-350 (peak D). Peak A is attributable to protein-like substances, peak B and peak C belonged to fulvic-like and humic-like substances respectively, while peak D to soluble microbial metabolites.

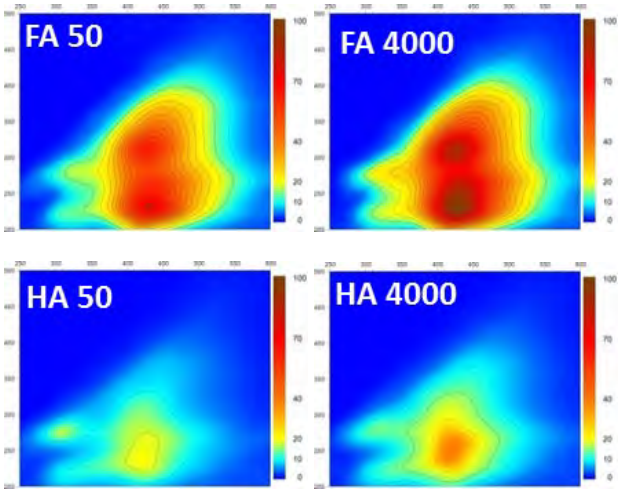



**Figure 2.** Contour maps (EEM spectra) of HA.

The positions of the observed peaks were essentially similar in samples from soils affected by the fly-ash and the control (Figure 2). However, there are clear differences in the intensity of the separated peaks.

## Conclusions

The fluorescence method is complementary tools and particularly suitable for fast determining the similarities or differences in the structure of humic substances and their degree of transformation. Obtained results indicated that alkalization caused by the influence of fly-ash dumping site contribute to creation of humic substances with a more complex structure containing the higher molecular weight fractions.

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# Mudstone and dump organic matter estimate by Rock-Eval pyrolysis

Z. MILAKOVSKA [(a)*], M. STEFANOVA [(b)], G. VLADISLAVOV [(c)], K. MARKOVA [(d)]

(a) Geological Institute, Bulgarian Academy of Sciences (BAS), 1113 Sofia, Bulgaria
(b) Institute of Organic Chemistry with Centre of Phytochemistry, BAS, 1113 Sofia, Bulgaria
(c) Mini Maritsa Iztok EAD, Mine Troyanovo-3 OOD, 6294 Mednikarovo, Bulgaria
(d) Department of Geology, Paleontology and Fossil Fuels, Sofia University "St. Kl. Ohridski", 1504 Sofia, Bulgaria
* E-mail: zlatkam@geology.bas.bg

**Keywords:** RE parametres and indices, organic matter weathering, Maritsa Iztok Basin

**Abstract.** A study of sets of fresh mudstone samples and dump materials by Rock-Eval technique shows a marked depletion in TOC and HI in the dump samples consistent with the mild continental weathering in Maritsa Iztok Basin area. Both $S_1$ and $S_2$ decrease proportionately by 26-29 % for dump samples compared to mudstone ones. The inert carbon content is high for both sample sets (*ca*. 42 % of the average TOC). $S_2$ *vs.* TOC graph indicates Type III kerogen for the sample sets. Expectedly, dump $T_{max}$ and OI show elevated values.

## Introduction

The understanding the mobility and fate of both organic and inorganic components in soils, sediments, water, and air is a challenge that requires complex study of the processes. For a successful humus remediation of dump areas after open-pit mining, it is of utmost importance to know organic matter (OM) basic characteristics as well as transformation processes that have proceeded. Rock-Eval (RE) technique offers indispensable options for such an estimate. In the case presented, the focus is directed to the environmental behaviour of organic components in Maritsa-Iztok Basin (MIB) dump area, e.g. organic constituents' interactions with the elements of the natural and man-made environment. As the source and maturation level of OM is known to influence the oxidation processes and intensities, it is first necessary to discuss carefully the geochemical characteristics of the starting material. No detailed organic geochemical studies of Late Miocene-Pliocene fresh lacustrine carbonaceous mudstones of MIB have been performed yet. The effects of mild continental weathering on their OM basic characteristics were also not studied up till now. In this way, a study was undertaken to better knowledge the cause of OM transformation and degradation and in particular to identify, if possible, the reactive species. It was also considered necessary to follow the mudstone OM evolution in dump environment.

## Materials and methods

The study is based on bulk rock analysis of 13 fresh core samples of carbonaceous mudstones chosen from boreholes from the three open-pit mines operating in Mini Maritsa Iztok EAD. RE data for seven anthropogenically weathered dump samples [1] were calculated and used for a comparison.

Bulk rock XRD study of the mudstones indicates presence of mixed-layered (illite/smectite) clay mineral, illite, kaolinite mineral, quartz, and feldspars as main constituents. Additionally, As-pyrite, pyrite, gypsum, and rarely muscovite, jarosite, lepidocrocite, goethite, and calcite were found in some of the samples. Qualitatively, dump samples mineral content is very similar to the fresh mudstones one [1].

The bulk rock samples were air-dried and ground to < 0.1 mm. Analyses were carried out on "Turbo" model RE6 pyrolyzer (Vinci Technologies, France).

## Results and Discussion

RE data for mudstone and dump samples have been averaged and compared having in mind, that a direct comparison of RE data for pair of fresh and naturally weathered samples from one sampling site is impossible.

TOC content varies significantly for both sample sets (Fig. 1). Mudstone TOC values range from 1.19 wt. % to 24.06 wt. % with an average of 7.64 wt. % (Table 1). These high, uncommon in lacustrine rocks TOC values are approaching the TOC measurements normally seen in coaly source rocks [2]. Dump TOC range is 1.18 - 10.73 wt. % and averages at 3.59 wt. % which is much higher compared to the $C_{org}$ submitted for Staroselets dump samples [3]. The marked TOC depletion for dump samples is consistent with the physical and chemical weathering in the mild continental climate [1] in dumps of MIB as oxidation of OM during weathering often results in TOC loss.

Low $S_1$ values (0.01-2.5 mg/g rock) for mudstone set indicate that few to no hydrocarbons were present at the start of the RE analysis. As concerns dump materials, they show decreased $S_1$ values (0.02-0.27 mg/g rock). The $S_2$ yield for mudstones ranges from 0.25 mg/g rock to 116.47 mg/g rock, and for the dump samples - the range is 0.51-28.34 mg/g rock, i.e. as it is characteristic for coals one order of magnitude greater than $S_1$ [2]. High $S_2$ results indicate that significant amounts of organic carbon were converted to hydrocarbons (HC) by pyrolysis. Both dump $S_1$ ("free" hydrocarbons) and $S_2$ ("bound" hydrocarbons) decrease proportionately by 26-29 % compared to mudstone ones. The depletion detected is lower than 60% decrease found for the leached zone of weathered outcrop samples of Mississippian Bowland Shale Fm. [4] and could be interpreted as there is no hydrocarbons preference for oxidation.

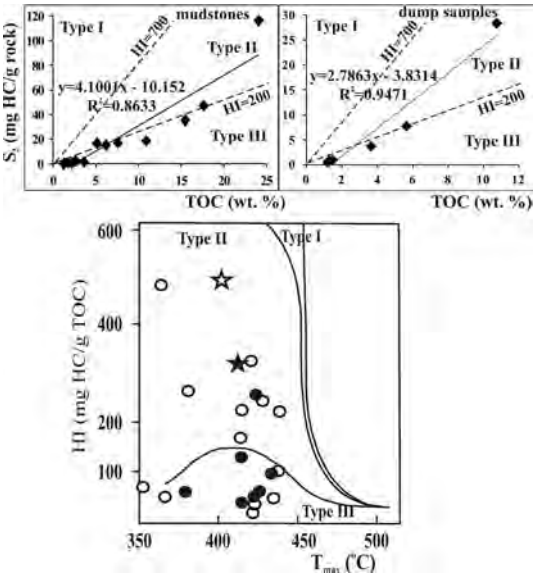



**Figure 1.** $S_2$ *vs*. TOC and HI *vs* $T_{max}$ diagrams (○ - mudstones, ● - dump materials, open and full asterisk - pyrolysable HI and average $T_{max}$).

**Table 1.** Calculated values for TOC and HI after [5]

| Parameter | Mudstones | Dump samples |
|---|---|---|
| $TOC_{av}$ (% wt) | 7.64 | 3.59 |
| $TOC_{inert}$ (% wt) | 3.18 | 1.50 |
| $TOC_{retained}$ (% wt) | 2.67 | 1.26 |
| $TOC_{dead}$ (% wt) | 0.51 | 0.34 |
| $TOC_{live}$ (% wt) | 4.46 | 2.09 |
| $HI_{obs}$ (mg HC/g TOC) | 178 | 105 |
| $HI_{live}$ (mg HC/g TOC) | 475.11 | 292.8 |

$TOC_{av}$ - arithmetic mean of TOC content; $TOC_{inert}$ - regression equation solved for x=0; $TOC_{retained}$ - y intercept of the regression line x α; $TOC_{dead}$=$TOC_{inert}$-$TOC_{retained}$; $TOC_{live}$=$TOC_{av}$-$TOC_{inert}$

By using the $S_2$ *vs*. TOC diagram (Fig. 1), the organic components of the sample sets were compared and mineral matrix effect estimated (2.48 % wt TOC for mudstones; 1.38 % wt TOC for dump samples). The intercept on TOC axis was attributed to a "clay matrix" effect, which turns out to be weak by comparison with the inertinite shift [6]. The $S_2$ *vs*. TOC graph is an effective tool to estimate the inert carbon - the intersection of the regression line with the TOC [5]. $TOC_{inert}$ is defined as OM that contributes nothing to the $S_2$ pyrolysate yield. The $TOC_{inert}$ values (Table 1) are high for both sample sets (ca. 42 % of the average TOC), and, also $TOC_{inert}$ is twice lower for the dump set. Correction of TOC to include only reactive TOC should lead to a reduction in the TOC intercept value and a less negative $S_2$ intercept value. $TOC_{live}$ (Table 1), i.e the proportion of the organic material that is pyrolysable [6], is which contributes to the $S_2$ value of RE data.

For both sample sets $S_2$ *vs*. TOC graph indicates Type III kerogen approaching Type II.

Pyrolysable hydrogen index ($HI_{live}$) calculated after quantifying the retention effect by mineral matrix and inert carbon increased the average HI for mudstone and dump samples by more than 297 and 188 mg HC/g TOC resp. In principle, samples containing inert carbon have a better source rock quality than those suggested by the RE pyrolysis [6].

$T_{max}$ averages at 408 ºC for mudstone set, and at 416 ºC - for dump set (Fig.1). The increased $T_{max}$ registered may be caused by a decrease in hydrocarbons weakly bonded to kerogen. Most of the pyrolysis temperatures ($T_{max}$) of the samples are <435 ºC indicating that the OM has suffered a very limited thermal alteration and is still immature. Plot HI *vs* $T_{max}$ (Fig. 1) indicates predominantly Type II kerogen for mudstones, and predominantly Type III kerogen - for the dump samples. The pyrolysable values for HI and the average $T_{max}$ values for both sample sets (Fig. 1) plot in the area of Type II kerogen and show a general tendency of decreasing hydrogen content from mudstone to dump samples.

The average values for OI are very high (89 and 127 mg $CO_2$/g TOC) for both sample sets being increased for dump samples. OI values greater than 10 mg $CO_2$/g TOC relate to the $TOC_{inert}$ or mineral matrix effects or mineral decomposition during the RE pyrolysis.

There is a very wide distribution of data in the pseudo van Krevelen (HI *vs*. OI) diagram (not shown) mostly in the low HI and high OI area and in general it drowns the weathering path of the samples.

It is accepted that OM weathering affects RE parameters leading in particular to an underestimation of HI values and overestimation of OI values. In a conclusion, based on the comparisons above, it appears that the impact of weathering for the dump samples is related to hydrocarbons ($S_1$, $S_2$, TOC) and HI depletion and to $T_{max}$ and OI elevation.

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# Topsoil alterations influence the genesis and composition of unusual organic-rich speleothems in volcanic caves from the Canary Islands

A. Z. MILLER [(a)*], J. M. DE LA ROSA [(a)], M. F. C. PEREIRA [(b)], J. A. GONZÁLEZ-PÉREZ [(a)], H. KNICKER [(a)], C. SAIZ-JIMENEZ [(a)]

(a) Instituto de Recursos Naturales y Agrobiologia de Sevilla (IRNAS-CSIC), 41012, Seville, Spain
(b) CERENA, Instituto Superior Técnico, University of Lisbon, 1049-001 Lisbon, Portugal
* E-mail: anamiller@irnas.csic.es

**Keywords:** Analytical pyrolysis, organic rich-speleothem, lava tubes, wildfires, soil organic matter

**Abstract.** This multidisciplinary study combines the use of mineralogy, chromatography, light stable isotopes and nuclear magnetic resonance spectroscopy to reveal the origin of black sludge-like speleothems found in a volcanic cave from La Palma Island (Spain). The combination of these analytical tools permitted the identification of specific biomarkers for tracing the potential sources of the organic compounds identified in these speleothems. Analytical pyrolysis revealed the presence of polysaccharides, plant lipids and specific terpenoids derived from the surface vegetation. In addition, polycyclic aromatic hydrocarbons and N-containing heterocyclic compounds were detected. They probably derived from the leaching of charred vegetation resulting from a wildfire occurred in the surface area in 2012. The $\delta^{13}C$ data of the cave speleothems, topsoil and overlying vegetation confirmed that the organic fraction of the speleothem samples is a combination of partially charred vegetation and organic compounds from the andic soil over the cave.

## Introduction

Speleothems, or secondary mineral deposits found in caves, are usually formed due to the dissolution of primary minerals from the host rock and subsequent precipitation of secondary minerals. Both limestone caves and lava tubes may contain a variety of speleothems, such as stalactites and stalagmites. Their formation is greatly prompted by water-rock interactions and climate conditions, which determine how much water drip into the cave [1]. In humid climates or during heavy precipitation a rapid speleothem growth may occur, whereas in arid climates or during drought the growth is moderated or ceased. Changes occurred on the soils over caves may also influence speleothem composition and formation. Hence, the composition, abundance and growth pattern of speleothems may be indicative of land use and climate changes, as reported by several authors [2, 3].

Soils in volcanic regions (including the Canary Islands, Spain) are mainly allophanic Andosols, which generaly composed of poorly crystalline clay minerals, such as allophanes and imogolite, and Fe and Al oxyhydroxides. These minerals are prone to form organo-mineral structures, which contribute to a stronger resistance of the soil to water erosion, an efficient protection of organic carbon against microbial decomposition and to the stability of the subterranean environment. However, these high amount of organic matter (OM) is easily combusted during wildfires [4]. Therefore, wildfire events over Andosols constitutes an additional risk to destroy the protecting topsoil and subsequently promotes erosion, leaching and transport of OM to the underlying cave environments.

Cave sedimentation typically results from the allogenic deposition of surface soils into caves. These sediments can provide information on local climate and other variables, such as possible changes in vegetation type, hydrology and geomorphology [5]. Similarly to sedimentary organic matter, cave speleothems are also key sites for organic matter (OM) deposition, offering the opportunity to study global climate changes and local environmental events [6]. Thus and taking into account that the soils overlying volcanic caves are a major source of carbon, the $\delta^{13}C$ of the organic fraction of speleothems can provide useful information on the origin of the OM of speleothems. Moreover, stable isotopes ($\delta^{13}C$, $\delta^{15}N$) and C/N ratios can be valuable indicators of OM origin and decay [6]. In an attempt to achieve an accurate characterization of the organic fraction of the ooze-like deposits found in MZ04 Cave in La Palma (Canary Islands) and unveil their nature and origin, analytical pyrolysis (Py-GC/MS), $^{13}C$ cross-polarisation magic-angle spinning nuclear magnetic resonance spectroscopy ($^{13}C$ CPMAS NMR) and stable isotope analyses were performed.

## Materials and methods

**Site description and sampling.** In a lava tube (MZ04 Cave) from La Palma Island, in Canary Islands (Spain), black deposits with a gelatinous texture were observed and collected from the wall and ceiling of the cave. In addition, the topsoil (0-5 cm) and charred vegetation over the cave were collected for comparison purposes.

**Analytical Pyrolysis (Py-GC/MS)** was performed using a double-shot pyrolyzer attached to a gas chromatograph. One mg of sample (speleothems, topsoil and surface vegetation) was pyrolysed at 500°C for 1 min and the evolved gases directly injected into the GC/MS. Chromatographic conditions were similat to those reported by Miller et al. [1]. The relative abundance of each pyrolysis product was expressed as a percentage (%) of the total chromatogram area, after the integration of peak areas.

**Solid-state $^{13}$C Nuclear Magnetic Resonance Spectroscopy (NMR)** was performed using a Bruker Avance III HD 400 MHz and zirconium rotors of 4 mm OD with magic-angle spinning of the rotor at 14 kHz and a contact time of 1ms. Over 60 000 scans were accumulated for each sample. The $^{13}$C chemical shifts were calibrated relative to tetramethylsilane (0 ppm) with glycine (COOH at 176.08 ppm). Spectra were quantified by integration of the following chemical shift regions: alkyl C (0-45 ppm), N-alkyl/methoxyl C (45-60 ppm), O-alkyl C (60-110 ppm), aromatic C (110-140 ppm),O-substituted aromatic C (140-160 ppm) and carboxyl/amide C and carbonyl C (160-220 ppm). The relative abundance of each typical C resonance region was expressed as relative percentage of the total C resonance area (assumed to be 100%) [7].

**Stable isotope analyses ($\delta^{15}$N, $\delta^{13}$C).** Bulk isotopic signature of light elements ($\delta^{13}$C and $\delta^{15}$N) was determined in triplicate by coupling the elemental micro-analyzer to a continuous flow Delta V Advantage isotope ratio mass spectrometer (IRMS) (Thermo Scientific, Bremen, Germany) (C/TC-IRMS) via a ConFlo IV interface unit.

## Results and Discussion

### Py-GC/MS

Analytical pyrolysis reported the presence of specific biomarkers (di- and triterpenoids) as well as abundant polysaccharides and plant lipids typical of the local vegetation (*E. arborea* and *P. canariensis*). Polycyclic aromatic hydrocarbons and N-containing heterocyclic compounds detected were related to the leaching of charred vegetation resulting from a wildfire occurred in the area in 2012. The lack of the typical pattern of odd-over-even in the series of n-alkanes observed for the black deposits from this lava tube suggested the alteration of the OM of the andosol over the cave probably due to the wildfire event.

### Solid-state $^{13}$C NMR spectroscopy

The $^{13}$C NMR spectra confirmed the abundance of polysaccharides in the OM fraction of the speleothem. They are typically derived from fresh soil OM and microorganisms. The peak corresponding to the aromatic-C region found in the speleothem is probably owed to the incorporation of charred residues which were leached from the topsoil.

### Stable isotope analyses

The $\delta^{13}$C value of the cave black deposits was similar to those measured for the topsoil and surface vegetation. The value of $\delta^{15}$N obtained for the black deposit was caracteristic of N fixation (from 0 to 7 ‰), being 4‰ generally considered the average of land plants (Maksymowska et al., 2000).

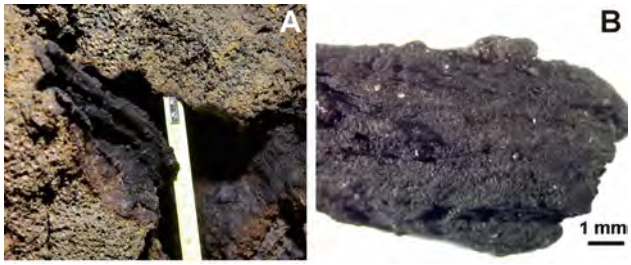



**Figure 1.** Black ooze-like deposits from MZ04 Cave, La Palma Island, Spain. A) Photography of the black deposits in the lava tube. B) Stereomicroscopy image showing their texture.

Stable isotope analysis confirmed that the organic fraction of the ooze deposits are a combination of partially charred vegetation and organic compounds from the andic soil located over the cave. Therefore, these cave deposits are the result of an input of plant OM and charred vegetation into the cave through rock fractures, which may constitute an important source of energy for cave organisms. Wildfire probably contributed to increase the alteration of the soil properties, such as porosity and water retention capacity, which promoted the leaching of OM and its input into the cave. Moreover, different depositional conditions occurred after the wildfire, which influenced the formation of the organic rich deposit.

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# Using natural organic matter (NOM) to synthesize magnetic materials applicable for removal of polycyclic aromatic hydrocarbons (PAHs) from seawater and produced water

R. V. M. OLIVEIRA [(a)], G. C. DA CUNHA [(a)], L. P. C. ROMÃO [(a)*]

[(a)] Federal University of Sergipe (UFS), 49100-000 São Cristóvão, SE, Brazil
* E-mail: luciane@ufs.br

**Keywords:** NOM, magnetic hybrid materials, remediation, PAHs.

**Abstract.** Polycyclic aromatic hydrocarbons (PAHs) exhibit high toxicity. This work describes the removal of PAHs using a magnetic hybrid adsorbent synthesized by a sol-gel route, with water containing a high amount of natural organic matter (NOM) employed to form the organic phase. XRD analysis revealed formation of the cobalt ferrite phase and, together with use of FTIR, confirmed the presence of NOM in the structure of the hybrid material with surface area of 108.2 $m^2 g^{-1}$. The material exhibited fast adsorption kinetics, with PAHs removal rates of 86.5-96.3% in only 30 min.

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds consisting of fused benzene rings, which have received attention due to their carcinogenicity, teratogenicity, and mutagenicity [1]. Several PAHs have been identified as priority pollutants to be monitored and controlled by environmental protection agencies in the United States and China [2].

PAHs are ubiquitous in the environment, with several studies reporting their presence in aqueous media, including seawater and produced water. The latter, which is one of the main wastes derived from oil and gas (O&G) extraction processes, consists of a complex mixture of several components that vary according to the geographic location and age of the well. PAHs are the most important pollutants found in produced water [3].

The removal of PAHs from aqueous media is a challenging problem, due to the presence of complex mixtures of compounds. Adsorption employing a new class of hybrid materials has emerged as a promising option for the remediation of environments contaminated with PAHs, especially when the adsorbent material possesses magnetic properties that enable it to be easily removed from an effluent by the simple action of an external magnetic field. Furthermore, these hybrids can be synthesized using precursors that have electronic structures similar to those of PAHs, resulting in adsorption processes that are governed by π-π interactions between the PAHs and the adsorbents.

The aim of this work was to synthesize a magnetic hybrid material by the sol-gel route modified using natural organic matter (NOM) to compose the organic phase, and to evaluate the effectiveness of this material in the removal of PAHs.

## Materials and Methods

### Synthesis of HMGS

The synthesis of the hybrid material employed an adaptation of the methodology proposed by Culita et al. [4]. The inorganic precursors used were $CoCl_2.6H_2O$ and $Fe_2(SO_4).6H_2O$, in a proportion of 1:2, which were dissolved in 200 mL of water with high NOM content (24 mg $L^{-1}$ DOC), under constant mechanical stirring. The pH of the medium was then gradually increased to pH 9 using aqueous NaOH, followed by agitation for 30 min. After gel formation, the system was heated at 100°C for 6 h to remove water and obtain a xerogel. The material was then washed with deionized water to remove the excess sodium chloride generated, followed by drying in an oven at 60°C and homogenization. The resulting material, denoted HMGS, was characterized by X-ray diffraction (XRD), infrared absorption spectroscopy (FTIR), and surface area analysis.

### Adsorption assays

In the evaluation of contact time, experiments were performed using 10 mL volumes of mixed PAHs solutions containing 100 μg $L^{-1}$ of each PAH: benzo[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), and dibenz[a,h]anthracene (DahA). After addition of 100 mg of HMGS, the mixtures were agitated at 300 rpm (Marconi MA 832/1 agitator) and 25 ºC. At predetermined times, the HMGS was removed from the solutions using a magnet, and the residual PAHs concentrations were determined by high performance liquid chromatography (HPLC). The analyses were performed using a Shimadzu Prominence system equipped with a fluorescence detector operated at excitation/emission wavelengths of 266/384 nm up to 20 min, followed by a switch to 290/430 nm for the remainder of the period. The assays were performed using seawater and produced water enriched with the mixed PAHs solution at a concentration of 100 μg $L^{-1}$ for each compound.

## Results and Discussion

### Characterization

It can be seen from Figure 1 that the most intense diffraction peaks of the hybrid material were in agreement with the diffraction data for the $CoFe_2O_4$ standard available in the Inorganic Crystal Structure Database (ICSD #192031). This confirmed the success of the route proposed in this work for synthesis of the $CoFe_2O_4$/NOM

hybrid, which was obtained at room temperature. This route uses NOM, a natural polymer, instead of the traditional solvents, which can provide the magnetic adsorbent matrix with differentiated properties.

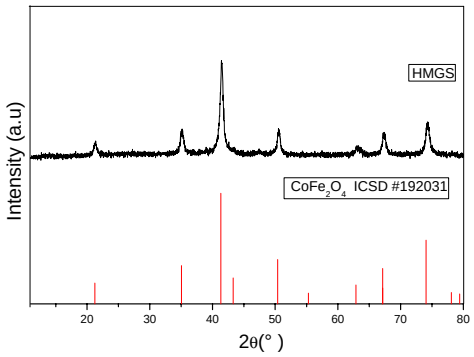



**Figure 1.** Comparison of X-ray diffraction patterns for the HMGS sample and the ICSD cobalt ferrite standard.

The FTIR spectrum of the HMGS material (Figure 2) showed broad and intense bands at around 3430 $cm^{-1}$, attributed to the stretching of O-H groups of water, alcohols, phenols, and/or carboxylic acids. Bands characteristic of NOM were present at 1626 $cm^{-1}$, corresponding to stretching of C=C bonds of aromatic groups, and at 1366 $cm^{-1}$, attributed to symmetric stretching of $COO^-$. A band at 596 $cm^{-1}$ could be assigned to the Fe-O bond characteristic of ferrites [3]. A band at around 1100 $cm^{-1}$, assigned to the stretching of S=O bonds, was indicative of the presence of sulfate derived from the precursor used in the synthesis.

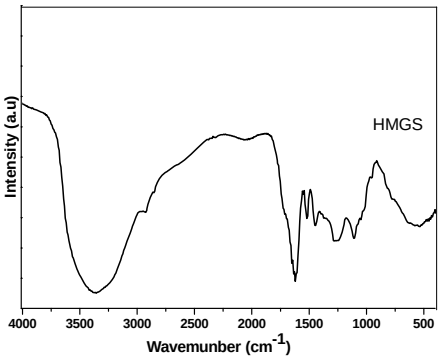



**Figure 2.** Infrared spectrum of the HMGS hybrid.

The HMGS sample showed a high surface area of 108.2 $m^2.g^{-1}$, which was superior to values reported in the literature. This indicated the technological potential of the eco-friendly synthesis route proposed here, which requires no heat treatment and uses water with high NOM content to compose the organic phase.

**Adsorption assays**

The adsorbent showed high PAHs removal rates in the range 90.3-95.6%, achieved in only 30 min (Figure 3). In environmental remediation, the time required is a very important consideration, since the faster a pollutant is removed, the less will be the environmental damage. These results highlight the great potential of the material for efficient removal of contaminants.

Adsorption tests with real samples (Figure 4) indicated that the high concentrations of salts in the seawater and produced water acted to increase the adsorptive capacity of the adsorbent, probably due to the greater ionic strength of the medium, which facilitated migration of the analytes to the adsorbent (the salting out effect) [1].

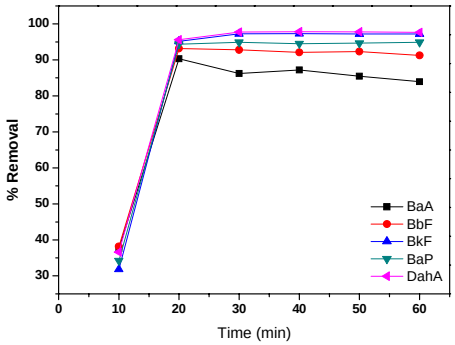



**Figure 3.** Effect of contact time on the adsorption of polycyclic aromatic hydrocarbons in mixed solutions. Conditions: $C_i$ = 100.0 µg $L^{-1}$; $m_{ads}$ = 100.0 mg; T = 25°C; agitation speed = 300 rpm.

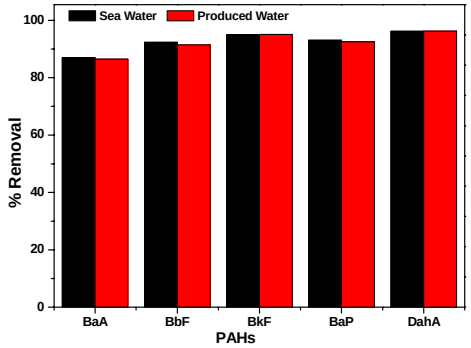



**Figure 4.** Removal of polycyclic aromatic hydrocarbons from real samples (seawater and produced water). Conditions: $C_i$ = 100.0 µg $L^{-1}$; $m_{ads}$ = 100.0 mg; T = 25°C; agitation speed = 300 rpm.

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# Optimized scheme for group analysis and fractionation of peat organic matters

S. SELYANINA, O. YARIGINA, T. PONOMAREVA, M. TRUFANOVA, V. TATARINTSEVA [(a)*]

(a) N. Laverov Federal Center for Integrated Arctic Research Russian Academy of Science (FCIArctic RAS), Severnoi Dviny Emb., 23, 163000, Arkhangelsk, Russia
* E-mail: leratatarintseva@gmail.com

**Keywords:** peat, humic acids, bitumens, extraction, scheme for group analysis of peat

**Abstract.** The diversity of methods of peat organic substances extraction that were developed not only in Russia but also abroad do not allow to extract the valuable organic components of peat and to estimate its properties correctly. Optimized "Method of gravimetrical measurement of group chemical composition of peat" allows using data to compare group composition of various types of peat and peat-forming vegetation; provides extraction of "pure" peat organic components.

## Introduction

Peat organic substances composition consists of various chemical matters. Also it is changeable and depends on different environmental conditions. This fact makes identification of peat components complex. Current methods do not allow characterize peat composition clearly.

Extractive substances (bitumens), water-soluble (polyoses and polyphenols), humic substances (fulvic acids, humic acids and on the occasion hymatomelanic acids), easy hydrolysable compounds and humus, heavy hydrolysable compounds and lignin are integral to peat organic part.

Different methods are used to study peat organic substance. These methods distinguish in terms of:

a. order of fractionating;

b. use of extracting agents;

c. extraction conditions (temperature, concentration, mass ratio, stages duration);

d. way of recovery degree determination (by concentration, using spectrophotometer, by gravimetrical method and etc.).

In particular mixture benzol : ethanol (1:1) were used to apply in processes of bitumens extraction in different methods [1-3]. Nowadays researchers use other extracting agents due to benzol toxicity: benzine, formyl trichloride, ethanol and hexane mixture and etc. Most of it is less selective and/or has low extractive power. Different methods of extraction using in the process (infusion with/without heating, reflux and reflux with infusion in Soxhlet apparatus) are also distinguished in terms of recovery degree. All these parameters make the results inaccurate and difficult to compare. For this reason method of researching should be unified to get the clear results.

## Materials and methods

On the basis of literature data and in vitro study "Method of gravimetrical measurement of group chemical composition of peat", particularity of which is described in this article, was developed and certificated [4]. Optimized scheme of group analysis and fractionating of peat organic matters is illustrated below (fig. 1).

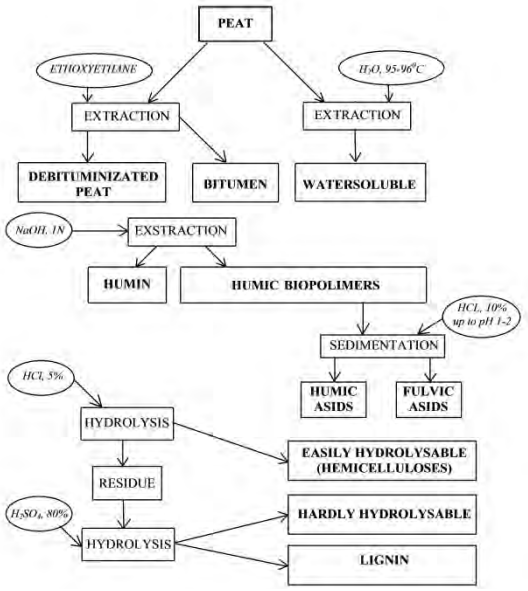



**Figure 1**. Optimized scheme for group analysis and fractionation of peat organic matters.

## Results and Discussion

The method of reflux with infusion was applied to debitumizing peat; extracting agent – ethoxy ethane. According to our researches to changing methods of extraction and extracting agents this method provides drastic removal of extractive substances and high selectivity [5]. The ethoxy ethane value as a solvent is the low boiling point (36.5ºC). This allows exclude the possibility of influence of high temperature on peat components transformation.

Alkaline treatment was conducted to extract humus substances. Conditions of the process: ambient temperature, duration of the extraction is 20 hours [2]. Contain of humus substances was calculated according loss of its weigh. Change of mineral compounds content of a solid body should be taken into account during calculations. It was detected alkaline and acid treatment conduce solubilization of part of mineral substances. This leads to over-estimation of weight content of humus substances. This imprecision could be about 50% and higher for high-ash peat.

Extraction of easy hydrolysable compounds should be conducted after humus substances removal to prevent

acid modification of the latter, particularly hydrolysis of etheric bonds.

In this method all group components except water-soluble components, concentration of which is usually low, are extracted sequentially.

We used both method of International Humic Substances Society and our modified scheme to compare the results of studying equal peat samples. Results are illustrated in **Figure 2**.

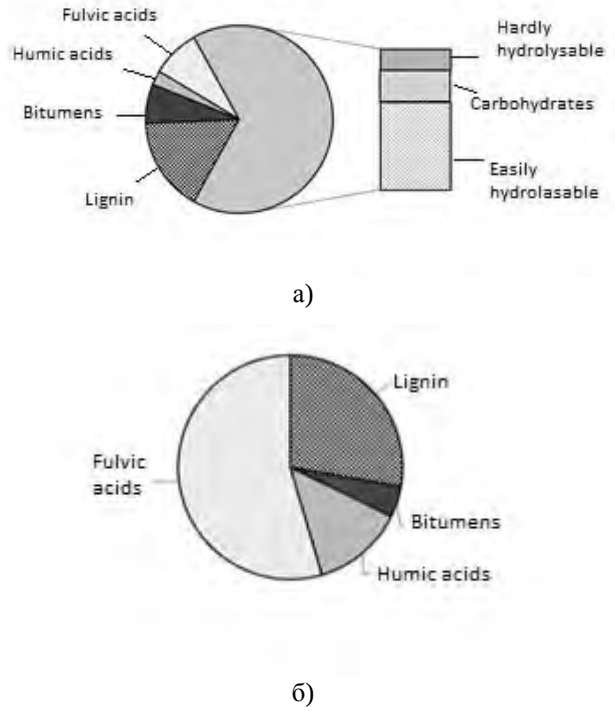



**Figure 2**. The group chemical composition of peat polymer matrices, studied with various methods:
*a* - the optimized technique; *b* - the traditional method

**Figure 2** illustrated that data on humic and fulvic acids contain are over-estimated using traditional method. Herewith carbohydrates are ignored in spite of the fact that it is the most stable part (especially heavy hydrolysable compounds) of low-decomposed peat in comparison with other parental plant components. Bitumens content is upward of 2 times using modified scheme. Selectivity of the removal is confirmed with spectrophotometer.

**Conclusions**

To conclude, using the developed scheme gives the possibility get more serious and real data of organic part of peat. In addition it provides inclusive and selective extraction of peat organic components and allows using data to compare group composition of various types of peat and peat-forming vegetation.

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# Mobil components of soil humus on the Barents Sea coast

E. SHAMRIKOVA [(a)]*, S. DENEVA [(a)], O. KUBIK [(a)]

(a) Institute of Biology, Komi Science Centre, Ural Branch, Russian Academy of Sciences
* E-mail: shamrik@ib.komisc.ru

**Keywords:** Arctic soils, low-molecular-weight organic compounds

**Abstract.** Composition of water and KCl-extracts from soil organic horizons (Cryic Folic Histosols Fluvic and Histic Reductaquic Cryosols Thixotropic) on the Barents Sea coast was studied. Weight concentrations of total organic carbon in water extracts make 0.2-0.5 g $dm^{-3}$. Total carbon in water extracts $\rho(C_{H2O})$ in two cases exceeds that in salt extracts $\rho(C_{KCl})$ and does not have true differences from the other organic soil horizons. Weight concentrations of low-molecular-weight organic compounds in extracts were identified by the GC/MS method. Sea water in flood area possibly lowers mobility of low-molecular-weight soil organic matter fraction.

## Introduction

Dissolved low-molecular-weight organic compounds influence almost every process in soil and aquatic environment. Sea coastal soils are regularly flooded with waters with a high ionic power. So, identification of organic compounds which pass into salt solutions is important for understanding coastal soil geneses. The purpose of the study was to identify composition of water and KCl-extracts from soil organic horizons on the Barents Sea coast.

## Materials and methods

The study region is located in the coastal part of the Khaipudyrskaya Bay (the Barents Sea). The area belongs to the north hypo-arctic tundra subzone. Organic horizons of two soil types served study materials. The first of them is Cryic Folic Histosols Fluvic and the second – Histic Reductaquic Cryosols Thixotropic [1] (N 68°20′05.7″, E 59°33′21.9″ and N 68°16′58.9″, E 59°54′49.5″, correspondingly, h = 8 m a.s.l.). Water and KCl-extracts ($c$ = 1 mol $dm^{-3}$) from organic soil horizons were prepared at a ratio of 1:25. Low-molecular-weight organic compounds in extracts were identified by the GC-MS method [2-5]. Chemical analyses were done at the Chromatographia Collective Usage Centre, also at the Soil Science Department and the Botanical Garden Department of the Institute of Biology Komi SC UrD RAS.

## Results and Discussion

Weight concentrations of total organic carbon in water extracts make 0.2-0.5 g $dm^{-3}$ which is similar to the previously obtained data on taiga and south tundra soils of the Komi Republic [2-5]. Total carbon values in extracts are interdependent. Total carbon value in water extracts ($\rho(C_{H2O})$) in two cases largely exceeds that in salt extracts ($\rho(C_{KCl})$) and does not have true differences from the other organic soil horizons. On the average, $\frac{\rho(C_{KCl})}{\rho(C_{H2O})}$ = 0.9 (Fig. 1A).

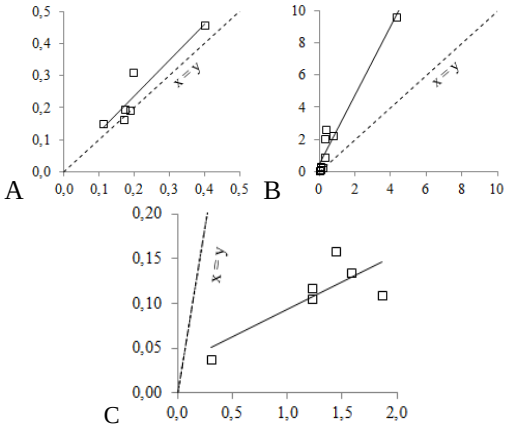



**Figure 1.** Relationships between the component concentrations in salt and water extracts from soil (A) weight concentration of total organic carbon, mg/$dm^3$. (B) weight concentration of carbon from low-molecular-weight compounds, mg/$dm^3$. (C) hydrogen ion activity, mmol/$dm^3$.

Consequently, salt solution, in contrast with water solution, tends to decrease total carbon content of KCl-extracts. We identified nine acids (mainly oxyacids) and seven carbohydrates.

Variety of low-molecular-weight acids and carbohydrates in salt extracts is less than that in water extracts (Table 1).

**Table 1.** Qualitative composition of extracts from organic soil horizons

| Com-pounds | Extragent | |
|---|---|---|
| | $H_2O$ | KCl |
| Acids | 2-oxypropionic, 2-oxybutanedioic, 2,3,4-trioxibutanoic, 2,3,4,5-tetraoxipentanoic | |
| | 2-oxyethanoic, 3-oxybutanoic, pentanoic, butanedionic, 1,2-dioxypropionic | – |
| Carbo-hydrates | galactose, arabinose, ribose, glucose, sucrose | |
| | xylopyranose, turanose | – |

Major water-soluble carbohydrates or acids pass into salt solution. *E.g.*, salt solution-extracted acids in water extracts make 42-94 and carbohydrates – more than 90 % of total content. Analogically with total carbon content, there exists a close correlation of low-molecular-

weight compounds (acids and carbohydrates) carbon in water and salt extracts. The ratio of $\frac{\rho(C_{KCl})}{\rho(C_{H2O})}$ = 0.5 (Fig. 1B) and is inferior to the analogous ratio for total organic carbon. So, salt solution decreases solubility of organic compounds, which is not the case with water extraction but decreases itself, is different. Found organic acids serve acidity source in extracts. Proton activities calculated using pH values of extracts also have high correlation values and the ratio of $\frac{a(H^+)_{KCl}}{a(H^+)_{H2O}}$ = 2.60 (Fig. 1C).

Increase of ion power in solution can cause decrease of compounds solubility. This process is called the "salting-out effect". Previous to salting out high-molecular-weight organic compounds (HMWCs), they change conformation. Change in ion power of solution destroys some weak bonds and forms others and so causes slight displacement of particular chain parts and appearance of new ionization-able functional groups on surface [6]. Thus, increase in ion power of solution can significantly lower solubility of low-molecular-weight compounds. But their part in composition of total organic carbon in soil water extracts is insignificant and so we do not see any serious decrease in total carbon content of soil water extracts. Salt solution extraction power of high-molecular-weight compounds decreases less or does not decrease at all compared with water extraction.

**Conclusion**

On sample of soil types on the Barents Sea coast, increase in extragent ion lowers solubility of low-molecular-weight organic compounds identified by the gas chromatography method. Low pH values of soil KCl-extracts compared with those of water extracts seem to be related to conformation changes of high-molecular-weight compounds. Sea water in flood area possibly lowers mobility of low-molecular-weight soil organic matter fraction.

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# Development of the theory of humic substances optical properties formation

E. SHIRSHIN [(a)*], A. ZHEREBKER [(b)], S. SHAKIROV [(a)]

(a) Department of Physics, Moscow State University, 119991 Moscow, Russia
(b) Skolkovo Institute of Science and Technology, 143026 Moscow, Russia
* E-mail: shirshin@lid.phys.msu.ru

**Keywords:** fluorescence spectroscopy, energy transfer, fluorescence lifetime, fluorophores

**Abstract.** Optical methods are routinely used to characterize humic substances (HS) in a wide range of applications. Based on phenomenological studies, optical descriptors have been introduced to assess HS properties. However, there is no theoretical model, which describes the mechanism of HS optical response formation and the reasons, which underlay similarity in the behavior of HS spectra regardless of their dramatic structural diversity. Here we present the results of theoretical modelling of HS optical properties of HS based on steady-state and time-resolved fluorescence spectroscopy and mass spectrometry data.

## Introduction

HS are natural ensembles of diverse organic compounds produced as a result of microbial and abiotic degradation of remnants of living organisms. They are characterized by extreme complexity, which brought to life the modern concept of supramolecular organization of humic molecular systems. HS demonstrate a dramatic variation of structural and molecular features depending on the source (water, peat, soil, coal etc). The ultrahigh resolution Fourier transform ion cyclotron mass-spectroscopy (FT-ICR MS) reveals that HS consist of numerous individual molecules, which may interact with each other, leading to unique chemical properties of the whole system. However, for some applications it is more convenient to characterize HS by integral parameters, the so-called descriptors, which express some property of the preparation (e.g. aromaticity, hydrophobicity, polarity etc) rather by a large set of data on all individual components, which comprise the selected substance.

Optical spectroscopy, namely, absorption and fluorescence spectroscopy, are among the most frequently used techniques for characterization of HS properties due to their simplicity, short acquisition time, low price of equipment, and sensitivity to structural alterations. For instance, such descriptors as SUVA254, $E_4/E_6$ and various spectral slopes can be extracted from the HS UV-Vis absorption spectra [1-2]. Fluorescence spectroscopy also provides important information about relative changes in HS structure, e.g. the humification degree, based on the spectral band shape of HS emission at a certain excitation wavelength. Also the PARAFAC-based approach to the analysis of HS fluorescence spectra is widely used to assess independent components, which are responsible for the overall HS emission signal [3].

The fundamental problem in HS analysis using optical spectroscopy is that optical descriptors of HS properties are empirical, i.e. there is no fundamental description of physical mechanisms underlying spectral and photophysical properties of natural organic matter. The advances in understanding the origin of HS optical properties are connected with the works of Del Vecchio and Blough [4-6], who demonstrated that HS cannot be considered as a system of independent chromophores/ fluorophores and that interaction between individual molecules in HS systems, presumably charge transfer interaction [6], is responsible for their specific optical properties. Although the existence of interaction between the HS molecular components was clearly shown, there was no theoretical description proposed with regard to the general mechanisms, which are responsible for similar behavior of optical properties of HS regardless of the dramatic diversity of their structure and constituents.

## Materials and methods

The investigated set of HS included collection of 30 preparations extracted from different sources (rivers, lakes, peat, soil and coal). The samples were investigated using picosecond time-resolved (Becker&Hickl, Germany) and steady-state (Horiba, Japan) fluorimetry. FT-ICT MS was performed using the Bruker 7T system.

## Results and Discussion

Similarly to the literature data, all the HS were characterized by the exponential decay of its absorption with wavelength. To ensure that the red edge (>600 nm) of optical density was due to electronic absorption, fluorescence emission at 635 nm was detected using the single photon counting technique. According to the literature data, exponential decay of absorption is typical for numerous systems in the solid state physics and is due to structural disorder. For molecular systems, this type of behavior can also be observed in several specific cases. First, this fact has been addressed for asphaltenes in the works of Mullins [7], and it was suggested on the basis of quantum chemistry calculations and electron microscopy studies that it originated simply from the superposition of spectra from polyaromatic hydrocarbons with different number of fused rings. Second, similar optical behavior is observed for melanin, which is a pigment responsible for the skin color. Numerous works aimed at theoretical description of mechanisms responsible for the broad absorption spectra of melanin, and it was concluded that it is originated from aggregation and excitonic interaction between aggregates [8]. These two examples demonstrate two opposite approaches to explanation of the observed phenomenon: in the first case we operate with different

independent molecules and their superposition, while in the second case we have a single monomer, which aggregates, and interaction between aggregates results in the appearance of new spectral features. To assess the possible mechanism in the case of HS, we analyzed the distribution of molecules within the samples obtained using FT-ICR MS aimed at evaluation of the distribution over the effective conjugation length. It was shown that this distribution is rather gaussian than exponential, and the absorption spectra can not be described by superposition of individual components. Next, introducing efective interaction between fluorophores/chromophores, absorption and fluorescence spectra were modeled based on the distribution of the aromaticity index for HS samples.

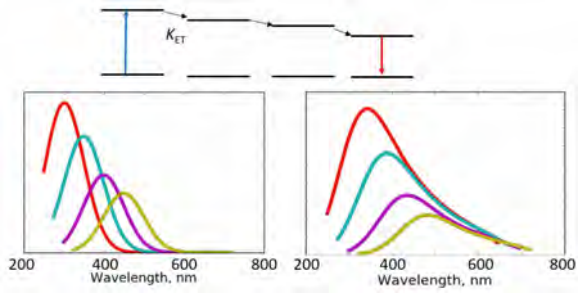



**Figure 1.** Modeled fluorescence spectra measured at different wavelengths (indicated by colors) for a subset of fluorophores in the HS structure without electronic interactions (left) and under the assumption of linear transport with similar energy transfer efficiencies for all neighbouring fluorophores.

Figure 1 demonstrates that the "embedding" of the HS fluorescence spectra for different can only be obtained when introducing electronic interaction between the molecules in the ensemble. Based on the modeling results, monotonous decrease of HS fluorescence lifetime with the detection wavelength, that was observed in experiments with all preparations under study, was also addressed. Summarizing, modeling of HS optical properties based on the assumption of interacting chromophores with certain distribution over the conjugation length was demonstrated to be promising for explaining the mechanisms responsible for the formation of HS optcal spectra.

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# Trace elements and organic matter composition in leached cinnamonic forest soil from Pernic region in western Bulgaria

T. SHISHKOV [(a)*], E. FILCHEVA[(a)]

(a) Department of Genesis, geography and soil classification, Institute of Soil Science, Agrotechnology and Plant Protection „Nikola Poushkarov", 7 Shosse Bankya Str., POBox 1369, Sofia 1331 tel./fax: +3592 824 6141
*E-mail: toma_shishkov@yahoo.com

**Keywords:** organic matter, trace elements, textural differentiation

**Abstract.** Study on trace elements, trends of their migration and organic matter were carried out. The following features of the distribution of the trace elements along the soil depth and organic matter characteristic were identified in the leached cinnamonic forest soil with textural differentiation of profile from Pernic region in western Bulgaria. It was established that the content of Co is accumulated at the surface and migration in profile was not observed. In this particular site, the fact of biogenic accumulation of Cr, Zn and Pb on the surface was not confirmed but some enrichment of deeper horizons was established. All trace elements as well as macroelements Ca and Mg increased significantly in the illuvial horizon. This enrichment could be explained with the advanced soil evolution where along with the textural differentiation of profile is also characterized with the distribution of clay minerals.

## Introduction

The content of trace elements as they are part of ferments, hormones, vitamins and their role in physiological and biochemical processes is important. Recently many studies on the trace elements in soils and the trends of their migration along the depth have been carried out [1, 2, 3]. The content of a given element depends not only on its initial amount in parent rock materials, the chemical properties and the features of the crystalline lattices of the bearing minerals but also on intensity and duration of weathering and soil forming processes [5]. The mineralogical composition of parent materials and processes during soil evolution has caused increased content in soils.

## Materials and methods

The studied soil is localized on the hill slope in a pasture in the Pernic region, with coordinates 42°31'14.01"N 23°07'59.79"E and elevation 886 meters. Parent rock is dolomite. In this region, the relief is hilly to low mountainous. Mean annual temperature is 10°C, -1.5°C in January, 20.2°C in July, mean annual precipitation is about 654 mm. Soil temperature regime is mesic and soil moisture is udic. According to the FAO-UNESCO (1990) classification is Moli-Chromic Luvisols. The texture is clay loam at the surface and clay at the depth. Data on the content of trace elements was determined from the genetic horizons after acid dissolving ($HF+HClO_4$) in the soil samples containing particles with size <1mm (Table 1). Their concentrations in the obtained solutions were determined by atomic absorption method (AAS).

**Table1.** Studied trace elements and Mg, Ca content in leached cinnamonic forest soil, in mg/kg

| Horizon, depth, cm | Pb | Co | Ni | Cr | Cu | Zn | Mn | Mg | Ca |
|---|---|---|---|---|---|---|---|---|---|
| AE 8-24 | 10.0 | 10.0 | 9.5 | 17.0 | 9.5 | 28.0 | 590 | 1600 | 152.5 |
| Bt1 24-42 | 18.0 | 11.0 | 9.5 | 21.0 | 9.0 | 30.0 | 520 | 1850 | 125.5 |
| Bt2 42-75 | - | - | - | - | - | - | - | - | - |
| Bt3 75-98 | 29.0 | 8.0 | 31.5 | 30.5 | 24.5 | 60.5 | 683 | 3300 | 307.5 |
| BCk 98-118 | 28.5 | 5.5 | 27.5 | 27.5 | 23.5 | 67.0 | 623 | 22750 | 24750 |
| Rk 118-138 | 21.5 | 3.5 | 12.0 | 12.5 | 14.0 | 38.5 | 398 | 45250 | 80750 |

## Results and discussion

The content of trace elements in soil is vitally important. The content of a given element depends on its initial amount in parent rock materials and its chemical properties of the intensity and duration of weathering, migration and other soil forming processes which result can be followed in the soil zones with enrichment [7]. The mineralogical composition of parent materials and processes during the evolution of soil have caused increased content in soils.

Special attention should be paid to the role of organic substances especially for copper, the role of clay minerals for zinc, different concentration in primary minerals with varying degree of resistance [2, 4].

Results for the content and profile distribution of heavy metals in the leached cinnamonic forest soils characterized with a texture-differentiated profile is shown in Fig. 1.

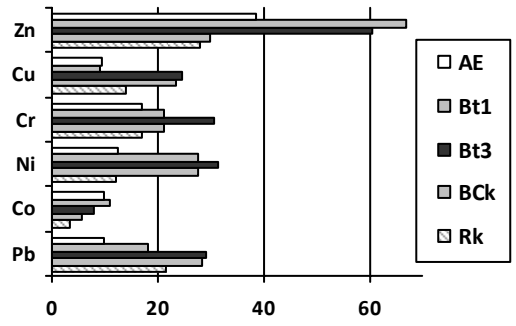



**Figure 1**. Content and distribution of some trace elements along the profile the leached cinnamonic forest soil.

Parent materials are of crucial importance for the trace elements content, particularly on the surface. However, due to the prolonged processes of migration up and down, the relationship between the content of trace elements in the surface and in parent rock may be somewhat erased. Due to this fact, AE horizon was prepared for the evaluation of trace elements trend of accumulation.

The results obtained for the studied content and profile distribution in leached cinnamonic forest soil of trace elements and macro elements Ca and Mg are presented in Table 1.

The distribution in depth of profile of trace elements is a consequence of the various soil formation processes and specific properties of the individual elements, which determines the enrichment or decreasing in the surface horizon with one or other elements. In the study, cobalt accumulates in the humus and humus eluvial horizon, while the other heavy metals studied have an elluvial, character of profile distribution similar to that of calcium and magnesium. The increased content of cobalt in most upper humus horizon is usually associated with the role of vegetation and some organics substances in the soil. Zinc exhibits a typical eluvial-illuvial characteristic of enrichment. The accumulation of manganese in the surface horizon can be explained only if it is accumulated as well in plant tissues. Due to its extremely poor migration capacity in the oxidizing conditions manganese normally exists in the well-aerated surface layer.

The importance of manganese oxides in the accumulation of cobalt and in many cases also on lead was reported [3,6]. It is necessary to take into account not only manganese but also iron oxides when explaining the distribution of individual trace elements. The significantly lower concentration of cobalt and lead was found under the same conditions in analogous iron enrichments, while copper, zinc and lead had approximately the same concentrations in manganese and iron enrichment. It is well-known fact that the iron oxides content is usually significantly higher in the soil.

Organic carbon content in humus horizon is high 1.4-1.9% (Figure 2). At the surface (0-8 cm) the type of humus is humic-fulvic where about 75% of humic acids are free or $R_2O_3$ bounded that means quite mobile. Despite the fact that the values of optical characteristic ($E_4/E_6$) are quite high, the humic acids molecule was less condensate and resembled fulvic acids. Deeper in humus horizon (8-24 cm) the type of humus rapidly changes to humic 100% bounded with Ca meaning lesser mobility in profile. Sharp decreasing of organic carbon content (more of 50%) was established in Bt horizon (24-98 cm).

Fulvic acids prevailing over the humic and type of humus gradually changes from humic-fulvic to fulvic one.

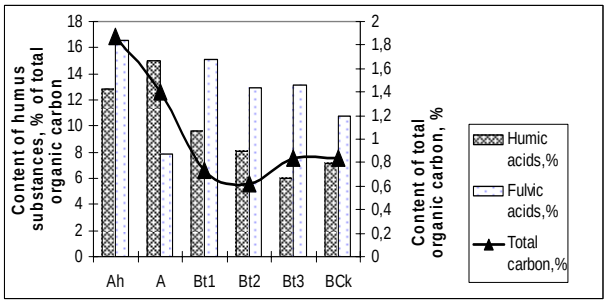



**Figure 2**. Content and composition of humic substances in (%) in leached cinnamonic forest soil textural differentiated.

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# Humic substances and physicochemical characteristic of leached chernozems from the region of Kavarna town in Northeastern Bulgaria

T. SHISHKOV [(a)*], E. FILCHEVA[(a)], E. DIMITROV [(a)]

(a) Institute of Soil Science, Agrotechnology and Plant Protection „Nikola Poushkarov”,
7 Shosse Bankya Str., POBox 1369, Sofia 1331
* E-mail: toma_shishkov@yahoo.com

**Keywords:** organic matter, cations, base saturation, humic and fulvic acids, chernozems

**Abstract.** This paper discusses interpretation of variables on study of organic matter and physicochemical characteristic of two soils - strongly leached and clayer chernozems (karasoluk), formed in the steppe zone region of Kavarna town in Northeastern Bulgaria. The composition of organic matter is used as the evidence of advanced and specific balance of soil processes as well biological activity. Total organic carbon (C%) was recognizable even at the depth of profiles.

## Introduction

The research site is located in Northeastern Bulgaria and covers the eastern part of the Dobrudzha plateau in an area with a pronounced influence of the Black Sea on the climate. It is bounded to the north by the village of St. Nicholas, to the northeast by the village of Balgarevo and the resort Rusalka, in the eastern part of the Black Sea. The unique feature of the Dobrudzha coastline is the alternation of wet and steppe areas.

Geomorphologically, the area is in the eastern part of the Misesian platform, as a major morpho-structural unit, and according to physical geographic zoning it falls into the Dobrudzha subregion of the Eastern Danube Plain. In hypsometric terms, the area is referred to the lowland up to 200 meters above the sea level. The terrain has an undulating relief plain to plain-hilly, with prevailing slopes from $3^o$ to $5^o$. In geological terms, this region of the Dobrudzha plateau is built on the lower and the middle-Sarmatian sediments made up of alternating sandy limestones and marls with the aquiferous flow that runs through in them. The most common surface karst forms are known in the region as kayryatsi. They are located at the edge part of the Dobrudzha plateau and raised up to 110 meters high near Kavarna town. Among the old abrasive flat forms, the one most well-known is the Sarmatian-Pontic with an altitude of 230-250 meter.

The survey area is referred to the temperate continental climate, which is formed under the direct influence of the Black Sea. The small amount of rainfall and the wind with cold continental northern air masses during winter are typical for the region. Winter seems harsh due to the constantly winding winds. The wind speed in the area is highest in winter and spring when it reaches 6.5-8.0 m/sec and the lowest is in summer and autumn less than 5 m/sec. The average annual air temperature is 11.8°C. The area is relatively cold for its geographic location, with average temperature of $0.8^oC$ in January and $22.3^oC$ in July. The annual temperature amplitude is 21.7-21.9°C. The region is one of the driest regions of Bulgaria. The total average annual rainfall is in the range of 411-480 mm. The maximum rainfall for the area is in autumn, the minimum in spring and summer. In hydrological terms, the area is poor on surface water there are no rivers and lakes.

The vegetation is characterized by the prevalence of grass species and scarce involvement of tree and shrub species. In the larger range, the plain terrains distributed on non-cultivated land dominated by the vegetation represented predominantly by xerothermic (dry-resistant) grass species [6]. There are separate forest-protection belts on chernozems, natural forest ecosystems occupy local areas and fields of protection.

In the area between Balgarevo village, Cape Kaliakra and Eni Kulak are the last and best preserved steppe habitats in Bulgaria. Zoning the territory referred it to the Euxin province of the European broad-leaved forest region and the Lower Danube province of the Eurasian steppe and forest-steppe region [1].

## Materials and methods

Soil organic carbon content was determined by modified Turin’s method [4, 7] (dichromate digestion at $125^oC$, 45 min, in presence of $Ag_2SO_4$ and $(NH_4)_2SO_4$ $FeSO_4$ $6H_2O$ titration, phenyl anthranilyc acid as indicator). According to the method of Ganev and Arsova [5] the total acidity is determined by the solutions of 1.0 N sodium acetate and 0.2 N potassium maleinate at pH 8.25, the value with maximum saturation of soil colloids with strong alkaline bases.

## Results and discussion

The area is entirely occupied by chernozems and with their varieties [2] and determined as izohumic soils [3]. Soils have a well developed humus horizon, with a weak alkaline to neutral soil reaction. In the northern part of the region, the most common soil type is strongly leached and clayey chernozems.

Rendzinas (or humus-calcareous soils) occupy a strip along the sea. These shallow skeletal soils on Sarmatian limestone parent rock are pastures of semi-steppe nature.

The difference in texture is significant. Thus in strongly leached chernozems clay fraction (<0.001mm) is 27-30% respectively in clayey chernozems (karasoluk) it is 44.5%, the so-called physical clay fraction (<0.01mm) in the first soil is 55% and in the second is 71%.

Both soils do not differ in content and composition of humic substances. In the strongly leached chernozems surface horizon can be determined as with high (2.59%) amount of organic carbon and decreases at the subsurface horizons to (1.81-1.97%). The amount of carbon decreases gradual downward, but it is about one and a half times less as compared to that in the surface horizons. In clayey chernozems (karasoluk) this amount is lesser about 1.20-1.50%. In the surface horizon of the first soil the amount of extractable organic carbon (extr. with 0.1M $Na_4P_2O_7$+0.1MNaOH) is 27% from the total organic carbon, beneath in (A) horizons that amount is respectively 30 to 33% and deeper inccreases to 38-41%. This difference shows that in the upper horizons the organic matter is more stronger bounded to the soil mineral part. In clayey chernozems (karasoluk) the extractable amount of humic substances is higher meaning complex bound with mineral part. Humic acids amount is 21-23% in the upper (A) horizons in the first soil determining it as such with low degree of humification, which gradually increases to 32.14% at the (AB) horizon at the depth 70-85 cm, determining as such with medium degree of humification. In clayey chernozems (karasoluk) this amount is higher 30-32% in the upper part meaning better conditions for humification and lesser mineralization. Both soils in (A) horizons to the depth of 40 cm show ratio $C_{ha}/C_{fa}$ about 2.5-5.0 determines the humic type of humus, according to Orlov [8]. Sharp change of this ratio in the first soil to 1.23 determines fulvic-humic type at the depth 84-105 cm in (AB) horizon, due to the evidence of slight increasing of fulvic acids from 6-8% to 18% at this depth. In the clayey chernozems the amount of 19% fulvic acids occurs only in Ck horizon (85-110 cm). Both soils perform 100% humic acids Ca-complexed, forming insoluble microstructure fragments. The unexrtactable organic carbon of 55-72% is bound strongly not only as organic-clay compounds in both soils but as well as $CaCO_3$ humate mixed sediments.

Both soils are with high colloidal reactivity $T_{8.2}$=44 (+)cmol.kg$^{-1}$ of soil in the upper horizons 0-40 cm. There is no differentiation of adsorption structures in the profiles.

The share of strongly acidic ($T_{CA}$) ion-exchange positions is also the same 80-88% at the depth which confirmed the absence of clay minerals with high isomorphic substitution. The base saturation is high, and values of exch. $H_{8.2}$=3.0-9.4% of $T_{8.2}$ indicates absence of acidification.

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# Study on barrier and transport properties of humic substances towards ionic compounds

J. SMILEK [(a)*], P. SEDLACEK [(a)], M. KALINA [(a)], M. KLUCAKOVA [(a)]

(a) Materials Research Centre, Faculty of Chemistry, Brno University of Technology, Czech Republic
* E-mail: smilek@fch.vut.cz

**Keywords:** humic acids, hydrogels, transport properties, interactions, organic dyes

**Abstract.** The immobilizing, barrier and transport properties of different humic substances (selectively blocking carboxylic functional groups) with ionic compounds (organic dyes) were studied by innovative diffusion cell techniques in combination with advanced spectroscopic techniques. The interactivity and barrier properties of humic substances with ionic substances were studied in supporting hydrogel media (non-reactive hydrogel), which can provide us more useful information about the immobilizing properties of humic substances in contrast with sorption batch experiments. The diffusion cell techniques can be used as universal reactive-mapping tool for different humic substances immobilizing various types of compounds (e.g. pesticides, surfactants, etc.).

## Introduction

Humic substances are one of the most important parts of soil organic matter (SOM) [1] and play irepreaceble role in binding and especially immobilizing of common pollutants (e.g. heavy, metal ions [2, 3], organic dyes [4, 5], surfactants [6], etc.). The complex structure of humic substances with enormous diversity of functional groups predict humic substances as universal binding agent which can be beneficial for e.g. self-cleaning of soil.

It is generally accepted that the positive affinity of humic substances towards ionic compounds is mainly given by the presence of polar structural moieties (carboxylic, phenolic and alcoholic functional groups). On the other, the affinity of humic substances towards non-polar compounds (e.g. atrazine [7] or PCB [8]) is often correlated with aromaticity and the content of aliphatic groups. The reactivity and immobilizing properties of humic substances are often studied by classical batch sorption experiments. Nevertheless, this approach does not address the real conditions of humic substances. Therefore new experimental approach to study on reactivity and barrier properties of humic substances has been developed. This alternative methodology has been detailed described in our previous publications [9, 10]. Humic substances are homogenously distributed in supporting hydrogel matrix (non-reactive thermoreversible polysaccharide – agarose) and then the transport of suitable solute (e.g. organic dyes, heavy metal ions) is realized. From the evaluation of experimental data, fundamental diffusion parameters such as effective diffusion coefficients, lag times and sorption capacities of hydrogel can be calculated and the barrier properties of humic substances based on these properties can be determined.

One of the most important parts of this research is the clarification of the influence of carboxylic functional groups on the immobilizing properties of humic substances. To question the role of different structural motifs of humic substances in the binding, selective modification of humic substances will be also utilized. The methylation of humic substances can in consequence help to verify the influence and contribution of these –COOH functional groups on overall reactivity of humic substances towards different ionic compound. The contribution of reactive carboxylic functional groups of HS on the interactions with ionic species will be studied mainly by comparison of the reactivity of original HS and their selectively modified equivalents (selectively methylated carboxylic groups) using transport experiments (diffusion).

## Materials and methods

### Modification of humic substances

Methylated humic substances were prepared from the native form (IHSS standard Leonardite humic acids 1S104H) using trimethylsilyl-diazomethane as a methylation agent and the alkylation procedure is described in our previous work [11].

### Preparation of agarose hydrogels

The diffusion experiments were realized in supporting hydrogel matrix based on linear polysaccharide agarose (1 wt. %) via thermoreversible processes. The hydrogel's network is formed by agarose chains which are interpenetrated by molecules of humic substances. The suspension of agarose and humic substances is heated up to 85°C. The hot solution of agarose with/without humic acids was poured into the pre-heated PTFE mould and the glass slides (also pre-heated) were placed on the opposite sides of mould. The mixture of agarose with humic acids gradually solidified into the cylindrical hydrogel plate sample (40 mm in diameter and 5 mm thick). This hydrogel sample (1 wt. % of agarose with/without addition of humic substances – max. 0.01 wt. %) was used in diffusion cells experiments.

### Diffusion cells experiments

The hydrogel in PTFE mould was placed between two chambers of diffusion cell. One chamber of diffusion cell was filled by 0.01 $g \cdot dm^{-3}$ of proper solute (organic dyes) and the second chamber was filled with deionized water. Both chambers of diffusion cell were filled by 60 $cm^3$ of solutions simultaneously. The change of

concentration of diffusion probe was determined by ultraviolet-visible fibre spectrometer USB 2000+ (Ocean Optics, Inc.) in the receiving part of diffusion cell as the time function. The ultraviolet-visible spectra were collected continuously at given time intervals (30 minutes). After the termination of the diffusion experiments, the absorbance in source part of diffusion cell was measured. The water-jacketed side-by-side diffusion cell purchased from Permegear Inc. Rhodamine 6G (positively charged organic dye) was chosen as the proper ionic solute.

**Results and discussion**

The rate of interaction between humic substances and organic dye was determined by fundamental diffusion parameters (ratio of effective diffusion coefficients (Figure 1) and increase in break-through time (lag time on Figure 2)).

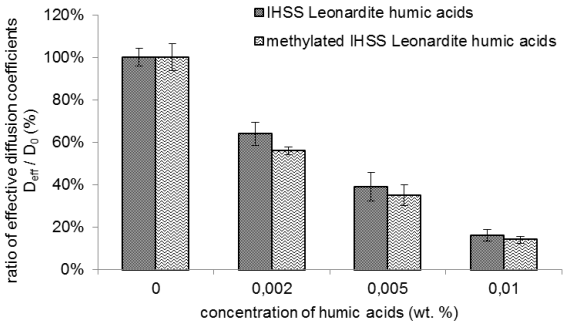



**Figure 1**: Decrease of effection diffusion coefficients of organic dye as a function of humic substances concentration in 1 wt. % agarose hydrogels ($D_0$ – effective diffusion coefficients in pure 1 et. % agarose).

It is obvious, that the presence of humic acids in the agarose hydrogels decrease the diffusion of chosen organic dye (effective diffusion coefficients are decreasing as a function of humic substances in hydrogel). This decrease is caused by positive affinity (interactions) of humic substances towards oppositely charged organic dye. The influence of methylation (selectively blocking of carboxylic functional groups) is quite unexpected. According to literature, the positive affinity of humic substances towards ionic compounds is given mainly by presence of carboxylic functional groups, therefore, it is expected that the effective diffusion coefficients (the rate of diffusion) will not be rapidly decreasing in comparison with native humic substances (non-modified). According to our results (Figure 1), the absolute values of effection diffusion coefficients are lower for methylated humic substances. It means, that these methylated humic substances slow down the diffusion of organic dye in comparison with their native equivalents. It could be given by structural changes of humic acids caused by methylation [12] or the presence of carboxylic functional groups is not so essential as indicated.

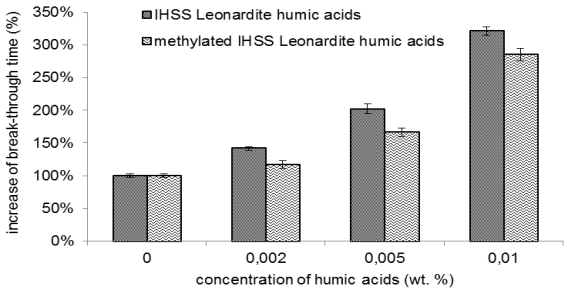



**Figure 2**: Percentage increase of break-through time as a function of humic substances concentration in 1 wt. % agarose hydrogels (related to pure 1 wt. % agarose hydrogel – 100 %).

On the other hand, the increase of break-through time (time needed for penetration of organic dye through agarose hydrogel with/without addition of humic substances) is higher for non-modified (native) humic substances (Figure 2). This finding is in contrast with effective diffusion coefficients dependence (Figure 1).

In summary, diffusion cells technique can be used as useful innovative techniques for study on immobilizing and barrier properties of different humic substances (e.g. origin of humic substances, modification, acidity, etc.) towards to ionic (e.g. organic dyes, heavy metal ions), non-polar (e.g. pesticides, PCB) as well as amphiphilic (e.g. surface active agents) compounds.

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# Maritsa East lignite humus matter study through AP-TPR-GC/MS

M. STEFANOVA [(a)*], S. P. MARINOV [(a)], J. CZECH [(b)], R. CARLEER [(b)], J. YPERMAN [(b)]

(a) Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
(b) Research group of Applied and Analytical Chemistry, CMK, Hasselt University, 3590 Diepenbeek, Belgium
* E-mail: maya@orgchm.bas.bg

**Keywords:** lignite humic acids, humovitrain, reductive pyrolysis

**Abstract.** Atmosphere Pressure–Temperature Programmed Reduction (AP-TPR) coupled "off-line" with thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS) was used to specify qualitatively/quantitatively organic components in volatiles from pyrolysis of lignite humic acids (HA) and humovitrain (HV). Determined amounts in flue gases were: HA– 55.9 mg/g$C_{org}$, HV– 13.1 mg/g$C_{org}$. Alkyl benzenes were dominant present in HA pyrogram, 46.3 rel. %, while methoxyphenols were highly abundant in HV volatiles (26.4 rel.%). The low samples maturity is reflected in high amount of phenols (~ 25 rel.%) and products of carbohydrates decomposition (<1%).

## Introduction

Bulgarian lignites are the main source for energy production of the country. During their exploration, incineration and storage of waste products from mining and combustion serious environmental problems can arise. One possibility for gradual reduction of lignites energy use and finding alternatives, is the direct application of lignites in other areas of the economy, i.e. production of humic acids, adsorbents, soil conditioners, etc. For a successful implementation it is necessary to study the organic matter compositions of the Maritsa East lignite humic acids and their parent material.

## Materials and methods

Two samples from Maritsa East lignites (Ro, %, < 0.2), including humic acids (HA) and humovitrain lithotype (HV) (Table 1) were studied. The last one is a macroscopically recognizable organic constituent of coal considered as the most representative on the coal organic matter composition. HA preparation was described in [1].

**Table 1.** Samples characteristics

| Characteristic | | HA | HV |
|---|---|---|---|
| Proximate analysis, wt. % | $Ash^{ar}$ | 4.0 | 9.2 |
| | $Moisture^{ar}$ | 6.5 | 6.0 |
| | $VM^{ar}$ | 38.4 | 52.8 |
| Ultimate analysis, wt.%$^{daf}$ | C | 58.5 | 65.8 |
| | H | 5.4 | 5.5 |
| | N | 1.3 | 1.0 |
| | S | 3.3 | 4.0 |
| | $O^{diff}$ | 31.5 | 23.7 |

$^{ar}$-as received
$^{daf}$-dry ash free basis
$O^{diff}$=100% - (C% + H% + N% + S% + ash%)

## AP-TPR-TD-GC/MS

Atmosphere Pressure-Temperature Programmed Reduction (AP-TPR) device coupled "off-line" with thermal desorption -gas chromatography/mass spectrometry (TD-GC/MS) apparatus was used [2]. Sample (*ca:*40 mg) and 20 mg of fumed silica were placed in the quartz reactor under a 100 ml/min flow of pure $H_2$. A linear temperature program of 5°C/min from ambient temperature up to 950°C was applied. The outlet of the AP-TPR reactor was connected to a set of two ice-cooled tubes containing Tenax (Sigma-Aldrich), a porous polymer of 2,6-diphenyl-*p*-phenylene oxide, as adsorbent. The volatiles were additionally diluted by adding inert gas to the $H_2$ flow in ratio 5:1 (v/v) in order to prevent the saturation of the adsorption tube. Volatiles were trapped in two separated temperature ranges: i) 250°C-550°C for the first tube; ii) 550°C-950°C for the second one.

Tenax tubes were separately desorbed and analyzed. The TD-GC/MS instrument was used with He as carrier gas at 85 kPa at the following conditions: a) Unity thermal desorbent (Marks): primary desorption 20 min at 275ºC; Cold trap at -10ºC, heated at maximum heating rate up to 320ºC, hold time 15 min; flow path temperature 200ºC; b) Trace GC Ultra-Gas chromatograph (Thermo Instruments): capillary column 30m ZB 5-MS x 0.25 mm x 0.25 µm (Phenomenex); temperature program – 3 min at 30ºC, heated 8ºC/min to 100ºC, heated 12ºC/min to 310ºC, hold time 5 min; c) DSQ-Mass spectrometer (Thermo Instruments): EI spectra; Ionization energy – 70 eV; Scan range – *m/z* 33-480 in 0.4s. Deuterated thiophene, 3 µg, was used as a standard.

## Results and Discussion

Reductive pyrolysis produced a complex mixture of volatiles with a multitude of classes of compounds. The set firmly corresponds to the already described pyrolysate composition of lignite humic acids [3]. These compounds might be present as such in HA or, more likely, have been formed *in situ* through secondary transformation from the HA building blocks. Compounds in pyrolysate flue gases are given in Table 2.

**Table 2.** Compounds in pyrolysate flue gases, in μg/g $C_{org}$

| Serie | HA | HV |
|---|---|---|
| *n*Alkenes/*n*Alkanes | 4672 | 183 |
| Alkyl benzenes | 25892 | 2480 |
| Furans/Benzofurans | 496 | 295 |
| Phenols | 11226 | 3023 |
| Methoxy phenols | 1504 | 3448 |
| PAHs | 7350 | 667 |
| Di-/Trisulphides | 1280 | 1589 |
| Thiophenes/Benzothiophenes | 2526 | 358 |
| Nitrogen containing compounds | 345 | ND |
| Sesquiterpenoids | 352 | 995 |
| Products of carbohydrates | 300 | 39 |
| Total | 55943 | 13076 |

ND: not detected

Abundance of methoxy phenols in pyrolysates gave evidence for the dominance of gymnosperm parent vegetation in the palaeomire. Methoxy phenols were the prevailing components in the volatiles of HV sample amounting up to 26.4% of the identified species (Fig.1) which is in contrast to HA (2.7%). This tremendous difference could be assigned to the samples origin. For HV, it is assumed that these materials are formed under somewhat drier surface conditions than other coal lithotypes. On burial, stagnant groundwater prevented the complete degradation of the woody plant tissue. Respectively, guaiacyl phenols from the coal-forming vegetation were preserved and were accessible for identification. The relative high presence of vinyl-containing phenols and methoxy phenols in volatiles were indicative of fresh organic matter.

Because of the high preservation potential of HV and the ability of the AP-TPR technique to track specific volatile compounds, sesquiterpenoids (Sesqui-T) could be identified (995 μg/g Corg, Table 2). The main species registered in HV sesqui-T were dihydrocurcumene, cedrane, cuparene, cadina-1,3,5-triene, cadalene, etc. The same Sesqui-T were identified in HA volatiles but in lower amounts, 352 μg/g $C_{org}$ (Table 2).

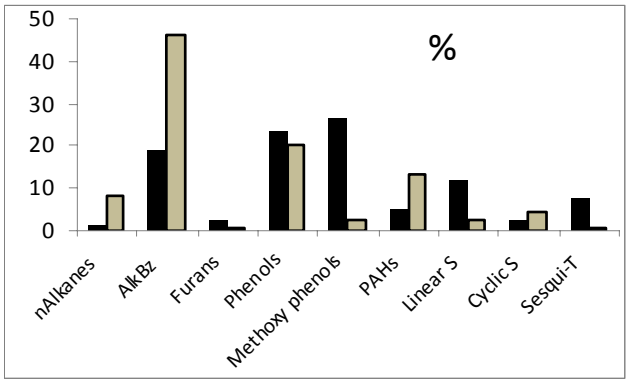



**Figure 1.** Relative compound classes distribution in pyrolysates, in % (black - HV; grey - HA).

Polysaccharides, representing undecomposed plant carbohydrates, can be converted to different furanic structures on prolonged hydrolysis. Furans and furaldehydes are considered as typical products of carbohydrates pyrolysis [4]. Different amounts of 2-cyclopentenones, $C_nH_{2n-4}O$, $C_5$-$C_7$; furandione, 4-methyl-5H-furan-2-one; 2-cyclopentene-1,4-dione, $C_5H_4O_2$, and furaldehyde, $C_5H_4O_2$, were registered (0.3-0.5%). Carbonyl products formed during cellulose pyrolysis are tracked as carbohydrate markers in flue gases. The occurrence of furans, furaldehydes and anhydrosugars in humic matter proves that hardly biodegraded polysaccharides are still susceptible to identification by AP-TPR.

The structural study by pyrolytic method of Miocene-aged HAs in comparison with HV lithotype of the same parent lignite has revealed some peculiarities related to coal-forming palaeovegetation and microbial activity in the palaeomire. Pyrolysis denoted similarities in the pyrolysate flue gases composition, i.e. volatiles dominated by aromatics, and certain peculiarities, i.e. relative amounts of aliphatics, PAHs and oxygen-containing compounds.

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# The existence and importance of humic substances and of humin

R. S. SWIFT [(a)*], M. H. B. HAYES [(b)]

(a) QAFFI, University of Queensland, Brisbane, Australia
(b) Chemical Sciences, University of Limerick, Ireland
* Email: r.swift@uq.edu.au

**Keywords:** Humic substances, organic matter fractions, decomposition models and processes

**Abstract.** The validity and the very existence of Humic Substances (HSs) have been questioned and a 'Soil Continuum Model" (SCM) proposed to describe the composition of soil organic matter (SOM)[1]. We disagree with these concepts. The SCM focuses on the access to SOM of decomposer organisms and the protection against decomposition provided by soil clays and regards SOM as a continuum from intact plant material to highly oxidised carbon in 'carboxylic acids'. In our view, SOM is composed of a wide range of discrete materials with different pathways and rates of decomposition; hence it is inappropriate to refer to it as a 'continuum'. In their paper, Lehmann and Kleber, demonstrate a very poor understanding of the composition of SOM and HSs and their degrees of resistance to biological transformations.

Lehmann and Kleber [1] have challenged well-established views and concepts about soil organic matter (SOM). They view "soil organic matter as a continuum spanning the full range from intact plant material to highly oxidized carbon in carboxylic acids". Their Soil Continuum Model (SCM) focuses on the ability of decomposer organisms to access soil organic matter (SOM) and on the protection of organic matter (OM) from decomposition provided by soil minerals. They regard the SCM as "a continuum of organic fragments (that) is continuously processed by a decomposer community from large plant and animal residues towards smaller molecular size. At the same time, greater oxidation of the organic materials increases solubility in water as well as the opportunity for protection against further decomposition through greater reactivity towards mineral surfaces and incorporation into aggregates". They state that the continuum "represents robust science and will facilitate the way we communicate between disciplines and with the public". This continuum does not take account of transformation products that have some innate persistence, other than that provided by protection from soil minerals. We agree that naturally occurring organic materials entering the soil will decompose eventually and, as Jenkinson [2] has stated, the surface of the earth would now be covered with any material that resisted degradation. There are classes of materials that have greater resistance to degradation than others.

Lehmann and Kleberstate that "the traditional 'humification' concepts limit observations of soil organic matter to its solubility in alkaline extracts, unlike the emergent view of organic matter based on solubility in water and its accessibility to microorganisms" They consider that humic substances (HSs) extracted by alkali from soil environments are incomplete and are artefacts of the extraction process, with humic acids (HAs) and fulvic acids (FAs) having an exaggerated chemical reactivity relative to "true isolates"(meaning the whole SOM and not just the alkali-extractable fractions.) We take a different view and regard isolation and fractionation procedures as opportunities to lead to a better awareness of the nature and compositions of the components of HSs. A procedure suggested by Hayes and Swift [3] for the extraction of HSs subdivides the components of SOM into:

(1) Unaltered materials that include fresh debris and non-transformed components of older debris; &

(2) Transformed products, or *humus*, that bear no morphological resemblances to the structures from which they were derived,

These transformed components are referred to as *humified* products. They consist of humic and non-humic substances and can, therefore, be subdivided as follows:

(2a) Amorphous, brown coloured humic components differentiated on the basis of solubility properties into humic acids (HAs), fulvic acids (FAs), and humins;

(2b) Other components belonging to recognisable classes, such as polysaccharides, polypeptides, altered lignins, etc. These can be synthesized by microorganisms or can arise from modifications of similar compounds in the original debris. Such materials, components of humus, would not be regarded as HSs.

We dispute the statement that aqueous base gives rise to artefacts. Swift and Posner [4] observed some oxidation when HAs were stored in alkaline solution for 30 days in an atmosphere of $O_2$, but none when under $N_2$. No evidence of oxidation of isolates was observed when HAs and FAs were processed by the resin procedure of the IHSS (involving base) from natural organic matter (NOM) obtained by reverse osmosis from river water [5].

There are techniques available that will extract all of the humified or microbially transformed or synthesized SOM. We have been involved in studies that, in exhaustive extractions, have isolated HSs at pH 7, pH 10.6, 0.1 M NaOH, and 0.1 M NaOH made 6 M with respect to urea [6, 7]. The extracts were further processed by the (poly)methylmethacrylate (XAD-8 or DAX-8) and styrene divinylbenzene (XAD-4) resins in tandem process (adapted from Malcolm & MacCarthy [8] The resin process gave 50% more hydrophilic materials Following the base-urea extraction. washing with water to remove the base and urea, and drying, the residual materials are isolated in dimethyl sulphoxide (DMSO) containing

6% (v/v) concentrated $H_2SO_4$, and that isolate when added to water gives the humin component as a precipitate [7]. Using this approach up to 95% of the SOM was extracted.

We regard the *humification* process as the transformations to HSs and humin by soil microorganisms of the organic residues that are present in or enter the soil environment. In their considerations of the fate of organic residues, Lehmann and Kleberquote literature suggesting that "upon cell death, materials that are synthesized in the course of microbial anabolism are released into the soil, where they are subject to further degradation. Throughout this process, these materials remain on an energetic downhill trajectory, as opposed to the hypothetical 'humic substances' whose 'secondary synthesis' would require energy investments. They have not taken account of the excellent work by Martin and his colleagues [9], which has shown, convincingly in our view, that soil fungi intracellularly synthesize products with the properties of HSs, and produce extracellular enzymes that catalyse the formation of HSs from phenolic precursors.

Lehmann and Kleber favour the concept that the humic extracts are composed of self-assembled aggregates rather than macromolecular substances. They base evidence for this on fractionation of organic amended HAs using HPSEC. They ignored evidence from gel filtration that fractionated HAs into discrete decreasing size macromolecular units. As the isolate sizes decreased the saccharide contents decreased and aromaticity increased [10].

There is a strong link between SOM and atmospheric $CO_2$and so it is important to model the behaviour of soil carbon in order to predict future changes in SOM levels. The RothC, CENTURY, and APSIM models are widely used. All of these models have similar constructs in that they utilise a combination of discrete pools of SOM. Each of these pools decays and turns over at different rates following first order kinetics, brought about principally through the actions of soil microorganisms. It has proved to be impossible to fit the observed data from many sites using a single pool with a single value for the rate of decomposition and first order kinetics. Yet, paradoxically, these are essentially the conditions which would appear to apply using the SCM.

Lehmann and Kleber [1] have stated that "to date 'humic substances' as extracted by alkali constitute the organic workhorse that is investigated by the community of aquatic chemists**.** They "argue that the persistence and movement of terrestrial derived organic carbon compounds entering aquatic ecosystems will rely on their protection by minerals, solubility in water and microbial degradation rather than primarily their chemical properties." We do not consider that alkali extracted HSs is the 'workhorse' for investigations by aquatic scientists because, since isolations from the Suwannee River in SE Georgia (USA) began nearly 40 years ago [11] numerous samples of HAs and FAs isolated by the IHSS resin procedure, as well as NOM (isolated by the RO process [5]), and HAs and FAs from it, have been distributed by the IHSS to the worldwide community of scientists. These samples have resulted in 781 published papers between 1980 and 2016, resulting in 30,162 citations (Web of Science, TOPIC: (Suwannee AND (fulvic OR humic OR natural organic matter (NOM), February 27, 2017)).

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# Humic substances and water-resistant macro-aggregates

E. TOBIAŠOVÁ [(a)*], G. BARANČÍKOVÁ [(b)], E. GÖMÖRYOVÁ [(c)], Š. KOCO [(b,d)], J. HALAS [(b)], R. SKALSKÝ [(b)], J. MAKOVNÍKOVÁ [(b)]

(a) Department of Soil Science, Slovak University of Agriculture in Nitra, 94976 Nitra, Slovakia
(b) National Agricultural and Food Centre – Soil Science and Conservation Research Institute, 82713 Bratislava, Slovakia
(c) Department of Natural Environment, Technical University in Zvolen, 96053 Zvolen, Slovakia
(d) Department of Geography and Applied Geoinformatics, 08116 Prešov, Slovakia
* E-mail: erika.tobiasova@uniag.sk

**Keywords:** humic acids, fulvic acids, soil macro-aggregates, soil organic carbon

**Abstract.** Humic acids (HA) and fulvic acids (FA) have a different influence on the proportion of fractions of water-resistant macro-aggregates (WSA). The higher the proportion of humic substances (HSs) in the soil is, the higher is the content of smaller (<1 mm) WSA and simultaneously lower is the content of larger (>3 mm) WSA. FA, mainly the fraction of free aggressive FA, had more marked influence on the contents of total organic carbon, labile carbon and non-labile carbon. The higher the content of clay was, the higher was a quality of HSs.

## Introduction

The specific influence of organic matter on soil aggregation is closely related to various fractions of organic matter, mainly polysaccharides, humic acids (HA) and fulvic acids (FA) [1]. Humic substances (HSs) are a key factor participating on the formation of stable soil aggregates, and they enter into mutual bonds with various soil components, therefore their fractions influence the formation of soil aggregates in a different way. HSs form physical-chemical bonds with clay soil particles [2] or with different cations [3] and contribute to the formation of aggregates of varying size and simultaneously influence the distribution of carbon and their fractions in these aggregates [4]. Humified organic material can promote permanent improvement of aggregate stability [5], but the functions of HSs are closely related to their quantity and quality in the soil. The objectives of this study were as follows: (i) to assess the influence of HSs and their fractions in the soil on the proportions of fractions of water-resistant macro-aggregates (WSA), (ii) to determine the differences between the amounts of carbon in WSA with dependence on the quantity and quality of HSs in the soil.

## Materials and methods

The experiment included 3 soils (Haplic Chernozem, Eutric Cambisol, Luvic Stagnosol), each in three types of ecosystems (forest, meadow, and agro-ecosystems). The soil samples of different localities were collected to a depth of 30 cm, and dried in a constant laboratory temperature of 25±2°C. The soil samples for determination of chemical properties were ground. To determine the fractions of soil aggregates, the soil samples were divided by the sieve (dry and wet sieve) to size fractions of the net aggregates [6]. The particle size distribution was determined according to the pipette method [7]. In the soil, the total organic carbon (TOC) by wet combustion [8], labile carbon ($C_L$) by $KMnO_4$ oxidation [9] and fractions of HSs according to the method of Ponomareva and Plotnikova [10] was determined.

## Results and discussion

HSs had influence not only on the proportion of macro-aggregates but also on the carbon content and its stability in these aggregates. The higher the proportion of HSs in the soil was, the lower was the proportion of larger (>5 and 3 – 5 mm) fractions of WSA ($r = -0.516$; $P < 0.05$ and $r = -0.692$; $P < 0.01$) and simultaneously higher was the proportion of smaller (0.25 – 0.5 and 0.5 – 1 mm) WSA ($r = 0.495$; $P < 0.05$ and $r = 0.627$; $P < 0.01$). Large aggregates are richer in a labile organic matter, while smaller contain mainly stabilized organic substances [11]. These larger (>3 mm) and smaller (<1 mm) fractions of WSA had different proportion also in the studied ecosystems, however in the case of optimal size (1 – 3 mm) of WSA, there were not observed any differences between the ecosystems. However, the most marked differences were observed in the case of WSA of the 0.5 – 1 mm size fraction (Fig. 1), which seems to be an important indicator of deterioration of soil structure [12].

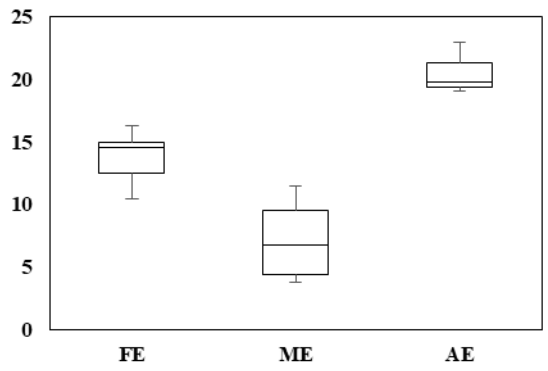



**Figure 1.** Content of water-resistant macro-aggregates (%) of the 0.5-1 mm fraction in FE –forest ecosystem, ME – meadow ecosystem, AE – agro-ecosystem.

In the case of TOC, the FA had more marked influence on its content in the WSA than HA (Fig. 2) and in the case of $C_L$, they were mainly free aggressive FA (FA1a) (Fig. 3).

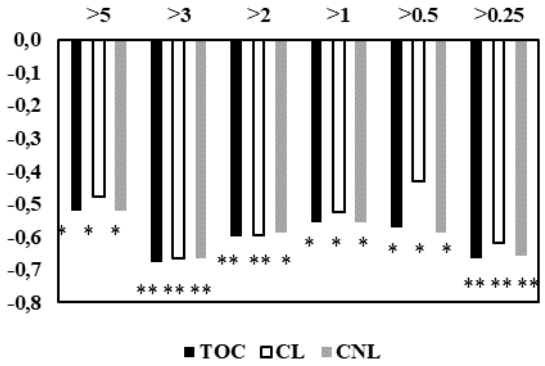



**Figure 2.** Correlation between the FA and carbon contents; TOC – total organic carbon, $C_L$ – labile carbon, $C_{NL}$ – non-labile carbon in the fractions of water-resistant macro-aggregates (size in mm).

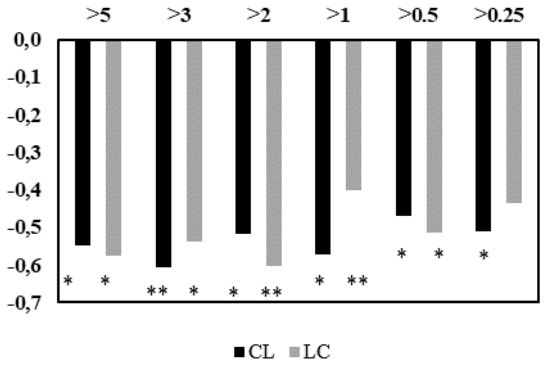



**Figure 3.** Correlation between labile carbon ($C_L$) and lability of carbon ($L_C$) and fractions of water-resistant macro-aggregates (size in mm).

Higher proportion of FA1a meant a total deterioration of soil structure. The higher the content of this fraction in the soil was, the lower was the stability of soil aggregates evaluated according to index of soil aggregate stability ($r = -0.549$; $P < 0.05$), and simultaneously the higher was the index of soil crusting ($r = 0.508$; $P < 0.05$). The higher the content of FA in the soil was, the lower were the contents of TOC, $C_L$ and $C_{NL}$ in the fractions of WSA, especially the higher the content of FA1a in the soil was, the lower was the content of $C_L$ in the WSA and simultaneously was also a lower lability of carbon. HA were mainly in the correlation with the proportion of TOC, $C_L$, $C_{NL}$ in the fractions of WSA in the coarse-grained soils and the FA were in the correlation with their amounts in the fine-grained soils [4]. The highest influence on the quantity and quality of HSs in the soil had the fraction of clay. The higher the clay content was, the higher was the content of HA ($r = 0.694$; $P < 0.01$) and the lower was the content of FA ($r = -0.609$; $P < 0.01$). The influence of clay was significant also on the quality of HSs. The higher the content of clay was, the more stabilized were HS ($Q_{HS}$; $r = -0.620$; $P < 0.01$) and also HA ($Q_{HA}$; $r = -0.535$; $P < 0.05$). The stability of humic substances was in the correlation with the proportion of $C_{NL}$ in WSA.

**Table 1.** Correlations between optical parameter of humus substances ($Q_{HS}$) and the contents of non-labile carbon ($C_{NL}$) in the fractions of water-resistant macro-aggregates

| $C_{NL}$ in WSA | $Q_{HS}$ |
|---|---|
| >5 mm | -0.483* |
| 3 – 5 mm | -0.572* |
| 2 – 3 mm | -0.492* |
| 1 – 2 mm | -0.441* |
| 0.5 – 1 mm | -0.471* |
| 0.25 – 0.5 mm | -0.567* |

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# Electrophoresis of soil humic acids – what are electrophoretic zones presents itself?

O. TRUBETSKOJ [(a)] *, O. TRUBETSKAYA [(b)]

(a) Institute of Basic Biological Problems, Russian Academy of Sciences,
142290 Pushchino, Moscow region, Russia
(b) Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences,
142290 Pushchino, Moscow region, Russia.
* E-mail: olegi03@yahoo.com

**Keywords:** soil HAs, fractionation by tandem SEC-PAGE, electrophoretic zones, structural features of soil humic acids

**Abstract.** Different soil HAs were fractionated by tandem size exclusion chromatography - polyacrylamide gel electrophoresis (SEC-PAGE) and the resulting electrophoretic fractions were studied using modern physical-chemical methods. On the basis of different analytical tools studies we can conclude that SEC-PAGE setup allowed for the first time separated soil HAs into fractions predominantly composed of aromatic or aliphatic compounds. Moreover data showed that soil HAs electrophoretic fractions consist mainly of primary humic structures compounds and highly likely that similar marked fractions have similar structural organization. Findings are consistent with the hypothesis that the structure of soil HAs arises through supramolecular assembly of different compounds.

## Introduction

The presence of negative charge resulting from the ionization of functional groups is a fundamental property of soil humic acids (HA) and electrophoretic techniques should be applicable for their investigation. One of the electrophoretic approaches, which have been successfully used in the last 40 years, is polyacrylamide gel electrophoresis (PAGE) where a few electrophoretic zones (EZ) were ordinarily observed. In biochemistry, a distinct EZ in PAG matrix ordinary means individual substances with a certain molecular properties. On the other hand, by using practically all known analytical techniques a huge number of individual molecular structural components were always detected in soil HAs and they are unlikely could represent only a few distinct EZ of soil HA. For this reason, the principal question concerning to EZ of soil HA is - what they are present itself?

## Materials and Methods

We have developed the new method of PAGE in the presence of denaturating agents (7M urea and 0.1% SDS) and used it in the combination with size exclusion chromatography (SEC). This procedure is called tandem SEC-PAGE [1-2].

## Results and Discussion

The PAGE of bulk HAs, isolated from Chernozem (Russia), Ranker (Spain) and Elliott soil (USA, IHSS standard 1S102H) is shown in Figure 1. All HAs gave several zones (fractions) with distinct EM. Fraction that did not move into the polyacry amide gel was named A, fraction in the mid part of the gel was named B, and all the fractions on the bottom of the gel were combined into the single fraction named C+D. Preparative quantities of electrophoretic fractions for their further studies were obtained by tandem SEC-PAGE. The novelty and advantage of this tandem rests primarily the presence of urea, which added at a level of 7M both in SEC and PAGE assist in the rapture of hydrogen bonds and prevents interaction between the fractionated humic material and the immobile phase. This is accompanied by the disaggregation of HAs, a process, we believe, is primarily related to the disruption of hydrogen bonds. The proposed procedure allows the separation of primary disaggregated structural humic components, thereby solving some of the key problems occurring in

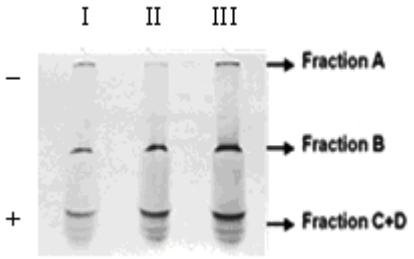



**Figure 1.** PAGE of ranker HA (I), chernozem HA(II) and IHSS soil HA standard 1S102H (III).

the fractionation of HS, e.g., irreversible/reversible chromatographic column adsorption. Using PAGE in combination with SEC makes possible to obtain preparative amounts of fractions differing in both EM and molecular size (MS). It should be stressed that only after application of tandem SEC-PAGE for fractionation of HS thorough study of a physical–chemical properties of soil HAs electrophoretic fractions was possible. We followed systematically this method over the last twenty years with different tools and it allowed us to obtain soil HA fractions independently from soil sources with clear differences in terms of:

1) electrophoretic mobility [ref. 1, 2]
2) molecular size [ref. 2]
3) UV-vis absorbtion spectra [ref. 3]
4) amino acid weight content [ref. 4]
5) plant and microbial long chain fatty acids (>C22) distribution [ref. 6, 9, 12]

6) aromatic/aliphatic components [ref. 10, 12]
7) hydrophobic components [ref. 12-14]
8) fluorescence intensity [ref. 5, 7, 11, 16]
9) photosensitizing activity [ref. 5, 8]
10) phytohormone-like activity [ref. 15]

On the basis of different analytical tools studies we can conclude that SEC-PAGE setup allowed for the first time separated soil HA into fractions predominantly composed of aromatic or aliphatic compounds. These soil HAs electrophoretic fractions consist mainly of primary humic structures compounds and highly likely that similar marked fractions have similar structural organization. The interactions between fractions are based mainly on the hydrogen and hydrophobic bonds.

Obtaining of these fractions with different chemical structure by itself does not lead to structure of soil HAs however, it is clear that such data needs to be interpreted by considering the precursors of HA molecules and their chemical organization. Considering that high molecular size fraction A consisted mainly of aliphatic components (e.g. long chain fatty acids and amino acids), portions of which (e.g. long chain fatty acids) possess amphiphylic character, it is reasonable to suggest that these compounds are capable to organize micelles or vesicular structures, and free amino acids or in form of short peptides and proteins physically encapsulated within the structure of fraction A and so preserved from their further degradation. Findings are consistent with the hypothesis that the structure of soil HAs arises through supramolecular assembly of different compounds.

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# Main chemical and diagnostic features of organic matter in soils from green urban areas of Sofia (Bulgaria)

V. TSOLOVA [(a)*], P. TOMOV [(a)], G. PETKOVA [(b)], I. NIKOVA [(a)]

(a) Soil Chemistry Department, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant Protection, 1080 Sofia, Bulgaria
(b) Soil Microbiology Department, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant Protection, 1080 Sofia, Bulgaria
* E-mail: venera_tsolova@abv.bg

**Keywords:** organic carbon, Mull humus, C:N, optical density, Technosols, Luvisols

**Abstract.** Three types of soils located in recreational areas of the Sofia city (Bulgaria) were studied to establish the basic humus features (chemical and diagnostic): overlapped (buried as a result of urbanization) soils (1); newly formed soils (2) and natural soils with unchanged profile (3). The intensive accumulation of organic carbon in modified soils resulted in a formation of the medium humous (2.59% in 2) and rich humous soils (5.52% in 1) regardless of their short genesis. The slightly alkaline pH and the carbonate matrix in both soils favour the formation of Mull (1) and Rhizomull (2) type of humus, wherein the content of very strongly condensed ($E_4/E_6$ from 3.50 to 3.87), respectively, hydrophobic and slightly mobile Ca-humates dominated. The natural soils are medium humous soils (2.86%) with more mobile Mull-type humus wherein the mobile fraction amounts to 18%. All humus systems are very old, deeply transformed and rich in N structures, that might suppress the biomes development accounting the C:N ratios (2.1-4.4).

## Introduction

Humic substances in soils play a vital role in sustaining life on the Earth. Plant ecosystems grown on humus soils are resistant to disease and climatic stress and therefore more productive. They also have a superior quality. Humic substances accomplish many of soil functions: nutrient storage and transportation, structure-formation, detoxification, source of energy, precursors and maybe the most important - the ability to hold water.

Having in mind the role of cities in providing a favourable living environment for humans, it is important to study the soils in urban areas that are subject to various in extent and type anthropogenic impacts. The scarce knowledge of urban soils evokes the interest in studying their humus status as a guarantor of soil health.

This article aims to present the main chemical and diagnostic features of the humus systems (morphological status, content, speciation and profile patterns) in soils distributed in the residential areas with recreational significance of the Sofia city (Bulgaria).

## Materials and methods

**Site description and field study.** Three soil profiles were localized in the direction of the increasing gradient of urbanization in the Sofia city:

- Overlapped moderately leached Smolnitsa, loamic (profile 1). This soil is buried as a result of urbanization and according to the WRB classification [3] is Urbic Technosol (Eutric, Loamic, Humic, Transportic) over Pellic Vertisol (Chernic, Endocalcaric). It is located in recreational green area provided for a child's playground of the Mladost residential district.
- Anthropogenic soil, moderately stony (15% coarse surface fragments content) - Urbic Technosol (Amphyskeletic, Calcaric, Mollic, Transportic). This is abandoned soil formed by deposition of terrestrial carbonate materials and municipal waste in the periphery of the city (profile 2).
- Moderately leached Cinnamon forest soils, loamic, slightly eroded - Chromic Endocalcic Luvisol (Clayic, Differentic, Humic, Profondic)). Virgin soil in the foot of the Vitosha Mountain, not affected by the excavation activities carried out nearby (profile 3).

The plant ecosystems are compiled of meadow-pasture vegetation wherein cereals dominated.

Field study and sampling is carried out by a trial pit technique [1].

**Chemical analyses.** The content of the extractable humus fractions was determined using the Kononova-Belchikova method [4] in four extracts at soil: solution ratio 1:20:

- Total carbon - after oxidation of the soil sample with 0.4 N $K_2Cr_2O_7$ and concentrated $H_2SO_4$ in a ratio 1:1 at 120 $^0$C for 45 min. in the presence of $Ag_2SO_4$ followed by a titration with 0.2 N Mohr's salt. Humus content is calculated by multiplication with the coefficient 1.724.
- Content of humic (HA) and fulvic (FA) acids – dissolved in a mixed solution of 0.1 M $Na_4P_2O_7$ and 0.1 M NaOH, and separated by 0.5 M $H_2SO_4$ as an acidifying agent;
- Content of free or linked to $R_2O_3$ humic and fulvic acids – extracted with 0.1 M NaOH (designated as a mobile HA and FA on the figures);
- Content of the low molecular (aggressive) fraction of fulvic acids – in 0.05 M $H_2SO_4$ extract.

Optical hallmarks ($E_4/E_6$) are determined in humic acid fraction as a ratio of its optical density at λ 465 and 665 nm.

Total nitrogen content is determined by Kjeldahl method [2].

**Results and Discussion**

A shallow Ah horizon with a very high humus content (5.52%) was formed in the Overlapped (Buried) Smolnitsa (profile 1, fig. 1), despite the soil recent creation (about 45 years ago). The humus is of Mull type with a strong predominance of humic acids ($C_{HA}/C_{FA}$ > 2,0) established along entire depth. Humic acids are strongly condensed ($E_4/E_6$ = 3.87), respectively hydrophobic and slightly mobile polymers. They are strongly bound to the mineral matrix due to the high content of Ca-humates. The low molecular (aggressive) fraction of fulvic acids is also present in downward concentrations (from 0,8 to 0,4 g/kg). The low ratios of C:N (2.8-2.1) in this epipedon is an indicator of high enrichment of hydrocarbons with N. These N-dressed compounds are a source of high ammonia emissions which may provoke the soil toxicity.

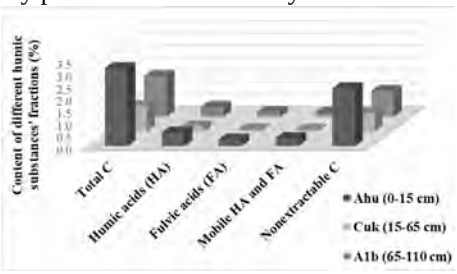



**Figure 1.** Content of main humus fractions in profile 1.

Deeper but less humous epipedon (2.59% in Ah) is formed in the medium humous Anthropogenic soils (profile 2, Fig. 2). The basic features of surface horizon are: homogeneous system formed by soil-biomes interactions with well humificated organic matter ($C_{HA}$: $C_{total}$ x 100, in % = 17.3%); the Rhizomull type of humus, wherein the highly condensed and stable humic acids ($C_4/E_6$ = 3.50) are absolutely prevalent ($C_{HA}/C_{FA}$ = 3.25).

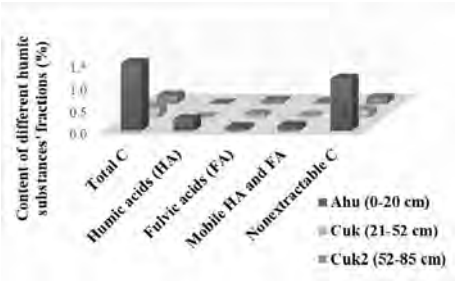



**Figure 2.** Content of main humus fractions in profile 2.

The content of organic carbon sharply drops (up to 1.8 g/kg) in subsoil, and DOM is only composed of FA (up to 33% of total C). The degree of OC enrichment with N is very high till 50 cm (C:N 4.4-3.0) and suppressing to biomes development.

The humus-accumulative horizon (Ah) of the Cinnamon forest soil (profile 3, Fig. 3) is shallow and moderately rich in organic matter (2.86% humus).

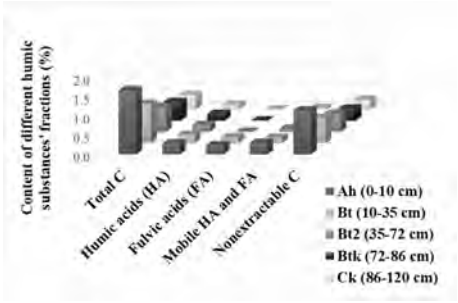



**Figure 3.** Content of main humus fractions in profile 3.

The prevalence of humic acids is the least pronounced ($C_{HA}/C_{FA}$ = 1.17) in this horizon and the degree of condensation of their aromatic nuclei - the lowest ($E_4/E_6$ = 4.08). This does not significantly change their hydrophobicity and low mobility - according to the results obtained fulvic acids predominantly compose the mobile fraction along entire soil depth. The increased content of mobile fraction evokes the naturally occurring leaching process and acidification of the pH (5.9). The interaction between the pH and the mobile fraction of humus acids, respectively fulvic acids is evidenced by the statistically significant correlation between them ($R^2$ = 0.73 and $R^2$ = 0.85 for fulvic acids).

C:N ratios (2.1-2.5) in this none affected epipedon indicates very high content of easily mineralized nitrogen known as unfavourable for soil biota. The low variation of C:N values by depth, especially in the $1^{-st}$ and $3^{-rd}$ profiles reveals the ancient age of humic substances and their stability in diverse soil environments.

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# Geospatial distribution of soil organic matter in Bulgaria. I. The Northwest region

V. TSOLOVA [(a)*], V. KRASTEVA [(b)], M. BANOV [(b)], V. KOLCHAKOV [(c)], N. MITEVA [(b)]

(a) Soil Chemistry Department, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant protection, 1080 Sofia, Bulgaria
(b) Department of Genesis, Geography and Classification of Soils, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant protection, 1080 Sofia, Bulgaria
(c) Soil Erosion Department, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant protection, 1080 Sofia, Bulgaria
* E-mail: venera_tsolova@abv.bg

**Keywords:** humus content, monitoring grid, Chernozems, Luvisols

**Abstract.** The content of soil organic matter is studied in the georeferenced points of the Bulgarian National Monitoring Network during 2011-2012. The study shows that the average humus content 2.67 ± 1.43% in the Northwest region is of medium rang. The prevailing part of the territory is occupied by medium humous soils: Calcic, Typical and Leached Chernozems, Gray and Dark grey forest soils with an average humus content 2,62%. Slightly humous soils with an average humus content of 1.64% are almost twice rarer. They are eroded varieties of Grey, Light and Dark gray forest soils, Moderately and Heavily leached Chernozems. Poor humous soils (containing < 1%), humous (4-5%) and the rich humous soils (5-6%) are the most rarely occurred in the region.

## Introduction

Soil monitoring is a modern tool for managing current degradation processes. Regarding organic carbon, it provides reliable information for preventing decline in soil fertility and limiting threats of soil function. Terrestrial ecosystems, including soils, are one of the major pools of organic carbon and accumulate ≈ 2200 Pg C. This positive balance is a result of a long-lasting evolution of terrestrial ecosystems but is fragile. Research over the recent years has shown that it can be significantly altered by human activity - deforestation, biomass burning, land use change and environmental pollution [2]. Nowadays we know that soil organic carbon is vulnerable to degradation and its content can be reduced in a short timeframe.

In view of the diverse applicability of soil monitoring data, this publication aims to present the geospatial variation of soil organic matter in Northwest region of Bulgaria generated by the pedological study of the points of the National Soil Monitoring Network.

## Materials and methods

**Description of the region.** The Northwest region of Bulgaria occupies 19 070 km² and includes 5 administrative districts (Vidin, Vratsa, Montana, Pleven and Lovech). The relief is characterized by a wide variety of forms: the Danube hilly plain with vast coastal valleys (Vidin, Archaro-Orsoiska, Dolnotsibarska and Kozloduy-Oryahovska) and loess plateau (Zlatya), the foot of the Stara Planina Mountain and the main mountain range. The distribution of soils is closely attached to the relief and also show a pronounced zonality in the North-South direction: after the azonal alluvial-meadow soils located in the Northernmost parts of the region follow the Typical and Leached Chernozems, which have formed on the loess, Gray, Light and Dark gray forest soils go after them and cover the foot of the Stara Planina Mountain followed by Brown forest and Mountain-meadow soils which are located in the upper part of the Stara Planina Mountain.

**Determination and mapping of organic carbon content.** The study of soil organic matter, displayed as humus content (HC), is carried out at the georeferenced points of the National Soil Monitoring Network with a density of 16x16 km [7]. Sampling is carried out by a trial pit technique [3] and the data on HC in the A-horizons are mapped. The total content of organic carbon was analyzed and calculated as a humus content by the modified method of Turin [4]. Software ArcGIS for Desktop 10.5 was used for mapping on organic matter distribution. World Terrain Base in a scale of ~1:10 m was used as a base map layer [1]. Polygonal layer providing administrative boundaries of the region and the Point layer containing monitoring data set on humus content overlap it (Figure 1).

## Results and Discussion

Humus content is a major qualifier in Bulgarian soil classifications and a subject to the multilateral research up to date. In view of the humus content, Bulgarian soils are assembled into several groups shown in Table 1 [4].

**Table 1.** Soil groups according to the humus content

| Soil groups | Humus content (%) |
|---|---|
| Poor humous soil | < 1 |
| Slightly humous soil | 1-2 |
| Medium humous soil | 2-4 |
| Humous soil | 4-5 |
| Rich humous soil | 5-6 |
| Amply humous soil | >6 |

Sixty nine monitoring points are located in the Northwest region, wherein the humus content ranges from 0.54 to 8.83%. The average value 2.67% ± 1.43 is estimated as a medium humus content and it's the most common value in the region [6].

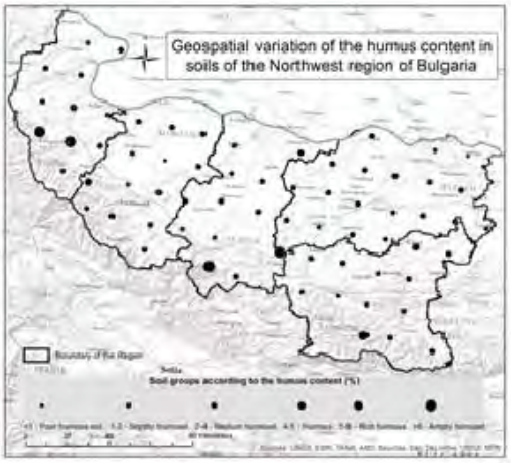



**Figure 1.** Geospatial distribution of the soil humus content in the Northwest region of Bulgaria.

The statistical analysis of data shows that Medium humous soils prevail in the region (occur in 58% of the monitoring points), and their average humus content - 2.62% is close to the average for the region (2.67%). This value also coincides with the mode around which the most values are concentrated (Figure 2). Slightly humous soil accounted for 28% of the points follow after them with an average humus content of 1.64%.

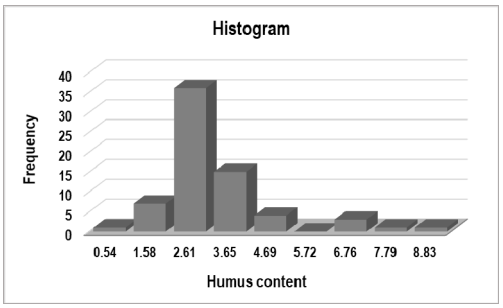



**Figure 2.** Frequency distribution of the soil humus content.

**Slightly humus soils** are spread as spots among the medium humous soils, and sometimes create typical associations with them. Their main representatives are 1) Gray forest soils, none to slightly eroded or moderately eroded. According to [5] they are: Haplic Luvisol (Epidystric, Siltic); Haplic Luvisol (Eutric, Siltic); Haplic Cambisol (Eutric, Siltic); Endoleptic Luvisol]; 2) Gray forest soils, slightly or moderately eroded, gleyic [Epigleyic Luvisol (Clayic); Endogleyic Luvisol (Siltic); Epigleyic Luvisol (Eutric, Clayic); Haplic Cambisol (Eutric, Clayic)]; 3) Light gray soils (pseudopodzilic) none to slightly eroded, gleyic [Endogleyic Planosol (Dystric, Siltic)]; 4) Dark gray forest soils, none to slightly eroded, gleyic [Endogleyic Phaeozem (Clayic)].

Several varieties of Chernozems also occur in this group, Moderately and Heavily leached Chernozems /Haplic Cambisol (Calcaric, Arenic); Haplic Cambisol (Eutric, Siltic) and Endocalcic Chernozem (Pachic, Siltic)/.

**Medium humous soils.** Chernozems with their basic subtypes calcic, typical and leached and varieties: none to slightly eroded, moderately deep dominate in this group [Endocalcic/ Epicalcic/ Bathycalcic or Calcic Chernozem; Haplic Cambisol; Haplic Regosol; Calcic Luvic Phaeozem]. Gray forest soils are predominantly none to slightly eroded as well as accumulated, gleyic. They belong to the Rs group of Luvisol usually as Haplic/ Endogleyic/ Epigleyic/ Endocalcic. Dark grey forest soils are known to slightly eroded [Bathycalcic Luvisol and Bathycalcic Phaeozem] or gleyic [Bathycalcic Bathygleyic Phaeozem and Luvic Phaeozem]. Alluvial-meadow soils are predominantly deep and belong to the: Calcic/Umbric/Haplic Fluvisol; Bathycalcic/ Epicalcic Phaeozem or Haplic Cambisol.

**Soils with humus content above 6%** are shallow and slightly developed Chernozems and rendzinas [Lithic or Rendzic Leptosols], or Brown forest soils [Haplic Leptosol and Epileptic Cambisol].

**The least organic soils** are cultivated sands from the Danube river valley, [Haplic Arenosol (Calcaric)] and Grey soils [Haplic Cambisol (Eutric, Arenic)].

**Humous soils** are moderately developed or eroded Chernozems [ Bathycalcic Chernozem (Pachic, Clayic), Haplic Regosol (Calcaric, Clayic)], or Dark Grey Forest soils, gleyic [Endocalcic Luvic Phaeozem (Pachic, Clayic)].

**Rich humous soil** is a shadow Dark mountain-forest soils [Mollic Leptosol (Eutric, Skeletic)].

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# Geospatial distribution of soil organic matter in Bulgaria. II. The North Central region

V. KRASTEVA [(a)*], M. BANOV [(a)], V. KOLCHAKOV [(b)], V. TSOLOVA [(c)], N. MITEVA [(a)]

(a) Department of Genesis, geography and classification of soils, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant protection, 1080 Sofia, Bulgaria
(b) Soil erosion Department, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant protection, 1080 Sofia, Bulgaria
(c) Soil Chemistry Department, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant protection, 1080 Sofia, Bulgaria
* E-mail: vkrasteva@abv.bg

**Keywords:** organic carbon, chernozems, monitoring, North Central region

**Abstract.** The content of soil organic matter in Bulgaria has been studied in the georeferenced points of the National Monitoring Network during 2011-2012. The 56 grid points in the North Central Region are set on the Carbonate, Typical and Leached Chernozems, Dark Gray and Gray forest soils. According to the data obtained, the average soil humus content 2.41 ± 1.49% is typical of "medium humus" soils. The medium humus soils (2-4% humus) prevail in 58.9% of the grid, 37.5% are "slightly humus soils" (1-2% humus), and 3.6% are classified as "Amply humus" soils (> 6%). Slightly humus soils are mostly eroded Gray, Light Gray and Dark Gray forest soils and Moderately and Heavily Leached Chernozems. Medium humus soils include the varieties of well developed Carbonate, Typical and Leached Chernozems, Dark Gray forest soils and Alluvial-meadow soils. "Amply humus" are Dark forest soils and a moderately eroded loamic variety of Grey forest soils.

There are no Poor humus (< 1%), Humous (4-5%) and Rich humous soils (5-6%) established in the region.

## Introduction

Soil monitoring is a systematic observation, assessment and prognosis of soil quality in a given territory over the time. It shows the results of anthropogenic activity and/or natural processes occurring in soils. The main factor and source of soil nutrients is the organic matter. Humus is a major constituent (85-90%) of the soil organic matter and is characterized by a specific nature. Individual compounds - proteins, carbohydrates, fats, lignin, amino acids, resins, tannins, organic acids and others compose the rest part (10-15%). Humus provides the most important features of the soil system, such as: structure and stability; fertility and water retention; limiting the soil erosion and the spread of pollutants, and protection of biomes.

The results presented in the article are a small part of a large-scale project related to the study and classification of soil varieties in points of the National Monitoring Network. Due to the versatility of the monitoring data and the information they provide, the purpose of this publication is to present humus content (%) in 56 monitoring points located in the North Central Region of Bulgaria.

## Materials and methods

**Description of the region.** The North Central Region occupies an area of 14 835 km$^2$ and includes 5 administrative districts (Veliko Tarnovo, Gabrovo, Razgrad, Ruse and Targovishte). The Northern border of the region is the Danube River and Southern - the ridge of the Balkan Mountain. The relief is mostly flat and hilly.

**Determination and mapping of organic matter content.** The study of soil organic matter, displayed as humus content (HC), is carried out at the georeferenced points of the National Soil Monitoring Network with a density of 16x16 km [7]. Sampling is carried out by a trial pit technique [2] and the data on HC in the A-horizons are mapped. The total content of organic carbon was analyzed and calculated as a humus content by the modified method of Turin [3]. Software ArcGIS for Desktop 10.5 was used for geochemical mapping of organic matter distribution. Map is compiled from the following layers: World Terrain Base in a scale of ~1:10 m was used as a base map layer [1], the Polygonal layer providing administrative boundaries of the region and the Point layer containing monitoring data set on humus content overlap it.

## Results and Discussion

The classification of Bulgarian soils according to the humus content is shown in Table 1 [3].

**Table 1.** Soil groups according to the humus content in Bulgaria

| Soil groups | Humus content (%) |
|---|---|
| Poor humous soil | < 1 |
| Slightly humous soil | 1-2 |
| Medium humous soil | 2-4 |
| Humous soil | 4-5 |
| Rich humous soil | 5-6 |
| Amply humous soil | >6 |

The humus content ranges from 1.25 to 12.06% in the 56 monitoring points located in the region. The average value, 2.41% ± 1.49 is almost equal to the average value in the Northwest region [5, 6].

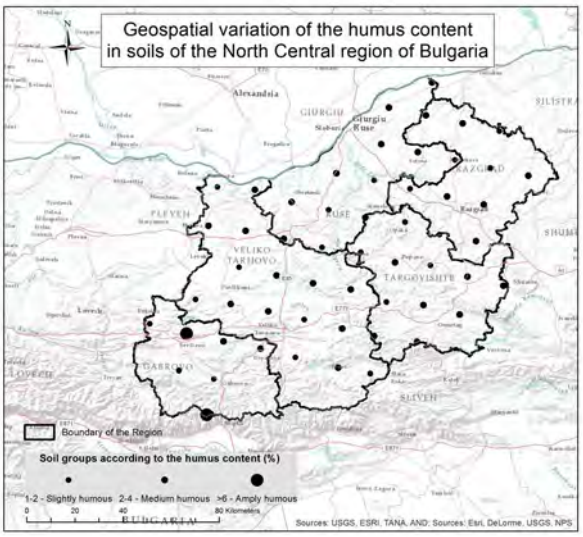



**Figure 1.** Geospatial distribution of the soil humus content in the North Central region of Bulgaria.

The data statistical analysis shows that medium humus soils prevail in the region (59% of the studied sites), and have an average humus content close to the average for the region - 2.48%. The concentrations vary from 2.02 to 3.62% but the most frequent interval is shown; Figure 2.

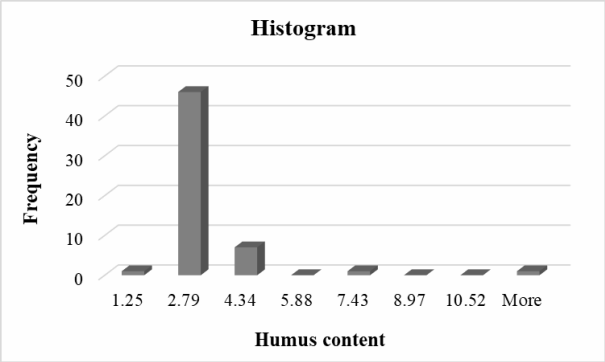



**Figure 2.** Frequency distribution of soil humus content.

**Slightly humous soils** take the second place with an average humus content of 1.67% and a concentration range of 1.25 to 1.98%. They are distributed in 38% of the grid and belong to the:

– Grey forest soils, none-eroded, gleyic /according to [4] they are: Epigleyic Luvisol (Eutric, Siltic Chromic)/; Gray forest soils, none-eroded and slightly eroded /Haplic Luvisol (Epidystric, Siltic)/; Gray forest soils, none-eroded and slightly eroded /Bathycalcic Luvisol (Siltic)/;

– Light grey forest soils, (Pseudopodzolic), slightly eroded, gleyic /Endogleyic Planosol (Eutric, Chromic)/.

– Dark grey forest soils, slightly eroded /Haplic Cambisol (Eutric, Siltic)/; Dark grey forest soils, moderately eroded /Haplic Cambisol (Eutric, Siltic)/;

– Chernozems, slightly leached, none-eroded and slightly eroded /Bathycalcic Chernozem (Siltic)/; Chernozems, moderately leached, slightly eroded /Endocalcic Chernozem (Siltic)/;

– Alluvial-meadow soils /Haplic Regosol (Dystric, Skeletic); Alluvial and Deluvial meadow soils /Haplic Phaeozem (Calcaric)/; Deluvial meadow soils /Calcaric Cambisol (Colluvic, Humic, Siltic); Epicalcic Phaeozem (Clayic)/.

**Medium humous soils**. In this group, Chernozems predominate, with subtypes calcareous and leached, and varieties: non-eroded and slightly eroded. They are classified as Endocalcic/ Epicalcic/ Bathycalcic or Calcic Chernozem; Haplic Cambisol; Haplic Regosol; Calcic Luvic Phaeozem. Dark grey forest soils are non-eroded and slightly eroded /Bathycalcic Luvisol and Bathycalcic Phaeozem/ or gleyic /Bathycalcic Bathygleyic Phaeozem and Luvic Phaeozem/.

Alluvial-meadow soils are predominantly deep soils, belonging to the: Calcic/Umbric/ Haplic Fluvisol; Bathycalcic/ Epicalcic Phaeozem; Haplic Cambisol.

**Amply humous soils.** Soils with a humus content of over 6% are Gray forest soils, moderately eroded /Endocalcik luvisol (Humic, Siltic)/ and Mountain-meadow soils, non-eroded /Endoleptic Umbrisol (Humic, Skeletic)/.

There are no Poor humus (< 1%), Humous (4-5%) and Rich humous soils (5-6%) established in the region.

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# Geospatial distribution of soil organic matter in Bulgaria Part III. The Northeast region

M. BANOV [(a)*], V. TSOLOVA [(b)], V. KOLCHAKOV [(c)], V. KRASTEVA [(a)], N. MITEVA [(a)]

(a) Department of Genesis, Geography and Classification of Soils, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant protection, 1331 Sofia, Bulgaria
(b) Soil Chemistry Department, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant protection, 1331Sofia, Bulgaria
(c) Soil Erosion Department, "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant protection, 1331Sofia, Bulgaria
* E-mail: banovm@abv.bg

**Keywords:** humus content, soil, monitoring

**Abstract.** The content of soil organic matter in Bulgaria has been studied in the georeferenced points of the National Monitoring Network during 2011-2012. The 56 grid points in the Northeast Region are set on the Carbonate, Typical and Leached Chernozems; Haplic Luvisol; Haplic Cambisol; Luvisol (Clayic) and Endogleyic Planosols. The medium humus soils (2-4% humus) prevail in 73% of the grid' points, 23% are "slightly humus soils" (1-2% humus), and 4% are classified as "amply humus" soils (> 6%). Slightly humus soils are mostly eroded Grey, Light grey and Dark grey forest soils and Moderately and Heavily leached chernozems. Medium humus soils include the varieties of well-developed Carbonate, Typical and Leached chernozems and Dark gray forest soils.

## Introduction

Soil monitoring is a systematic observation, assessment and prognosis of soil quality in a given territory over the time. It shows the results of anthropogenic activity and/or natural processes occurring in soils. The main factor and source of soil nutrients is the organic matter. Humus is a major constituent (85-90%) of the soil organic matter and is characterized by a specific nature. Individual compounds - proteins, carbohydrates, fats, lignin, amino acids, resins, tannins, organic acids and others compose the rest part (10-15%). Humus provides the most important features of the soil system, such as: structure and stability; fertility and water retention; limiting the soil erosion and the spread of pollutants, and protection of biomes.

The results presented in the article are a small part of a large-scale project related to the study and classification of soil varieties in points of the National Monitoring Network. Due to the versatility of the monitoring data and the information they provide, the purpose of this publication is to present humus content (%) in 56 monitoring points located in the Northeast Region of Bulgaria.

## Materials and methods

### Description of the region and monitoring grid

The Northeast region of Bulgaria occupies 14 781 km² and includes 4 administrative districts (Varna, Dobrich, Silistra and Shumen). The territory includes the Eastern parts of the Danube hilly plain and the Foot of the Balkan. The relief is characterized by a wide variety of forms: the Danube hilly plain with vast coastal valleys and loess plateaus, the foot of the Stara Planina Mountain and the main mountain range.

The distribution of soils is closely attached to the relief and also show a pronounced zonality in the North-South direction: Typical and Leached chernozems, which have formed on the loess, Gray, Light and Dark gray forest soils go after them and cover the foot of the Stara Planina Mountain and Brown forest and Mountain-meadow soils which are located in the upper part of Stara Planina Mountain.

The study of soil organic matter, displayed as humus content (HC), is carried out at the georeferenced points of the National Soil Monitoring Network with a density of 16x16 km [6]. It is designed to provide a regular assessment of soil chemical composition, its properties and degradation in modern land use conditions [5]. Sampling was done by digging profiles [2].

**Determination and mapping of organic matter content.** The total content of organic carbon was analyzed and calculated as humus content by the modified method of Turin [3].

Geochemical map of organic matter distribution is compiled from two layers: World_Terrain_Base, provided in a scale of ~1:10 m was used as a base map layer [1], overplayed by the layer of monitoring data.

## Results and Discussion

Humus content is a very important feature in Bulgarian soil classifications. Depending on the humus content, the soils in Bulgaria are grouped in several groups shown in table 1.

**Table 1.** Soil groups according to the humus content

| Soil groups | Humus content (%) |
|---|---|
| Poor humous soil | < 1 |
| Slightly humous soil | 1-2 |
| Medium humous soil | 2-4 |
| Humous soil | 4-5 |
| Rich humous soil | 5-6 |
| Amply humous soil | >6 |

Monitoring grid in the Northeast Region is compiled of fifty six points. The humus content varies from 1.28 to 5.33%. The average value, 2.62% (SD ± 1.43), is close to the established average values in monitoring points presented for the Northwest and North Central Regions of Bulgaria and other studies as well [4].

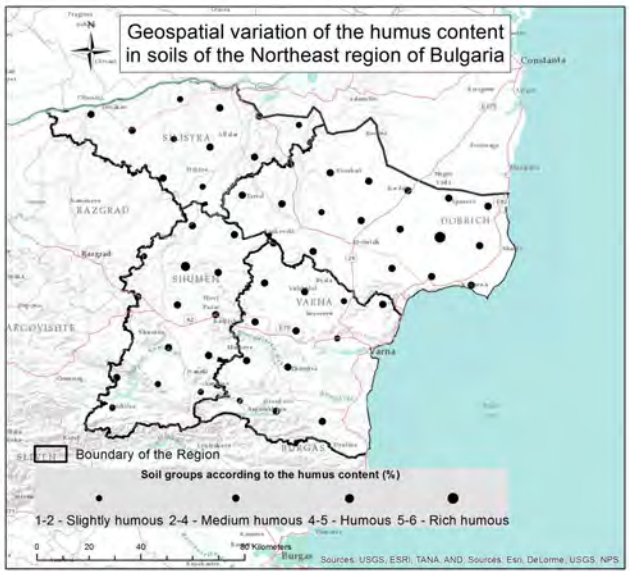



**Figure 1.** Geospatial distribution of the soil humus content in the Northeast region of Bulgaria.

The statistical analysis of the data shows that medium humus soils prevail in the region (73% of the surveyed sites), which have an average humus content of the order of 2.75%. It also coincides with the value at which most values are concentrated (Figure 2). They are followed by Slightly humous soil, which form 23% of the data and have an average humus content of 1.61%.

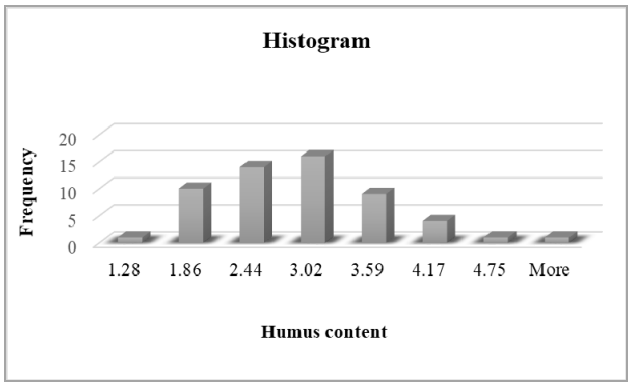



**Figure 2.** Frequency distribution of the soil humus content.

**Slightly humous soils**

These soils are located as spots in average humus soils. The main representatives in this group are the varieties of Gray, Light gray and Dark gray forest soils:

- Grey, none-eroded, slightly and moderately eroded - Haplic and Endoleptic Luvisol; Haplic Cambisol.
- Grey, slightly and moderately eroded, glayed - Epigleyic Luvisol; Endogleyic Luvisol.
- Light gray forest soils, (Pseudopodzolic), none-eroded and slightly eroded, gleyic - Endogleyic Planosol.
- Dark grey forest soils, none-eroded and slightly eroded, gleyic - Endogleyic Phaeozem.
- Chernozems, medium and strong leached - Endocalcic Chernozem.

**Medium humous soils**

In this group. Chernozems predominate with subtypes Calcareous and Leached, and varieties - non-eroded and slightly eroded. According to WRB they are classified as Endocalcic/ Epicalcic/ Bathycalcic or Calcic Chernozem; Haplic Cambisol; Haplic Regosol; Calcic Luvic Phaeozem.

Gray forest soils are predominantly none-eroded and slightly eroded or accumulated, gleyic. They are assigned to the group of Luvisol.

Dark grey forest soils are non-eroded and slightly eroded /Bathycalcic Luvisol and Bathycalcic Phaeozem/ or gleyic /Bathycalcic Bathygleyic Phaeozem and Luvic Phaeozem/.

Alluvial-meadow soils are predominantly deep soils, belonging to the: Calcic/Umbric/ Haplic Fluvisol; Bathycalcic/ Epicalcic Phaeozem; Haplic Cambisol.

Soils with a humus content of over 6% are generally shallow – Lithic or Rendzic Leptosols.

Soils with the lowest organic content are agricultural sands /Haplic Arenosol (Calcaric)/ from the Danube river valley.

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# The effect of organic matter composition on PAHs accumulation in soils

A. UKALSKA-JARUGA [(a)*], B. SMRECZAK [(a)]

(a) Institute of Soil Science and Plant Cultivation – State Research Institute, 24-100 Pulawy, Poland
* E-mail: aukalska@iung.pulawy.pl

**Keywords:** humic substances, black carbon, organic contaminants

**Abstract.** The effect of different SOM fractions on PAHs content in agricultural soils form industrialized region was examined. Soils have differed substantially in BC (1.6-27.1% of SOC) and humic substances (FA:0.3-21.9%; HA:4.6-60.1%; HM:32.4-94.53% of SOC). High HA/FA ratio indicates on high-polymerized form of SOM while BC/SOC ratio on their inert character. The Σ16PAHs differed between soils (0.38 to 224.13 $mg \cdot kg^{-1}$) with the majority of high molecular weight hydrocarbons. PAHs exhibited significant correlation with HM (r=0.73) while the more efficient with BC (r=0.87). BC and HM exhibited the strongest effect on PAHs accumulation in soil.

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants of hydrophobic nature. They are formed during incomplete combustion of fossil fuels such as coal, oil, and natural gas [1, 2]. PAHs are composed with two or more fused aromatic (benzene) rings and represent group of persistent organic pollutants (POPs). Low molecular weight PAHs (two and three rings) occur in the atmosphere predominantly in the vapour phase, whereas multi-ringed PAHs (five rings or more) are largely bound to particles [1]. Intermediate molecular weight PAHs (four rings) are partitioned between the vapour and particulate phases, depending on the atmospheric temperature [1, 2]. PAHs are continuously deposited in soils by dry or wet precipitation processes. Some of these compounds origin from, transportation activities and runoff from roadways [3]. Furthermore, they can be introduced into soils with materials containing these compounds in higher amounts like composts, biochars, or sewage sludges [1].

It has been widely accepted that a remarkable proportion of PAHs entering soils would tightly associate with the soil organic fraction and persist in soil. This phenomenon is referred to as ageing or sequestration [4]. Mechanisms accounting for this include strong association with soil organic matter (SOM), especially with a portion of its constituents: the carbonaceous components such as combustion-derived black carbon (BC) including soot, tar, chars, as well as humic substances [5, 6]. It was found that SOM contained predominant sorption domains for hydrophobic organic contaminants and was positively correlated with their content [6, 7]. Humic substances including fulvic acids (FA) humic acids (HA) and humins (HM) represents the major carbon-containing components of SOM. They were demonstrated to have considerable high sorption capacity for hydrophobic organic contaminants due to their abundant hydrophobic carbon domains including macromolecular aliphatic chains and aromatic carbon components as well as significant amount of small pores, which facilitated retention of hydrophobic organic contaminants in soils [7].

The main objective of this study was to evaluate the impact of SOM fractions on PAHs accumulation in soils from south-western part of Poland, a typical industrial area with high anthropopression level.

## Materials and methods

Soil samples were collected from the upper layer of agricultural soils (n=22) located in Silesia region, Poland. The SOC content was determined by sulfochromic oxidation of organic carbon followed by titration of the excess $K_2Cr_2O_7$ with $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ (PN-ISO 14235, 2003).

The assessment of the humic substances (HS) content, containing humic acids (HA), fulvic acids (FA) and humins (HM) were carried out by an adapted method approved by International Humic Substances Society. The obtained extracts of individual humic substances were centrifuged (4000 rpm for 10 min), decanted, filtered (through a 0.45 µm membrane filter on Büchner funnel) and analyzed for a carbon content by using liquid C-N analyzer (Multi N/C 2100/2100S Jena Analytics). To evaluate stability of SOC and innertness of SOM the humification index: HI=HA/FA and BC/SOC index were calculated

The content of BC was analysed by combustion of samples in access of air at 375°C for 24 h (CTO375) according to the method described by Gustafsson et al. (1997), and then the remaining carbon was measured by high temperature combustion in an elemental analyser (Vario Macro Cube CN; Elementar Analysensysteme GmbH). Before BC analysis carbonates were determined.

## Results and Discussion

All soils were characterized by a high content of SOC (12.7-130.0 $g \cdot kg^{-1}$) which exhibited the diversity of the fractional composition.

The heterogeneity of humic substances composition was evident, as indicated by the carbon content in the individual fractions. The mean concentrations of the organic carbon in humic substance fractions were 2.6 $g \cdot kg^{-1}$ (CoV=57%), 4.7 $g \cdot kg^{-1}$ (CoV=38%), and 8.3 $g \cdot kg^{-1}$ (CoV=72%) for FA, HA and HM respectively. Low FA+HA/HM ratio ranged from 0.1 to 2.8 constituted the largest and more diversified part of humic substances what indicates the high stability of SOM in an alyzed soils. Additionally, relatively high proportions of HA/FA (from

1.2 to 7.2) confirm that humic substances occur mainly in high-polymerized organic forms (8).

BC content was in the range 0.7-42 $g \cdot kg^{-1}$ (CoV=86%) with BC/SOC ratio from 0 to 12.6. The high BC/SOC ratios in some soils may suggest a more inert character of carbon pool, hence higher stability in soils [7]. Additionally, soils under high anthropopressure often receive large quantity of atmospheric deposition (dust), soot and pollutants. The study confirms other findings [7] indicating on high BC content and BC/SOM ratio upper layer of soils form industrial areas [10].

Generally, higher molecular weight PAHs (≥4 rings) accounted for a higher proportion (up 75%) of the total PAH pool. Low-ring PAHs, occurring mainly in gaseous form, are subject to long-range transport and degradation, whereas high-ring PAHs are prone to rapid deposition, which could be in short distance from the local emission sources. Reported in the study a minor contribution of lower molecular weight PAHs (<4 rings) to the total loads of those compounds (∑16PAHs) is may be related to their lower persistence due to a depleted sorption and sequestration potential as well as a relatively high volatility and biodegradability (9). The noted pattern of PAHs implies a domination of combustion over petrogenic sources [10].

Concentration and the pattern of PAHs in soils are related to their input from emission sources but also associated with soil properties, mainly SOM content and character, influencing their sorption and desorption. Our results showed significant relations between PAHs and total SOC ($r=0.82$, $p<0.005$)., ∑16PAHs was positively correlated among SOC fractions only with HM ($r=0.73$, $p<0.005$) and also BC ($r=0.87$, $p<0.001$). The links between HM and PAHs were stronger for low molecular (<4 rings) weight PAHs ($r=0.87$, $p<0.001$) than with high molecular (≥4 rings) PAHs ($r=0.77$, $p<0.005$). BC fraction contrary to HM exhibited a higher correlations with high molecular weight PAHs ($r=0.87$, $p<0.001$). FA and HA did not show significant relationships with hydrocarbons. According to Chen et al (2017) they participate in the transport of pollutants in the soil profile and between resisitant SOM factions, such as HM and BC.

Our findings illustrated that SOM different fractions might possess divergent binding capability and dissimilar binding strengths to PAHs. Diversified sorption capacity of BC and HM in relation to PAHs of different molecular weight results from various structure, i.e. polar and apolar functionalities in sorption domains and pore size structures of these fractions [4, 7, 9]. Furthermore, high BC correlation with high molecular PAHs may arise from their co-emission. Emitted PAH can be sorbed by BC particles in the gaseous phase and partitioning during atmospheric transport or sorbed and partitioned in soil after deposition [6, 10].

**Conclusions**

The BC and HM fractions were the most strongly related to the amount of PAHs in soil, while HA and FA were of less importance These findings are of great significance for understanding the influence of individual SOM fractions on PAH retention and persistence in soils.

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# Effect of land use on organic matter composition in soil

A. UKALSKA-JARUGA [(a)*], B. SMRECZAK [(a)]

(a) Institute of Soil Science and Plant Cultivation – State Research Institute, 24-100 Pulawy, Poland
* E-mail: aukalska@iung.pulawy.pl

**Keywords:** arable lands, grasslands, soil organic matter

**Abstract.** The objective of this study was to investigate the differences in the content of organic matter fractions (fulvic acids:C-FA, humic acids:C-HA, humins:C-HUM) and dissolved organic including cold:C-CWE and hot:C-HWE water extractions) in arable and grassland soils. The results indicated significant differences in SOC content among soils with HA and HUM as dominant fractions. SOC content was higher in grassland a in arable soils where lower C-CWE and higher C-HWE were noticed. The results showed that humified and labile organic matter fractions are protected under the grasslands and less vulnerable to mineralization.

## Introduction

Soil organic matter (SOM) content and its composition play a crucial role in the soil quality assessment [1]. It is often assumed, that the SOM contains humic and non-humic substances. Due to complex interactions of biological, chemical and physical processes in soil, the turnover of different SOM components varies continuously [1, 2]. Humic substances (HS) represent more stable organic carbon fraction which make up a significant portion of the total SOM. The nature of HS: fumic acids (HA), fulvic acids (FA) and humins (HU) contributes to their persistence in soils influencing carbon sequestration-emission balance and nutrient flows through ecological systems [2-5]. Various indicators describe the stability of SOC in soil ecosystem. The ratio between concentrations of humic and fulvic acids (HA/FA) indicating on the potential mobility of C in the soil system, while the (HA+FA)/HUM ratio pointing out on lower intensity of organic matter humification associated with their slight transformation into the stable forms of the SOM [6].

Nonhumic substances, such as dissolved organic carbon (DOC) consists of a wide range of molecules, reflecting the composition of the total soil organic carbon. The soluble phase tends to be in equilibrium with the solid phase of SOC [7, 8]. The DOC responds more quickly to changes in the agriculture ecosystem than HS but is characterized by rapid turnover times [7, 9]. It is documented that dissolved C is more sensitive to alterations in moisture, temperature and plant species in comparison with carbon of HS [8].

Land use and related management practices can contribute to negative changes in the SOM content and quality. Therefore their influence should be investigated and monitored in order to reduce environmental impacts of agricultural activities [4]. Due to the complexity of the SOM, the relationship between its fractional composition and land use is still insufficiently understood. Moreover, adaptation of agriculture to climate changes and implementation of best agricultural practices will need indicators confirming sequestration and mobility of SOC in soils. The main objective of the study was to evaluate SOC characteristics as influenced by land use. The research covered a typical agricultural area with high production potential from central-eastern Europe.

## Materials and methods

Soil samples representing arable (n=18) and grassland soils (n=15) were collected form the upper layer of 0-30 cm from Polish territory

The total organic carbon content (C-TOT) was determined by sulfochromic oxidation followed by titration of the excess $K_2Cr_2O_7$ with $FeSO_4(NH_4)_2SO_4$ $6H_2O$ (PN-ISO 14235, 2003). The assessment of the humic substances (HS), containing humic acids (C-HA), fulvic acids (C-FA) and humins (C-HUM) were carried out by method approved by International Humic Substances Society.

Dissolved organic carbon (DOC), cold water extractable carbon (C-CWE) and hot water extractable carbon (C-HWE) were determined according to Ghani (2003) and Landgraf et al. (2006) method, respectively. CWE-C was determined by shaking of soil with distilled water (1:10 w/v soil to solution ratio) for 24h in 20$^o$C, while HWE-C by heating (in a water bath) of soil with distilled water (1:10 w/v soil to solution ratio) for 24 h in 80$^o$C.

The obtained soil extracts from both extraction methods covering HS and DOC were centrifuged (4000 rpm for 10 min), decanted, filtered (through a 0.45 µm membrane filter on Büchner funnel) and analyzed for a carbon content by using liquid C-N analyzer (Multi N/C 2100/2100S Jena Analytics).

To evaluate stability and mobility of SOC under various type of land use the humification index: HI=C-HA/CFA and degree of humification: DH=(C-HA+C-HA)/C-HU were calculated.

## Results and Discussion

The C-TOT content was significantly higher ($p<0.05$) and much more varied in grasslands (Me=17.5 g·kg$^{-1}$; CoV=99%) in comparison to arable lands (Me=9.9 g·kg$^{-1}$; CoV=33%). The differences in C-TOT observed across sample set can be attributed to differences in land-use practice, such as tillage, plant-derived, fertilization and/or soil properties influencing turnover of SOM but also the various amount and

character of root residues of grasses and crop plants [2, 3, 4, 6].

Generally, arable soils were characterized by significantly lower ($p<0.05$) content of individual fractions of HS comparing to grassland soils. Content of HS fractions in both land use systems were as follow: C-HA(3.9g·kg$^{-1}$) >C-HUM(3.5g·kg$^{-1}$) >C-FA (2.3g·kg$^{-1}$) for arable lands and C-HUM(8.3g·kg$^{-1}$) >C-HA(7.2g·kg$^{-1}$) >C-FA(3.0g·kg$^{-1}$) for grassland. Contrary to expectations, C-HA fraction was predominant in arable lands soils, while in grassland soils C-HUM carbon exhibit the highest concentrations. Higher variability in the share of individual HS were observed in arable lands: C-FA(34%) <C-HA(45%) <C-HUM(62%) than in grasslands: C-HA(43%)<C-FA(47%)=C-HUM(47%).

The C-HA to C-FA ratio expressed by the humification index (HI) indicates the quality of organic matter and is very important for soils of agricultural use. The results indicated that HI varied very widely from 0.3-10.3 for grasslands and 0.3-3.9 for arable lands but average values did not differ significantly. The broad range of values could be related to high heterogeneity of organic residues remaining at different sites, as well as differences in the organic carbon humification process intensity [4, 6]. HI below the value of 1.0 (indicating on prevailing SOM mineralization processes) exhibited 7% of soil samples representing grasslands and 17% from arable lands. Higher proportion of C-HA/C-FA for grassland soils showed their higher humification rates. The intensity of organic matter humification expressed by DH indicates that land use did not significantly differentiate the transformation processes of organic matter. Nevertheless, DH values were more diversified in the arable lands (0.2-20.1; Me=1.81) than in grasslands (0.2-6.2; Me=1.35). A low DH values can be related to a strong interaction between the SOC and the soil mineral phase, as well as predominant in soils, more recalcitrant humin fractions [6]. The high HI and low DH indexes are expected to indicate relatively fertile soil with good water infiltration capacity, aggregate stability, microbial activity, and nutrient supply. The higher HI and DH indexes impliys, that humic substances occur mainly in high-polymerized organic forms [4].

The land usage significantly affected the content of DOC. C-CWE carbon concentrations were significantly ($p<0.05$) lower compared to C-HWE. The highest difference between DOC fractions was recorded in soils from arable lands, in which C-HWE content (Me=2.59g·kg$^{-1}$) was 64% higher than C- CWE (Me=0.75g·kg$^{-1}$). Grasslands exhibited higher C-CWE (Me=1.39g·kg$^{-1}$) and lower C-HWE (Me=1.97g·kg$^{-1}$) concentration than arable lands. The relative average content described as % of SOC was 7.4% and 6.8% in grasslands, and 7.8% and 28% in arable lands, for C-CWE and C-HWE, respectively.

High of C-HWE in arable lands may be caused by organic fertilization, fast turnover of SOM and higher amount of microbial populations forming part of DOC [7,10].

**Conclusions**

The study investigated the organic matter fractional composition (total SOC, DOC and HS) in relation to land use. The results showed that land use significantly affected all organic carbon fractions, their quality, quantity and stability as described by HI and DH indexes. On the basis of the above findings, there is a need to develop proper land use policies and sustainable soil management and cropping practices to promote humification processes leading to the organic carbon sequestration especially in mineral soils that dominate in rural areas of eastern and central Europe. The proper characterization of SOM is essential in maintaining agricultural soils quality.

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# Molecular composition of humic substances from permafrost peats of European North of Russia as climate change markers

R. VASILEVICH[(a)*], E. LODYGIN [(a)]

(a) Institute of Biology, Komi Science Center, Ural Branch, Russian Academy of Sciences, 167982 Syktyvkar, Russia
* E-mail: vasilevich.r.s@ib.komisc.ru

**Keywords:** forest-tundra, permafrost, peat, humic acids, fulvic acids, Histosols

**Abstract.** Humic substances (HSs) from the mire peats of the forest-tundra zone of the European North of Russia have been characterized in terms of molecular composition. This was accomplished using solid-state $^{13}C$ nuclear magnetic resonance ($^{13}C$ NMR) techniques. The composition depended on the intensity of cryogenic processes in the active layer, the quality of the humification precursors (the degree of peat material transformation), and the biochemical selection of aromatic fragments during humification. Humic acids (HAs) and fulvic acids (FAs) of the peat soils showed the presence of compounds with a low extent of condensation and a low portion of aromatic fragments, which increased with depth. A higher proportion of aliphatic carbon species was found in the HAs, indicating a low degree of organic matter stabilization. Based on the data from the two types of peat soils, we suggest that particular changes in the proportion of aromatic and unoxidized aliphatic fragments on the border of the bottom of the active layer and permafrost layers can be used as markers of current climatic change.

## Introduction

Relict permafrost peatlands in the tundra and forest-tundra zones attract major interest in the study of climate changes. The stability of the stored carbon under changing climatic conditions is the subject of the concern due to the expected climate warming, which is predicted to be the most pronounced in northern continental regions [2]. Permafrost degradation in the peatland ecosystems affects global and regional carbon balances [5]. Peat profiles can serve as "archives" indicating conditions in past environments. Peat HSs are of key importance in the context of the ongoing process of climate change [3, 4]. The ratio of aromatic to aliphatic HSs components is related to the type of peat material and content of oxygen-containing carboxylic and phenolic groups. This ratio also reflects the degree of humification [3, 4]. Conditions of the permafrost affect soil formation, and together with low microbiological activity in Arctic, they leads to cryogenic denaturation and the transformation of highly dispersed HSs. Thawing-freezing cycles result in intensive changes in the composition of humus and its colloidal physicochemical properties. These changes are manifested in increased HSs mobility because of increased polydispersity of the molecules [1]. Thus, short- and long-term climatic changes could affect the quality of HSs. The alteration should be most pronounced in the supra-permafrost layers.

## Objects and methods

The two peats – Hemic Folic Cryic Histosol (peat with vegetated plots) and Hemic Folic Cryic Histosol (Turbic) (peat with bare spots) have been studied from a peat plateau-thermokarst complex located in the north forest-tundra subzone, west of the Ural Mountains, the Komi Republic, Russia (Fig. 1). Sampling was conducted to a depth of 2.0 m. The two Histosols differ in their active layer thickness and thermal regime. In the summer season, the permafrost table is fixed at a depth of ca. 40–60 cm. Analysis of the temperature regimes showed that during the summer, peat with bare spots was heated and insolated more intensively than the peat with vegetated plots. Thus, from the studied parameters, the bare peat spots are analogous to the peat with vegetated plots and differ only by the temperature conditions of the seasonally thawing layer, which can form a model basis for predicting changes in the structural and functional parameters of HSs under the current varying climate.

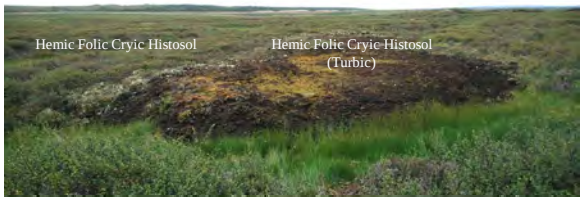



**Figure 1.** The sampling site.

HSs were characterized for their elemental composition (C, N and H) using an EA-1110 analyzer. $^{13}C$ NMR measurements were carried out using a JNM-ECA 400 (JEOL) NMR spectrometer (100.53 MHz for $^{13}C$). The cross-polarization sequence of excitation pulses was used for the registration of $^{13}C$ spectra (CP/MAS).

## Results and Discussion. Elemental composition.

A decrease in the $x$(H) : $x$(C) ratio of HAs is revealed down the profile from 1.05 to 0.82 in bare peat spot and from 1.16 to 0.90 in peat with vegetated plots, which is due to the natural selection of biothermodynamically stable molecular structures and determines the high degree of organic matter humification in lower peat layers (Fig. 2). The degree of peat degradation increases down the profile from 20 % for moss peats in the upper part of the profile to 35–40 % for sedge and trefoil–sedge species in the central part and to 35–50% for wood-sedge

peat in the lower layers. A reliable relationship is observed between the *x*(C) : *x*(H) ratio in HAs and the degree of humus degradation ($r = 0.578$, $n = 18$, $P = 0.95$, $rcr = 0.468$).

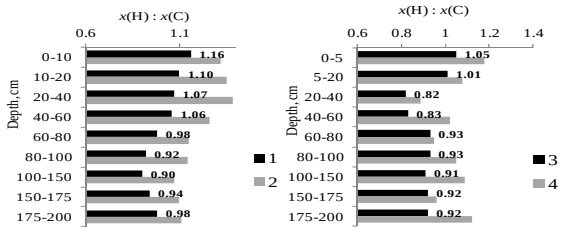



**Figure 2.** The *x*(H) : *x*(C) molar ratios in (1, 3) HAs and (2, 4) FAs from the Hemic Folic Cryic Histosol (1, 2) and (3, 4) from the Hemic Folic Cryic Histosol (Turbic).

The peat with vegetated plots are worse heated in summer because of the buffer effect of moss litter. This can be a reason for the lower degree of condensation of HA and FA molecules in the peat horizons down to the permafrost table (Fig. 2). From a depth of 80 cm, the *x*(H) : *x*(C), *x*(O) : *x*(C) ratios of HAs are almost similar for two profiles and indicate the relict character of humus, which is a result of diagenesis of the Atlantic period with warmer climate conditions. In the peat layer at a depth of 80–150 cm, the most humified HSs correspond to the Holocene optimum 7022–6508 Cya (IGRAN 4644, 4645).

**Structural molecularcomposition (13C NMR)**

The quantitative compositions of $^{13}C$ NMR spectra revealed a high portion of unoxidized aliphatic fragments and a low portion of aromatic carbon in the HAs and FAs (15.2–36.3 and 1.6–12.8%, respectively) (Fig. 3).

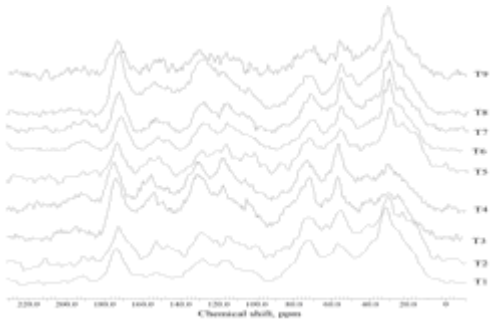



**Figure 3.** CP/MAS $^{13}C$ NMR spectra of HAs isolated from Hemic Folic Cryic Histosol (Turbic).

The highest indexes of biological activity from bare permafrost peatlands indicate possible climate changes in cases of increased soil temperatures. This results in a stronger alteration of the SOM content in the solum active layer (Fig. 2, 4).

The content of functional groups was different in seasonally thawed layers and permafrost layers. Specifically, the aromatic fragments and methoxy groups show increasing trends and paraffin groups show decreasing trends with respect to depth (Fug. 4). This directly reflects the interrelationships between the structural composition of the HAs and climatic conditions of the Holocene period, and it could be related to periods of warm climate.

In the active layers of the unvegetated permafrost peat soils, the portion of aromatic fragments of HA showed a substantial increase with depth. Dynamic thawing and freezing on the border of the permafrost results in increased aromaticity and a decreased portion of unoxidized aliphatic fragments (Fig. 4c, 4d). This is most pronounced on the border of permafrost. The indexes correlated with the elemental composition data. For the relic peat sampled from depths of 150–175 cm, intense maximums of aromatic carbon were revealed for both HAs and FAs. These peaks are related to the Holocene climatic optimum, which is dated at about 7000 cal BP (IG RAS 4645, 4646).

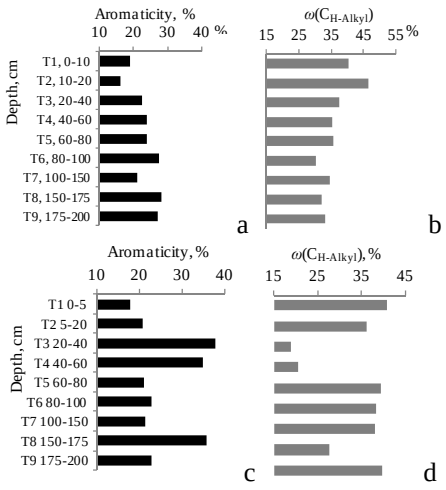



**Figure 4.** The aromaticity (a, c) and mass fraction of the paraffin fragments (b, d) in HAs from the Hemic Folic Cryic Histosol (a, b) and (c, d) from the Hemic Folic Cryic Histosol (Turbic) (according to $^{13}C$ NMR).

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# Computational modelling of metal binding by complex organic systems – humic substance models

E. A. VIALYKH [(a)*], R. L. COOK [(b)], C. H. LANGFORD [(a)*], D. SALAHUB [(a)], G. ACHARI [(c)]

(a) Department of Chemistry, University of Calgary, 2500 University Dr NW, Calgary, AB. Canada T2N 1N4
(b) Department of Chemistry, Louisiana State University, Baton Rouge, LA, 70810 U.S.A.
(c) Schulich School of Engineering, University of Calgary, 2500 University Dr NW,
Calgary, AB, Canada T2N 1N4
*E-mail: elena.vialykh@ucalgary.ca

**Keywords:** complex organic systems, metal ions, complexation, humic substances

**Abstract.** One of the most important interactions of complex natural organic systems, such as humic substances (HS), is their interaction with metal ions. In this work, we have used computational modelling to study the behaviour of humic and fulvic acids in the presence of $Cu^{2+}$ and $Mg^{2+}$ ions. The new paradigm of HS organization adopted in this work treats HS as supramolecular labile aggregates, allowing us to study the binding of metal ions by HS and the emergent properties of the system.

## Introduction

Traditionally, the studies of metal (Me) binding by HS has been focused on the chemistry of the processes that take place at the binding site. To-date, the effect of the metal binding on the structural rearrangement within the HS system has attracted less attention, except for some exploration of how the HSs, viewed as weakly associated molecular assemblies (WAMA) [1, 2], affect the chemical properties of the HS system [3]. This leads to the larger question of how environmental conditions, including the presence of metal ions, influence the chemistry of HS and generalizing to complex organic systems, what is the effect of the presence of metal ions in solution on the emergent properties of such systems.

Modern computational tools allow one to investigate such WAMA and the influence of environmental conditions on these assemblies. In this work, we apply the recently developed ReaxFF force field [4] to study Me-Ligand binding interactions in model WAMA HS systems. The main advantage of this force field resides in its ability to model covalent bond formation and breaking. In doing so, this force field allows one to study the Me binding processes, emergent properties of the HS WAMA system and changes to these properties as a function of the Me bonding and other interactions within the HS WAMA.

## Materials and methods

### Computation method

Leveraging the available data from the International Humic Substance Society (IHSS) [5] elemental composition, functional group content, aromaticity, "carbohydrate-like" fragments content and molecular size for the humic and fulvic acid standards, three organic systems were created. These were: two fulvic acid systems and a humic acid system composed of 22, 6, and 6 molecular entities, respectively.

Each organic system was modeled in aqueous solutions, with organic matter concentrations of 2.4, 1.2, 0.49, 0.24 % (g/g) (or 100, 200, 500 and 1000 water molecules). Metal ions, $Cu^{2+}$ or $Mg^{2+}$, were added to each system (solution) at a concentration from 0.0011-0.14 %. $Na^{+}$ was added to keep the solutions neutral. Molecular dynamics (MD) simulations on each generated system were carried out for 0.5 ns under NPT conditions, and for another 1.5 ns under NVT conditions. The trajectories of the last 0.25 ns of MD simulations were analyzed, and the potential energies, number of hydrogen bonds, and hydrophilic/hydrophobic surface areas were calculated. Metal ion locations were also investigated.

## Results and Discussion

The MD simulations of the three organic systems with different metal ion concentrations and water content revealed some common patterns. When only one ion, $Cu^{2+}$ or $Mg^{2+}$, was in the systems, it was found that in the presence of (i) 500 or 1000 water molecules, the metal ions remained solvated, and there was no Me-Ligand binding, (ii) 200 water molecules, one or two Me-Ligand bonds were formed, and (iii) 100 water molecules, typically three to four Me-Ligand bonds were formed.

The system's indifference to the metal ion type, $Cu^{2+}$ or $Mg^{2+}$, with respect to Me-Ligand binding continued until the system consisted of 8 and 16 metal ions and 500-1000 water molecules. At this point, the $Cu^{2+}$ and $Mg^{2+}$ systems diverged. $Mg^{2+}$ tended to form mainly outer sphere complexes i.e., it was bound to organic molecular entities via water bridging, which was attributed to $Mg^{2+}$ properties [6]. On the other hand, some $Cu^{2+}$ ions in the system formed inner sphere mono/bidentate complexes with the organic moiety, while others were fully hydrated ions, which was in agreement with experimental data [7].

It was also observed that as the number of metal ions increased, while the number of water molecules remained the same, the ratio of complexed metal ions (Me-Ligand) to hydrated ions increased for both $Cu^{2+}$ and $Mg^{2+}$. In an 8 $Cu^{2+}$ and 1000 water molecules system, four $Cu^{2+}$ ions were bound directly to the organic molecules, whereas four $Cu^{2+}$ ions were in solution. In the same system with $Mg^{2+}$ ions, two ions were directly

bound to organic molecules, two ions formed an outer sphere complex, and four ions were in aqueous solution. Increasing the number of $Cu^{2+}$ ions in the system to 16 resulted in seven ions being directly bound to organic molecules, with three ions forming an outer sphere complex, and six ions being fully solvated. In the case of $Mg^{2+}$, five ions formed Me-ligand bonds, four $Mg^{2+}$ ions formed an outer sphere complex, and seven ions were only associated with water. Though the exact values depend on the organic system under investigation, the main pattern remains similar. In all organic systems, carboxylic groups were the main metal binding sites.

The presence of different metal ions at various concentrations affected the organic systems' characteristics as well. Increasing the number of $Cu^{2+}$ ions in the system with 1000 water molecules increased the potential energy and the number of hydrogen bonds, whereas in systems with 100-500 water molecules or with an increased number of $Mg^{2+}$ ions, a decrease in potential energy and a slight increase of the number of hydrogen bonds were observed.

Changes in hydrophobic/hydrophilic surface areas were also observed (Table 1). Increasing the number of water molecules present led to an increase in hydrophilic, and a decrease in hydrophobic, surface areas for all systems. The systems containing $Cu^{2+}$ ions and 1000 water molecules yielded larger hydrophilic surface areas compared to the $Mg^{2+}$-containing counterpart systems. Increasing the number of metal ions present resulted in more compact aggregates with smaller hydrophilic and hydrophobic surface areas, with the exception being the $Mg^{2+}$ and 1000 water molecule system. This could be explained by the fact that $Mg^{2+}$ tends to form outer sphere complexes with organic ligands, and the $Mg^{2+}$ concentration was low enough to ensure that all $Mg^{2+}$ ions were surrounded by water molecules. Increasing $Cu^{2+}$ ion concentration led to the formation of an increased number of Me-Ligand bonds and, consequently, causing the more compact aggregates with hydrophilic groups, such as $COO^{-}$ located inside the aggregate, to bond with $Cu^{2+}$ ions. The decrease of the hydrophobic surface area in the systems containing 100 water molecules, compared with those having 200-500 molecules, could be explained by the formation of more compact aggregates that are comparable to the dry state of HS [8]. On the other hand, in the systems with 200-500 water molecules, the organic matter formed more open aggregates, comparable to the HS in the wet, or solvated, state [9].

**Table 1.** Hydrophilic/phobic surface areas ($Å^2$) of the Fulvic acid-22 system after MD simulations with different water and metal ions content

| Number of water molecules in the system | Amount of $Cu^{2+}$ ions added | | | | | | Amount of $Mg^{2+}$ ions added | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 8 | | 16 | | 1 | | 8 | | 18 | |
| | philic | phobic | philic | phobic | philic | phobic | philic | phobic | philic | phobic | philic | phobic |
| 1000 | 1832 | 495 | 1799 | 505 | 1707 | 532 | 1670 | 455 | 1655 | 491 | 1696 | 457 |
| 500 | 1765 | 594 | 1690 | 601 | 1632 | 609 | 1615 | 592 | 1585 | 616 | 1526 | 613 |
| 200 | 1694 | 716 | 1629 | 694 | 1453 | 561 | 1617 | 588 | 1526 | 577 | 1395 | 584 |
| 100 | 1355 | 532 | 1344 | 564 | 1362 | 552 | 1556 | 499 | 1418 | 503 | 1184 | 563 |

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# The fate of terrigenous organic matter from the mississippi river delta to the canyon of the continental shelf, gulf of mexico

S. WARE [(a)], D. WAGGONER [(a)], P. HATCHER [(a)*]

(a) Department of Chemistry, Old Dominion University, 4402 Elkhorn Ave., Norfolk, VA 23529, USA
*E-mail: Phatcher@odu.edu

**Keywords:** humic acid, organic matter, ESI-FTICR-MS, lignin

**Abstract.** Humic acids were extracted from samples extending from the Mississippi River Birdsfoot Delta to the canyon of the continental shelf of the Gulf of Mexico. Characterization of the humic acids was performed by ultrahigh resolution mass spectrometry and NMR. Data indicates condensed aromatics (CA) and carboxyl rich alicyclic molecules (CRAM) present at the mouth of the river, with CA diminishing and CRAM persisting with seaward progression. The shift from CA to CRAM like structures is explained herein by the selective oxidation of terrigenous organic matter resulting in rearrangement via ring opening.

## Introduction

Marine organic matter (OM) is believed to mainly be derived from autochthonous material, with terrigenous OM thought to be degraded through microbial and photochemical processes prior to reaching the open ocean, as measured by lignin biomarkers used to identify terrigenous OM [1]. Recent studies, however, have shown that much of the organic carbon in oceanic systems likely has terrestrial origins, by investigating the export of OM from major river systems [2]. These studies use isotopic abundances combined with radiocarbon dating methods, showing depleted $^{14}C$, in surface sediments to validate the presence of terrigenous OM. The isotopic signatures present strongly indicate that the OM is considerably older than expected from autochthonous OM, having undergone significant aging prior to export. Stable isotope signatures additionally suggest that major river systems, such as the Amazon and Congo, export C3 plant OM to offshore depocenters in the deep ocean. [2, 3]

Experimental evidence has lead us to support the hypothesis that soil-derived carbon export contributes significantly to sedimentary OM stemming from the Mississippi River system into the Gulf of Mexico. By employing electrospray ionization coupled to a 12T Fourier transform ion cyclotron resonance mass spectrometer (ESI-FTICR-MS) and solid-state $^{13}C$ NMR, we examine whole samples as well as humic acid (HA) fractions. These samples were previously studied by Sampere et al., focusing on lignin phenols, markers for terrigenous material, plant pigments, markers for autochthonous material, and stable isotopic compositions. The goals of our study were to show molecular evidence of markers for terrigenous allochthonous carbon as well as autochthonous carbon with NMR and ultrahigh resolution mass spectral data.

## Materials and methods

Sediment samples analyzed in this study were retrieved as described in Sampere et al [4]. Briefly, a transect was taken in July 2003 in the Gulf of Mexico originating at the Mississippi River birdsfoot delta and extending to the continental shelf. Humic acids were isolated from sediment samples via NaOH base extraction, cation exchange clean up, and acid precipitation with HCl. The resultant HAs were dissolved in 1% ammonium hydroxide, diluted in a methanol/water solution, and analyzed by FTICR-MS in negative mode electrospray ionization as described by Sleighter and Hatcher [5]. Peaks were assigned unique elemental formulas via in-house written MATLAB code. Solid humic acids from 3 points along the transect were analyzed via solid-state $^{13}C$ NMR, using a multiple cross polarization magic angle spinning pulse sequences. $^{13}C$ spectra were acquired on a 400 MHz Bruker AVANCE II NMR spectrometer, equipped with a 2.5 mm H-X MAS probe. The samples were spun at 15K Hz with 5005 scans acquired during the entirety of the run for each sample.

## Results and Discussion

To verify that humic acid fractions of the sediment are a sufficient representation of the whole sample, we analyzed a whole sediment, collected separately from a site, site 8064, near the mouth of the river. Figure 1 shows the solid-state $^{13}C$ NMR spectra of 3 points along the transect, the first point in the river, a mid-point, and the end-point in the canyon. The figure also contains spectra for site 8064, a whole sediment sample, for comparison to the humic acid extract transect samples. The spectra for site 8064 and the transect correlate exceedingly well, suggesting that the humic acid fraction is representative of the whole sediment in this system. Humic acid analysis times were found to be substantially shorter than the whole sediment, 5 hours vs 72 hours respectively, and were therefore used for all analyses. Comparing the peak intensities across the transect, the persistence of the aromatic carbon peak in each sample is a strong indicator of soil-like material close to the surface, as buried, more humified samples would show less aromatic functionality. Lignin from terrestrial vascular plants, for example, typically shows characteristic signals in solid-state $^{13}C$ NMR spectra at 56 and 150 ppm. Peaks associated with lignin are weak in spectra shown in Figure 1, consistent with observations for highly-oxidized soil humic acids and soil organic matter. Additionally supporting these findings, Sampere et al [4] noted low amounts of lignin phenols in offshore Mississippi sediments. To further test

our hypothesis, soil samples from Catlin Il, USA, a Midwestern area of the United States that feeds into the Mississippi River, were analyzed and shown to correlate strongly with the HAs analyzed as part of the offshore Mississippi transect [6].

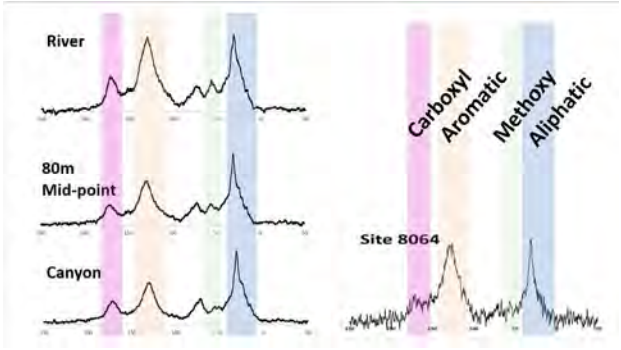



**Figure 1.** Solid state $^{13}C$ NMR spectra for 3 sediment humic acid samples collected along an offshore Mississippi transect (left) compared to 1 whole sediment sample (right). Regions are shown to highlight changes and similarities in peak shape and intensities.

Data obtained from FTICR-MS analysis were plotted on van Krevelen (vK) diagrams. The H/C and O/C ratios populate formulas in similar areas to that of NMR, corroborating the results from NMR peak intensities. However, the lignin region of the vK is more visible and diminishes over the seaward track trending to undetectable levels. A strong similarity exists between the studied sediment HAs and several soil HA samples from the Mississippi River flood basin analyzed by our group, [6] indicating a comparable origin. An observed increase of the aliphatic region and decrease in the condensed aromatic region can be observed on the vK diagram, as can be seen in Fig. 2. This supports our hypothesis that lignin degrades through selective oxidation by hydroxy radical, [7] which decreases the intensity of the lignin region while populating the aliphatic region.

Experiments are underway to synthesize labeleed lignin and subject it to hydroxyl radical attack simulating the processes occurring in oxidative riverine and oceanic regimes. These experiments will enable us to molecularly trace labeleed atoms of lignin and verify the proposed pathway for the environmental fate of lignin.

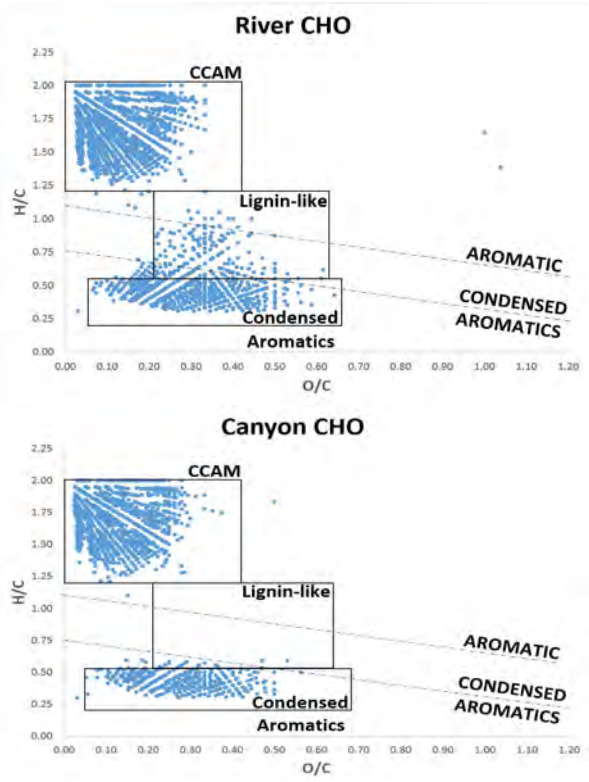



**Figure 2.** van Krevelen CHO diagrams of the first (river) and last (canyon) sites of the Mississippi transect samples, clearly show the loss of lignin and diminished condensed aromatics as one progresses from river to offshore.

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# Chemical characterization of dissolved organic matter from oilfield produced brines in the western Qaidam Basin, China

Y. ZHANG [(a)*], K. YANG [(a)], Y. DONG [(a)], Z. NIE [(b)], W. LI [(a)]

(a) Qinghai Institute of Salt Lakes, Chinese Academy of Sciences, Xining 810008, PR China
(b) Institute of Mineral Resources, Chinese Academy of Geological Sciences, Beijing, P. R. China
* E-mail: zhyl@isl.ac.cn

**Keywords:** dissolved organic matter, oilfield, brine, Qaidam Basin, FTIR, $^1$H NMR, Py-GC/MS

**Abstract.** Four samples of dissolved organic matter (DOM) were isolated from oilfield produced brines in different well depths of the western Qaidam Basin by solid phase extraction method. The compositions and features of the DOM samples were characterized by a combination of techniques. The results indicated that the studied DOM samples were dominated by aliphatic compounds, with a small amount of alkenes. The contents of purely aliphatics, the carboxylic-containing compounds, and phenols were found negatively correlated with the oil well depths, while carbohydrates, halogen-containing compounds, cycloparaffins, and PAHs were positively correlated with the well depths.

## Introduction

Oilfield produced brines (OPB) in the western of the Qaidam Basin, Qinghai Province, China contain tons of useful inorganic components, such as halite, lithium, magnesium, boron, cesium, rubidium, strontium, etc., which are important raw materials for industry, agriculture, and medicine [1]. Until recently, the oilfield brine resources in the areas have been explored and utilized. Howeve, r with the procedures of multi-stage evaporating ponds to concentrate and isolate the inorganic salts, the high content of dissolved organic matter (DOM) exist in the brines is found to be also concentrated and exerts adverse effects on the quality of the salt products [1, 2]. Moreover, DOM in oilfield produced waters is known to contain a number of potentially toxic organic components, including polycyclic aromatic hydrocarbons, heterocyclic compounds, and aromatic amines, and those compounds may have a risk of mortality in humans [3, 4]. Therefore, it is crucial to find an effective way to treat or remove DOM in oilfield brines for both production concerns and environmental concerns.

DOM in oilfield waters has been treated by using various technologies with DOM removals ranging from 20% to 90% [3, 4]. This high variation is likely due to the recalcitrance of some fraction of the DOM to be chemical and microbial degraded, or physically removed. Relatively little research has been performed on DOM from oilfield brines [3, 4], so their chemical characteristics and susceptibility to treatment remain less well understood. In this study, four samples of DOM were isolated from OPB in the Qaidam Basin by solid phase extraction method. A combination of techniques, i.e. Fourier transform infrared spectroscopy (FTIR), $^1$H nuclear magnetic resonance ($^1$H NMR), and pyrolysis gas chromatography-mass spectrometry (Py-GC/MS), were used for systematical study the composition and features of the isolated DOM.

## Materials and methods

**Chemical analysis of OBP.** The pH, densities, the concentrations of dissolved organic carbon, dissolved nitrogen, major cations and anions were determined by chemical and instrumental analysis.

**Extraction and purification of DOM.** The filtered oilfield brine samples were acidified to pH 2.0 with HCl and passed through an XAD-8 and XAD-4 resins (Amberlite, USA) in series [5]. The DOM adsorbed on each XAD resin column was back-eluted separately using 0.1 M NaOH. Then the acidified eluates from the two columns were re-concentrated with smaller XAD-8 and XAD-4 columns, respectively. The 0.1 M NaOH eluates from the two smaller columns were separately passed through $H^+$-saturated cation exchange resin column. Then the two subsamples from the cation columns were combined together and freeze-dried for further analysis.

## Results and Discussion

### Chemical characteristics of OPB

The well depths of the studied four samples were between 1645 to 3600 m (3600 m for N-1, 3180 m for N-2, 2500 m for N-3, and 1645 m for N-4, respectively), which belonged to Paleogene-Neogene strata. The pH's of the samples were all weak acidic. The concentrations of total dissolved solids ranged from 222 to 291 g/L. The distribution of the inorganic salts for the studied samples was similar, in which $Cl^-$, $Na^+$, and $Ca^{2+}$ were dominant ions. For the four samples studied the amounts of dissolved carbon ranged from 15.0 to 83.1 mg/L. The contents of total dissolved nitrogen were very high, and the most of the nitrogen was in inorganic forms. In general, the concentrations of the carbons were negatively correlated with the well depths, while the contents of nitrogen were positively correlated with the depths for the studied samples.

### FTIR Characterization

In Figure 1, we observed that the spectra for the DOM samples exhibited similar spectroscopic features due to the overlapping of functional groups. All samples show adsorption peaks of aliphatics, carboxylic-containing compounds, aromatics, amides, aliphatic alcohols, and carbohydrates. More aliphatics were found in the shallower oil wells, while relatively abundance of branched and small aliphatic structures existed in the

deeper wells. The content of carboxylic-containing compounds is negatively correlated with the well depth, while that of carbohydrates is positively correlated with the depth.

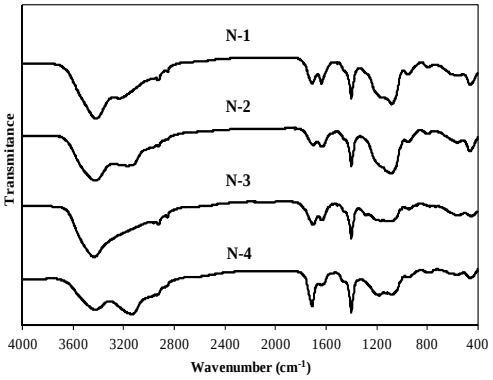



**Figure 1.** FTIR spectra of the isolated DOM samples.

### $^1$H NMR Characterization

The $^1$H NMR spectra, as well as the percentage of the total integrated peaks in each region of the spectra, of the four DOM samples, were presented in Figure 2 and Table 1, respectively. The purel aliphatics were found negatively correlated with the well depths; however, the abundance of carbohydrates were positively correlated with the depth. The contents of functionalized aliphatics and aromatics presented no obvious changes with well depths. Only a small amount of alkenes were detected in all the DOM samples.

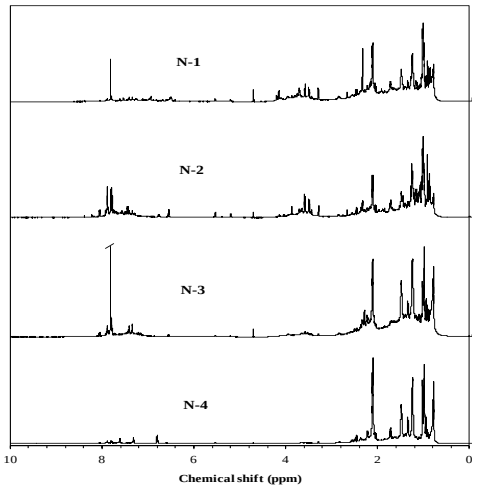



**Figure 2.** $^1$H NMR spectra of the isolated DOM samples.

**Table 1.** Percentages of the different types of hydrogen in the studied DOM samples

| Samples | HC-C-C | HC-N, HC-C-O | HCO | HC=C, HCO$_2$ | H$_{ar}$ |
|---|---|---|---|---|---|
| | 0.5-1.95 ppm | 1.95-3.1 ppm | 3.1-4.6 ppm | 4.7-6.0 ppm | 6.0-10.0 ppm |
| N-1 | 54.13 | 24.07 | 12.22 | 1.04 | 8.52 |
| N-2 | 56.36 | 16.45 | 11.02 | 1.05 | 15.11 |
| N-3 | 60.99 | 21.71 | 4.88 | 0.59 | 11.83 |
| N-4 | 65.54 | 25.01 | 2.66 | 0.58 | 6.21 |

### Py-GC/MS Characterization

The total ion current pyrograms of the Py-GC/MS analysis of the studied samples were presented in Figure 3. A total of 188 compounds was assigned and further classified as cycloparaffins, carbohydrate products, monocylic aromatic hydrocarbons, methylene chain compounds, nitrogen-containing compounds, polycyclic aromatic hydrocarbons, phenols, sulfur-containing compounds, and halogen-containing compounds.

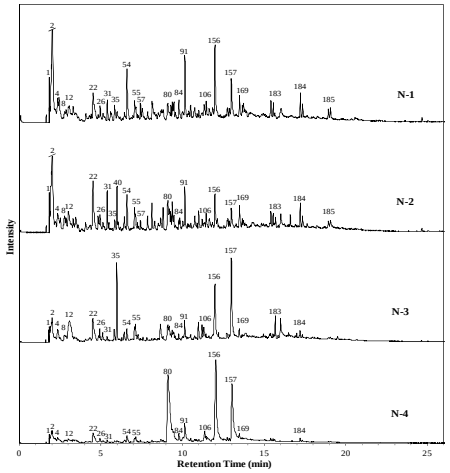



**Figure 3.** Py-GC/MS total ion current pyrogram for all the studied DOM samples.

Aliphatics were the dominant components of the studied DOM samples which was in line with the $^1$H NMR results. The contents of halogen-containing compounds, cycloparaffins, carbohydrates, and polycyclic aromatic hydrocarbons positively correlated with the well depths, while that of methylene chain compounds and phenols negatively correlated with the depths. Other compounds have no obvious variations with the depths.

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# Structural investigation of humic substances and natural organic matter using 2D NMR, FTICR MS and optical spectroscopy

A. ZHEREBKER [a,b,c], Y. KOSTYUKEVICH [a,b], E. SHIRSHIN [d], O. KHARYBIN [a], A. KONONIKIN [b], I. V. PERMINOVA [c], E. NIKOLAEV [a,b]*

(a) Skolkovo Institute of Science and Technology, 143025, Skolkovo, Moscow region, Russia
(b) Institute for Energy Problems of Chemical Physics of RAS, 119334 Moscow, Russia
(c) Department of Chemistry of Lomonosov Moscow State University, 119991, Moscow, Russia
(d) Department of Physics of Lomonosov Moscow State University, 119991, Moscow, Russia
* E-mail: e.nikolaev@skoltech.ru

**Keywords:** humic, structure, FTICR MS, deuteromethylation, fluorescence, fractionation

**Abstract.** Here we report an application of fractionation, 2D NMR, FTICR MS and optical measurements for mapping phenols, aliphatic and aromatic carboxylic acids and ketoacids on Van Krevelen diagram for HS. We applied sequential SPE on PPL cartridges with gradual lowering of the pH. 2D NMR and fluorescence revealed a shift in nature of COO-groups and changes in terrestrial/DOM components proportions. Application of ultra-high resolution FTICR MS showed interconnection between molecular composition and structure of components. Using deuteromethylation it was also shown the connection between structure and a number of carboxylic groups.

## Introduction

Humic substances (HS) are composed of a number of organic acids, which carry different amounts of carboxylic and phenolic units [1]. Application of Fourier transform ion cyclotron resonances mass-spectrometry (FTICR MS) with electrospray ionization (ESI) [2] reveals thousands of molecular compositions in every HS sample. This hinders the determination of structural fragments of HS. Even most powerful NMR approaches cannot unambigously resolve peaks of individual compounds.

In our previous work devoted to deuteromethylation coupled to FTICR MS, we found that HS from different sources displayed similar distribution of carboxylic acids on Van Krevelen diagram characterized by a gradual shift from non-carboxylated lignins, condensed tannins and carbohydrates to carboxylic rich unsaturated oxygenated compounds [3]. The observed pattern pointed out the possibility of preparative separation of HS ensemble using differences in pKa values of their major acidic compartments: phenols, aliphatic and aromatic carboxylic acids and ketoacids [4].

Recently, Dittmar et al. [5] proposed a use of SPE cartridges to isolate both hydrophilic and hydrophobic compounds of dissolved organic matter (DOM). This open new opportunitity to effectively separate HS components with different acid-base properties. Sorption on PPL cartridges is implemented by hydrophobic interaction. So, compounds with different pKa values will interact with hydrophobic resin at different pH: phenols (pKa>7), carboxylic acids (pKa ~ 5 - 4), aromatic carboxylic acids (pKa >3), β-keto carboxylic acids or polycarboxylic acids (pKa<2.5). Here we report about using sequential extraction at different pH values on the SPE PPL cartridge for isolation of distinct molecular constituents of HS with similar acidity or proton-accepting ability. This was confirmed by a combination of analytical techniques: 2D NMR, FTICR MS, selective labeling and fluorescence measurements. The developed approach was applied for separation of two humic materials from different sources - coal hymatomelanic acid and the Suwannee River humic acid (SRHA) and for 2 soil DOM.

## Materials and methods

**Fractionation of HS and DOM using SPE on PPL cartridges.** Fractionation was performed by four subsequent extractions at different pHs: 7, 5, 3 and 2. For the purpose, initial solutions were adjusted to pH 7, passed through the activated cartridge, and the exiting solution was collected and used for the follow-up fractionation by adjusting pH to 5 and discharging it through the second PPL cartridge. The procedure was repeated by adjusting pH to 3 and finally – into 2. The isolated fractions were assigned the indexes with the corresponding pH value (e.g., CHM-7 for pH 7). The obtained fractions were divided in 2 portions. First one was used for FTICR MS analysis. Second portion was dried and redissolve for fluorescence and NMR spectroscopy

**Fluorescence measurements.** Fluorescence spectra were recorded using the FluoroMax-4 fluorometer (Horiba Hobin Yvon) in a quartz cuvette; the excitation wavelength was set to 280 nm, while the emission was detected in the 300-700 nm spectral range, and the excitation and emission slit widths were set to 5 nm.

**FTICR MS**. All experiments were performed on FT MS Bruker Apex Ultra with harmonized cell (Bruker Daltonics) equipped with a 7 T superconducting magnet and electrospray ion source (ESI) in negative ionization mode located at the facilities of the Institute of Biomedical Chemistry of RAMS. Data were processed using the lab-made “Transhumus” software with sensible chemical constraints (O/C ratio $\leq$ 1, H/C ratio $\leq$ 2, element counts (C $\leq$ 120, H $\leq$ 200, O $\leq$ 60, N$\leq$ 2, S $\leq$ 1) and mass accuracy window < 0.5ppm).

**Deuteromethylation.** General procedure is described in our previous study [3].

**NMR spectroscopy**. Experimental conditions of $^{13}C$ NMR spectroscopy are described in [6] and for acquisition of HMBC and HSQC spectra are reported in [7].

## Results and Discussion

### NMR spectroscopy

HMBC experiments provide long-range $^{1}H$-$^{13}C$ connectivities. We observed series of cross peaks of aliphatic protons with carboxyl moieties ($^{13}C$ > 170 ppm) in fraction isolated at pH 5. This indicates the aliphatic character of carboxylic acids. However, unlike riverine dissolved organic matter we explored a correlation of the same protons with aliphatic (<50 ppm) and aromatic carbons (110-150 ppm). This is likely 3 bonds correlation, which indicates the presence of phenylpropanoic acids and matches the predicted spectrum (Fig. 1).

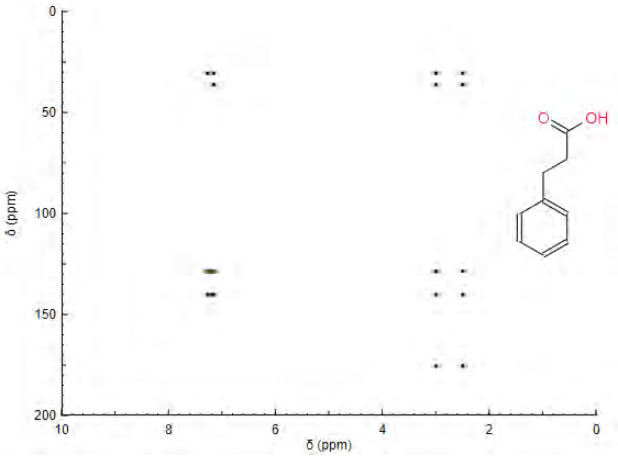



**Figure 1.** Predicted HMBC spectrum of phenylpropanoic acid matching pH 5 fraction.

This supports the contribution of lignin-like structures in hydrophobic fractions. pH-3 fraction is depleted with aliphatic carboxyls. We observed correlation of only aromatic protons with COOH moieties. Aromatic acids possess lower pKa, which explains the isolation at pH 3. These aromatic protons are also connected to aromatic carbon. Compared to pH-5 we did not observe 2-bond correlation of aliphatic $^{1}H$ and $^{13}C$ on pH-3. Therefore, NMR results show a change of major type of carboxylic acids corresponded to decrease of pKa. The results are supported by quantitative $^{13}C$ NMR and measurement of humification indexes determined by fluorescence measurements.

### Determination of molecular composition

Application of FTICR MS to study showed clear shift in molecular composition form lipids and lignins isolated at pH-7 to highly oxidized condensed compounds, often referred to hydrolyzable tannins. Comparison of FTICR MS data with 2D NMR spectroscopy allowed to attribute lignin components to phenols or aliphatic carboxylic acids, while most oxidized species correspond to aromatic and keto-acids, which is also supported by $^{13}C$ NMR. In addition, we explored that shift of molecular composition is accompanied by changes in a number of carboxylic groups carried by individual molecules. Hydrophobic fractions are composed of non-carboxylic compounds, while hydrophylic fractions consist of polycarboxylic compounds as is shown in Fig. 2.

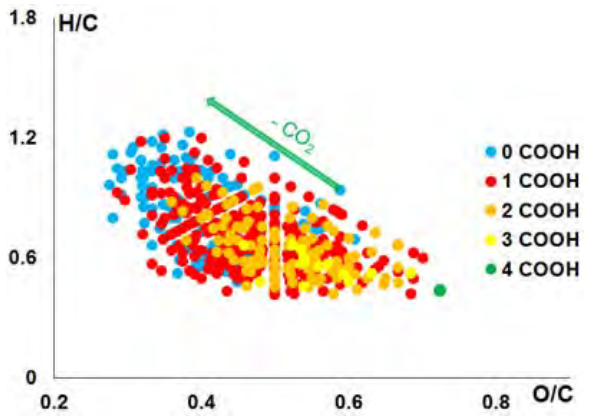



**Figure 2**. Van Krevelen diagrams for 500 most abundant CHO molecules determined by FTICR MS with a number of carboxylic groups highlighted in blue (0), red (1), orange (2), yellow (3) and green (4).

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# TOPIC 2.
# “BIOTIC INTERACTIONS - TOXICITY - ROLE OF HUMIC SUBSTANCES AND NATURAL ORGANIC MATTER”

# Conformational and structural changes caused by Laccase-enzyme modify the bioactivity of a sedimentary humic acid

J. ARANAZ [(a)], D. DE HITA[(a)], O. URRUTIA [(a)], M. FUENTES [(a)], G. MONREAL[(a)], R. BAIGORRI [(a,b) *], J. M. GARCÍA-MINA [(a)]

(a) Department of Environmental Biology, University of Navarra, 38008, Pamplona, Spain
(b) Department of Technical Development, TIMAC AGRO INT, Lodosa, Spain
* E-mail: roberto.baigorri@timacagro.es

**Keywords:** Bioactivity, Laccase, humic acid, structural arrangement

**Abstract.** Although the ability of humic substances to enhance plant growth is linked to their effects on some hormonal and nutritional pathways; more explanation is needed about the HA structural features that provide these biomolecules with this biological activity. Here, we show the structural changes caused by an enzymatic (Laccase) treatment (HA enz) in a sedimentary humic acid (HA), and their effects on the ability of HA to increase the growth of two different plant species (*Arabidopsis Thaliana* and *Cucumis Sativa*). The enzymatic treatment caused structural changes in HA, which included a decrease in the molecular size distribution accompanied by a re-organization of fluorophores indicating relevant conformational changes. These structural changes induced by Laccase led to a main reduction in the HA ability to promote plant growth.

## Introduction

Bioactivity of complex molecules is linked to both their chemical structure and molecular conformation in solution. Among the natural biomolecules present in soil, HAs are one of the most important molecular family in relation to plant nutrition in natural ecosystem as well as in agricultural crops [1].

Functionality in HA may be reflected by carboxylic and phenolic groups among others. And probably these groups are the most abundant in humic structures. In soils, phenolics are mainly degraded by fungi (e.g., Basidiomycetes and Ascomycetes) and bacteria (e.g., Pseudomonas) [2]. These microorganisms release extracellular enzymes into soils that break down phenolic compounds. One of the most common and used enzyme is Laccase that may be released by *Trametes versicolor*, Basidiomycete PM1 and *Pycnoporus sanguineus*. Using Laccase as breakdown-enzyme, we have studied structural changes in HA and the effects of these changes on the ability of HA to promote the growth of two different plant species: *Arabidopsis Thaliana* and *Cucumis Sativa*. As bioindicators we have used the fresh and dry weight of the shoot (FW Shoot, DW Shoot) and the root (FW Root, DW Root).

## Materials and methods

Seeds of cucumber and arabidopsis were germinated in darkness in a seed germination chamber. After germination, plants were transferred to hydroponic solution. The pH of the nutrient solutions was held at 6.0 and did not change significantly during the experiment. Experiment was performed in a growth chamber at 25/21 ºC, 70-75% relative humidity and with 15/9 h day/night photoperiod (irradiance: 250 µmol $m^{-2}$ $s^{-1}$) [3].

Synchronous fluorescence spectra were recorded in a Jasco FP-8200 Spectrofluorometer from 250 to 600 nm with $\Delta\lambda$ = 18 nm following Peuravuori et al. [4].

Solid-state $^1$H-NMR experiment was measured at 11.7 T in a Bruker Avance XDR-500 spectrometer operating at 500 MHz. The spectra were processed and the signals were assigned with MestreNova software v10.0.

Functional groups were measured by potentiometry. HP-SEC molecular size distributions were assessed by means of a chromatographic system consisted of a Waters 2795 Alliance followed by a Waters 2996 Photodiode Array Detector set at 280 nm. Size exclusion separation occurred through a XBridge BEH 200 Å SEC 3.5 µm 7.8x150 mm column (Waters).

## Results and Discussion

Lacasse effect was studied by measuring the decrease in the phenolic content expressed as very weak acidity measured by potentiometry.

**Table 1.** Measurement of functional groups

| mmol $g^{-1}$ HA | HA | HA enz |
|---|---|---|
| Strong Acidity | 0.07 | 0.13 |
| Weak Acidity (Carboxylic) | 1.72 | 2.50 |
| Very Weak Acidity (Phenolic) | 1.28 | 0.78 |
| Total Acidity | 3.07 | 3.41 |

HA enz showed, a decrease in the phenolic content but also showed an increase in the strong, weak and total acidity.

Phenolic groups loss also was observed by $^1$H-NMR. Signal around 8 ppm (and related to -OH) decreased in HA enz thus supporting potentiometric data.

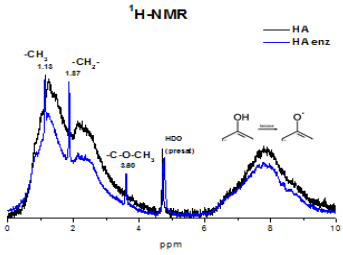



**Figure 1.** $^1$H-NMR of HA and HA enz showing a phenolic groups loss.

In Figure 2 we can observe the enzyme effect on size distribution. HA enz presented an increase in the lower size-related peak and a decrease in the higher size-related peak. This fact implies a slight disaggregation of higher size populations to lower size populations.

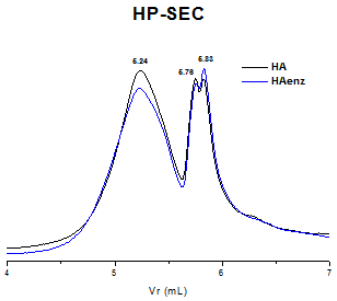



**Figure 2.** HP–SEC of HA and HA enz showing a disaggregation.

Different molecular conformation is showed by the synchronous fluorescence study. As for HA enz, we observed an increase in the intensity of fluorophores corresponding to more complex and condensate (450-600 nm) conformations. This in increase may be caused by a concomitant increase in structural rigidity. However, the interpretation of these changes is complicated since, as Burke and Cairney [5] pointed out, "…laccases can mediate both in depolymerization and polymerization and that, without the knowledge of redox mediators for these enzymes, predicting the direction of phenolics processing may be difficult". Moreover, laccase action is also substrate-dependent [6]. This fact also suggests that the amount and distribution of phenol groups in HA structure might play a key role in the nature of the reactions induced by Laccase.

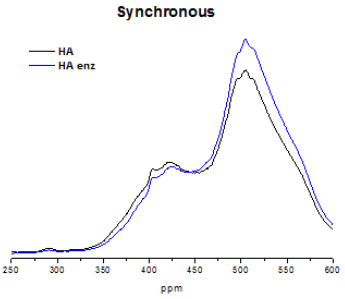



**Figure 3.** Synchronous fluorescence of HA and HA enz.

As for the consequences of Laccase on the plant growth promoting action of HA, the results showed that HA enz has a lower ability to stimulate both shoot and root growths. showed in both plants a biostimulant effect in growth. This fact suggest that the native structure and molecular conformation play a crucial role in the beneficial action of HA on plant growth

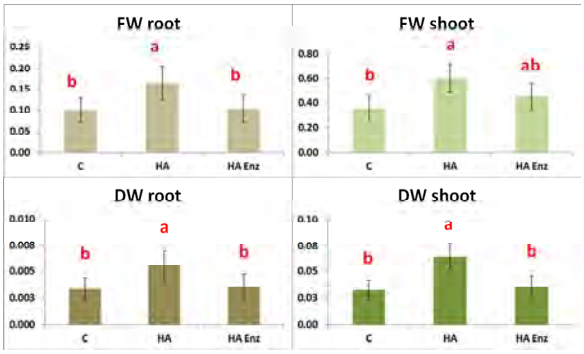



**Figure 4.** Biological activity in Arabidopsis.

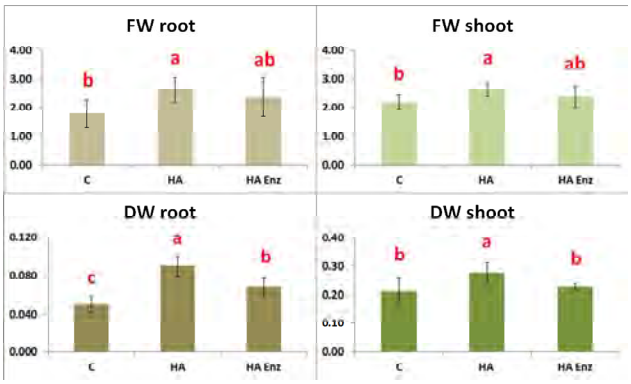



**Figure 5.** Biological activity in Cucumber.

As a conclusion, these results indicate that not only the type of functional groups but also their distribution and influence on the molecular conformation of HA in solution, play a crucial role in the HA ability to enhance plant growth.

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# The effect of organic carbon content on mobility and potential bioavailability of trace elements in bottom sediments

A. BARAN [(a)*], M. SZARA [(a)], M. TARNAWSKI [(b)], T. KONIARZ [(b)], M. MIERZWA-HERSZTEK [(a)], K. GONDEK [(a)]

(a) Department of Agricultural and Environmental Chemistry, University of Agriculture, Krakow, Poland
(b) Department of Hydraulic Engineering and Geotechnics, University of Agriculture, Krakow, Poland
* E-mail: Agnieszka.Baran@ur.krakow.pl

**Keywords:** trace elements, organic carbon, mobility, sequential fractionation, etocoxicity

**Abstract.** The aims of the studies were to assess a possible relationship between the observed content of organic carbon and mobility and potential bioavailability of trace elements in bottom sediments. Sediment organic matter significantly affected the distribution of the metals among different geochemical fractions.

## Introduction

Trace elements are a significant part of bottom sediment contaminants, which, at some concentrations, may be toxic to the aquatic ecosystem. Total content of trace elements might serve as useful indicators for assessment of sediment contamination. However, they cannot provide important information to identify the environmental impact of contaminated sediments because trace elements are present in different chemical forms in sediments. Forms of elements determine their mobility, and potential bioavailability [1, 2]. Content of organic matter is the chief measure of the capacity of bottom sediments to accumulate of traces elements [3]. Organic matter has a high capacity to complex and adsorbs cations due to the presence of numerous negatively charged groups.

The aims of the studies were: (i) to assess the mobility of trace elements in sediments by using chemical fractionation, and, (ii) to analyze a possible relationship between the observed content of organic carbon and mobility and potential bioavailability of trace elements.

## Materials and methods

### Study area and sampling

The reservoir is located in one of the most industrialized areas of Poland, which affects the contamination of the sediments. The samples of bottom sediments form Rybnik reservoir were collected from 33 stations.

### Laboratory analyses

The fraction analysis of metals was performed using the three-step method of sequential fractionation by means of the modified BCR technique: fraction I – exchangeable and acid-soluble fraction, fraction II – forms associated with free Fe and Mn oxides; fraction III – forms bound to organic matter.

Metal concentrations were assessed using ICP-OES method by Optima7300 DV PerkinElmer. Moreover**,** total organic carbon (TOC), carbon humic acid (Cha), fulvic acids (Cfa) and carbon hydrolysing (Cnh) and dissolved organic carbon (DOC) were analyzed.

## Results and Discussion

Fractionation of trace elements showed that organic matter is likely to be an important factor which controls metal distribution and mobility in the sediments. The highest amount of trace elements was associated with fraction III (organic), which indicates that this form of metals was dominant in sediments of the Rybnik reservoir. The results can provide a better understanding of environmental risks of heavy metals in sediments.

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# The role of organic matter on pollution content and ecotoxicity of bottom sediments

A. BARAN [(a)*], M. MIERZWA-HERSZTEK [(a)], K. GONDEK [(a)], M. SZARA[(a)], M. TARNAWSKI [(b)], T. KONIARZ [(b)]

(a) Department of Agricultural and Environmental Chemistry, University of Agriculture in Krakow, Poland
(b) Department of Hydraulic Engineering and Geotechnics, University of Agriculture in Krakow, Poland
* E-mail: Agnieszka.Baran@ur.krakow.pl

**Keywords:** bottom sediments, organic matter, pollutions, toxicity, biotest

**Abstract.** The aims of studies were to assess content of organic matter fractions in bottom sediments and to determine their influence on content of pollution and toxicity of sediments. In order to identify the degree of pollution of sediments, it is important to assess content and composition of organic matter.

## Introduction

Bottom sediments are important part of aquatic ecosystems [1]. They are a site affluent in nutrients for microorganisms, plants and animal organisms, therefore constituting an important component of matter and energy cycle in water reservoirs. On the other hand, they are a place of various substances deposition, whereas their structure makes them a natural geosorbent, in which pollutants introduced to the aquatic environment accumulate [2, 3]. Organic matter is one of the most important components of bottom sediments, and is mainly responsible for the sorption properties of bottom sediments [4]. Humic substances have an important influence on the fate of environmental pollution (heavy metals, PAHs, dioxin, pesticides).

The aims of studies were as follows: to determine the content and composition of organic matter in bottom sediment collected from the Rożnów reservoir, and to determine their impact on the content of selected pollutants.

## Materials and methods

**Study area and sampling**. The bottom sediments were collected from Rożnów reservoir located in South Poland. The samples of bottom sediments were collected from 53 stations.

**Laboratory analyses**. Following compounds of carbon were analyzed: total organic carbon (TOC), carbon humic acid (Cha), fulvic acids (Cfa) and carbon hydrolysing (Cnh), black carbon (Cbc). The total trace element content was assessed using the ICP-OES method with the use of Optima 7300 DV PerkinElmer. Content of the PAHs was determined by gas chromatography-mass spectrometry on a Varian GC/MS/MS 4000 equipped with an ion trap. The sediment ecotoxicity was carried out using biotests: Phytotoxkit, Ostracodtoxkit, and Microtox.

## Results and Discussion

The fraction of Ckf was dominated in samples of sediment 5. The contents of heavy metals and PAHs in sediments demonstrate that these pollutants have a good affinity for organic carbon in the Rożnów reservoir sediments. Bottom sediments were non-toxic or low-toxic to organisms, and there was observed a significant relationship between the content of TOC and Ckf and the reaction of *H. incongruens* and *S. alba.* Organic carbon is an important factor controlling the content of pollutants in bottom sediments, and thus their bioavailability and toxicity.

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# Effect of sugars on the structure and morphological features of mixed biofilms

I. GANCHEV [(a)*]

(a) Department of General Microbiology, The "Stephan Angeloff" Institute of Microbiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. Bldg. 26, Sofia 1113, Bulgaria
*E-mail: ivotg@abv.bg

**Keywords:** biofilms, *B.subtilis*. *E.coli* K-12, exopolysaccharides

**Abstract:** *Bacillus subtilis* is a plant-beneficial Gram-positive bacterium widely used as a biofertilizer. In contrast, much is known about how this bacterium forms matrix-enclosed multicellular communities (biofilms) in their assosciation with another microbial species *in vitro*. The morphological features of biofilms formed by joint cultivation of *B. subtilis* 170 and *E.coli* K-12 1655, *B. subtilis*168 and *E.coli* K-12 1655 strains were established in the presence of different kind of sugars in the medium in this study.

## Introduction

The rhizosphere, the portion of soil surrounding plant roots, is a nesting site for a rich and dynamic community of microorganisms that are plant pathogens or live with plants as mutualists. Bacteria that thrive in the rhizosphere, colonize the roots and promote plant growth are known as plant growth-promoting rhizobacteria. One such plant growth-promoting rhizobacterium is the Gram-positive bacterium *Bacillus subtilis* that are also found in association with roots of many different plants and promotes plant growth via the secretion of cytokinin hormones and volatiles that modify plant hormone homeostasis [1].

The Gram-positive bacterium *Bacillus subtilis* forms surface attached matrix-enclosed multicellular communities known as biofilms. The matrix, which provides structure to the biofilm, is composed of two very different components: an amyloidl-ike protein, TasA, and an exopolysaccharide (EPS). The presence of EPS is a nearly universal feature of biofilms and in some cases accounts for 90% of the colony mass. In *B. subtilis*, a 15-gene operon, *epsA* to *epsO* (*epsA-O*), encodes the proteins responsible for the synthesis of this polysaccharide. While the structural role of the EPS in the biofilm has been well established, the precursor metabolites used in its synthesis and its final composition are not known [2]. In this respect, the aim of this study is the evaluation of the relationship between the type of sugars in the medium for a cultivation and morphological features of mixed biofilms formed at the association of *B. subtilis* and *E. coli* strains.

## Materials and methods

**Bacterial strains.** The bacterial strains used in the present study are *B. subtilis* 170, *B. subtilis*168 and *E.coli* K-12 1655. The strains were cultured in LB broth. All strains were inoculated into 9 ml of liquid broth and incubated at 37 ° C for 18 hours before each experiment.

**Method for cultivation of mixed biofilms.** The cultivation of mixed biofilm of pairs *B. subtilis* 170 and *E.coli* K-12 1655, *B. subtilis*168 and *E.coli* K-12 1655 and the estimation of their morphological features were made on microscope cover glasses described by Vacheva and co-workers [3].

## Results and Discussion

Exopolysaccharide is a key component of the biofilm matrix in *B. subtilis* since mutants deficient in EPS production are unable to form biofilms. Their biosynthesis is indirectly controlled by the master regulator Spo0A via the expression of the antirepressor SinI. Glucose has been shown to be essential for the synthesis of various exopolysaccharides, such as colanic acid in *E. coli*. It had been demonstrated that GtaB, which catalyzes the formation of UDP-glucose from glucose-1-P and UTP, is necessary for biofilm formation in LB medium by the *B. subtilis* domesticated strain 168. The requirement for this molecule meant that UDP-glucose is either involved as a signalling molecule or as a precursor for synthesis of EPS [2]. Figures 1 and 2 present microscope data of morphological characteristics of mixed biofilms after 24 h cultivation in this study. The association of *B. subtilis* 170 and *B. subtilis* 168 strains with strain *E. coli* K-12 1655 leads to the formation of structures in the present study which are characterized by an average thickness of 10.35±0.41 and 10.78±0.37 μm at roughness ratio of 0.02-0.03 in the presence of glucose, which is changed to values of up to 8.83±0.45 and 9.98±0.33 μm at the roughness coefficient of 1.22-2.11 at the replacement with lactose. The presence of maltose at a concentration of 100 M ensures the formation of biofilms with mean thickness 0.35±0.41 μm resulted by the interaction of *B. subtilis* 170 and *E. coli* K-12 1655 strains and 0.78±0.37 μm at the co-cultivation of *B. subtilis* 168 and *E. coli* K-12 1655 strains with an unevenness factor in the range of up to 0.02-0.03, which represents 52.23 and 81.25 % of their values when exchanged with xylose and in the presence of lactose (Table 1). The result of Chai and co-workers [2] suggests a coordinated regulation of the *galE* gene and the *epsA-O* operon mediated by Spo0A, but independent of *sinI* activation, during biofilm formation. It is notable that the basal level of *galE* gene expression was quite significant.

The relative volume per unit area varies in the wide range over of biofilms resulting from the interaction of the *B. subtilis* 170 and *E. coli* K-12 1655, *B. subtilis* 168 and *E. coli* K-12 1655 strains depending on the nature of sugars contained in the medium for cultivation in this study ($p < 0.05$).

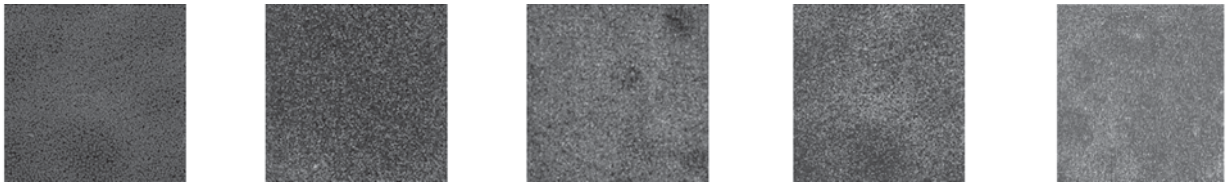



**Figure 1.** Microscopic study of biofilms of strains *Bacillus subtilis* 170 and *Escherichia coli* K-12 1655 in the presence of different sugars (100 mM) in the medium. Growth of biofilms was performed on microscope cover glasses for 24 hours at a temperature of 20 ° C. The staining was done with a Congo Red complex fluorescent dye, and observations were made using a confocal laser scanning electron microscopy at a wavelength of 540 nm.




**Figure 2.** Microscopic study of biofilms of strains *Bacillus subtilis* 168 and *Escherichia coli* K-12 1655 in the presence of different sugars (100 mM) in the medium. Growth of biofilms was performed on microscope cover glasses for 24 hours at a temperature of 20°C. The staining was done with a Congo Red complex fluorescent dye, and observations were made using a confocal laser scanning electron microscopy at a wavelength of 540 nm.

**Table 1.** Morphological characteristics of mixed biofilms of *Bacillus subtilis* 170 and *Escherichia coli* K-12 1655, *Bacillus subtilis* 168 and *Escherichia coli* K-12 1655 strains in the presence of different sugars (100 mM) in medium

| Sugar concentration, 100 mM | Mean thickness, μm | Robust coefficient | Relative area, $\mu m^2$ | Relationship volume to area, $\mu m^2.\mu m^{-3}$ |
|---|---|---|---|---|
| Biofilm of *Bacillus subtilis* 170 and *Escherichia coli* K-12 1655 strains | | | | |
| Maltose | 0.35±0.41 | 0.02 | 0.49±0.12 | 0.74±0.33 |
| Xylose | 0.67±0.11 | 0.95 | 0.68±0.04 | 0.78±0.07 |
| Saccharose | 0.52±0.05 | 1.02 | 0.44±0.01 | 0.39±0.02 |
| Lactose | 8.83±0.45 | 1.22 | 0.75±0.04 | 1.48±0.08 |
| Glucose | 10.35±0.41 | 0.02 | 0.89±0.12 | 1.74±0.33 |
| Biofilm of *Bacillus subtilis* 168 and *Escherichia coli* K-12 1655 strains | | | | |
| Maltose | 0.78±0.37 | 0.03 | 0.47±0.21 | 0.78±0.23 |
| Xylose | 0.96±0.15 | 0.56 | 0.61±0.03 | 0.99±0.05 |
| Saccharose | 0.60±0.02 | 1.12 | 0.58±0.01 | 0.55±0.02 |
| Lactose | 9.98±0.33 | 2.11 | 0.80±0.03 | 1.64±0.05 |
| Glucose | 10.78±0.37 | 0.03 | 0.87±0.21 | 1.78±0.23 |

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# Role of biosurfactants of Bacillus subtilis 170 and 168 on mixed biofilm formation

I. GANCHEV [(a)*]

(a) Department of General Microbiology, The "Stephan Angeloff" Institute of Microbiology, Bulgarian Academy of Sciences, "Acad. Bonchev" Str., Bldg. 26, 1113 Sofia, Bulgaria
* E-mail: ivotg@abv.bg

**Keywords:** biosurfactant, biofilm, *B. subtilis*, biomass of biofilm

**Abstract.** The plant growth-promoting bacterium *Bacillus subtilis* is frequently found associated with plant roots where it protects plants from infection and forms bio films. These microbial strains secrete surfactin that acts as an antimicrobial agent against pathogens. The aim of this study is the evaluating of impact of the concentration of biosurfactants isolated from *Bacillus subtilis* 170 and 168 strains on their biofilm capacity in association with *E.coli* K-12 1655 strain.

## Introduction

*Bacillus subtilis* is a flagellated bacterium ubiquitously distributed in the environment and recognized as a potent agent for the biological control of plant diseases. It possesses remarkable metabolic and physiological versatility that allows its growth in diverse settings within the biosphere, including solid substrates in many different environments [2] and can directly prevent infection of the plant by bacterial pathogens by releasing the AiiA enzyme. AiiA is a lactonase that functions by inactivating acylhomoserine-lactone molecules that regulate the expression of virulence genes by many plant pathogens [1]. *B. subtilis* also secretes surfactin that acts as an antimicrobial against pathogens such as *Pseudomonas syringae*. Finally, *B. subtilis* can indirectly protect plants by inducing systemic resistance, which affords the plant protection against a broad range of pathogens [2]. However, in contrast to biofilm formation, in which surfactin acts as an autoinducer or a quorum-sensing signal leading to the derepression of genes involved in matrix synthesis [1, 2], nothing is known about the activity of the lipopeptide with respect to mechanism of dispersion of biofilms formed by different microbial species.

The aim of this study is the evaluating of impact of the concentration of biosurfactants isolated from *Bacillus subtilis* 170 and 168 strains on their biofilm capacity in association with *E.coli* K-12 1655 strain.

## Materials and methods

**Bacterial strains.** The bacterial strains used in the present study are *B. subtilis* 170, *B. subtilis*168 and *E.coli* K-12 1655. The strains were cultured in LB broth. All strains were inoculated into 9 ml of liquid broth and incubated at 37 ° C for 18 hours before each experiment.

**Isolation of biosurfactants and preparation of media.** The isolation of biosurfactants and prepare of mediums were made according to methods described by Ghelardi and co-workers [2].

**Method for cultivation of mixed biofilms.** The cultivation of mixed biofilms of pairs *B. subtilis* 170 and *E.coli* K-12 1655, *B. subtilis*168 and *E.coli* K-12 1655 was made on microliter plate described by Vacheva and co-workers [3]. The number of colonies of B. subtilis and E.coli strains was evaluated according to method described by Vacheva and co-workers [3].

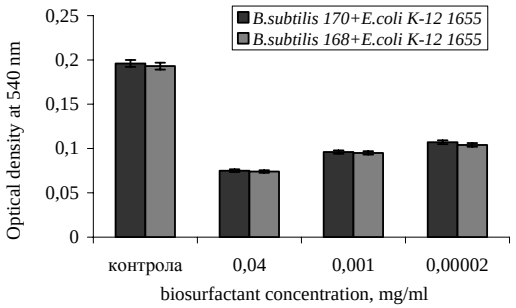



**Figure 1.** Impact of the biosurfactant concentration on the optical density at 540 nm of the mixed biofilms after staining with Crystal violet.

**Table 1.** Impact of biosurfactant on the number of colonies of *B.subtilis* 170 and *E.coli* K-12 1655 strains and the spores in the structure of mixed biofilms

| № | Biosurfactant concentration, mg/ml | Number of colonies in mixed biofilms, cfu/ml | | Spores, cfu/ml |
|---|---|---|---|---|
| | | *B.subtilis* 170 | *E.coli* K-12 1655 | |
| 1. | 0 | $(15.3\pm0.16).10^6$ | $(14.2\pm0.10).10^6$ | $(5.8\pm0.2).10^2$ |
| 2. | 0.0002 | $(2.17\pm0.02).10^4$ | $(0.72\pm0.03).10^4$ | $(0.8\pm0.00).10^2$ |
| 3. | 0.01 | $(1.57\pm0.01).10^4$ | $(0.27\pm0.03).10^4$ | $(0.6\pm0.00).10^2$ |
| 4. | 0.04 | $(0.52\pm0.02).10^4$ | $(0.12\pm0.00).10^4$ | 0 |

**Table 2.** Impact of biosurfactant on the number of colonies of *B.subtilis* 168 and *E.coli* K-12 1655 strains and the spores in the structure of mixed biofilms

| № | Biosurfactant concentration, mg/ml | Number of colonies in mixed biofilms, cfu/ml | | Spores, cfu/ml |
|---|---|---|---|---|
| | | *B.subtilis* 168 | *E.coli* K-12 1655 | |
| 1. | 0 | $(10.7\pm0.25).10^6$ | $(10.36\pm0.32).10^6$ | $(15.4\pm0.6).10^2$ |
| 2. | 0.0002 | $(2.38\pm0.05).10^4$ | $(0.42\pm0.02).10^4$ | $(4.6\pm0.4).10^2$ |
| 3. | 0.01 | $(1.66\pm0.02).10^4$ | $(0.06\pm0.00).10^4$ | $(4.2\pm0.30).10^2$ |
| 4. | 0.04 | $(0.6\pm0.03).10^4$ | $(0.02\pm0.00).10^4$ | 0 |

**Results and Discussion**

Decreasing of the concentration of biosurfactant in the medium from 0.04 to 0.0002 mg/ml leads to the proportional reduction of the biomass of the biofilms formed by establishing a linear relationship between the two elements according to results of the study ($r^2$=0.9685). The optical density at 570 nm after staining with crystal violet differs from the value of 0.107 ± 0.003 for the *B.subtilis* 170 and *E. coli* K-12 1655 at a biosurfactant content of 0.0002 mg / ml, which slightly decreases to a value of 0., ± 0.002 due to the increase in concentration to 0.04 mg / ml. Analogical results were observed during the formation of biofilms formed by the co-cultivation of B. subtilis 168 and E. coli K-12 1655 strains with a change in the biosurfactant content in the culture medium in the range of 0.0002 to 0.04 mg/ml, the optical density decreasing to 0.074 ± 0.003 with the addition of a biosurfactant at a concentration of 0.04 mg/ml (Fig. 1).

*E.coli* K-12 1655 strain has the greatest sensitivity to the action of biosurfactants, their population decreases to (0.12 ± 0.00).$10^4$ cfu/ml in association with *B.subtilis* 170 strain and (0.02 ± 0.00). $10^4$ cfu/ml as result of his interaction with *B.subtilis* 168 strain in the structure of the mixed biofilms formed at a biosurfactant content of 0.04 mg/ml. The number of colonies of B.subtilis 170 and B.subtilis 168 strains did not differ significantly at a concentration of the biosurfactant in the range of 0.0002 mg/ml to 0.01 mg/ml and was significantly decreased by increasing of the value to 0.04 mg/ml (Table 1 and 2). Sporeforming forms of bacteria are distinguished by greater resistance to adverse environmental factors and stress factors, which are at the root of their proliferation in the appearance of favourable environmental conditions and are preserved as resting cells upon sucking of the nutrient content in the medium [1, 2].

The established relationships correlate with the presence of spores in the structure of biofilms, the number of which reaches (0.6 ± 0.00).$10^2$ cfu/ml for *B.subtilis* 170 and *E. coli* K-12 1655 strain and (4.2 ± 0.30).$10^2$ cfu/ml for *B.subtilis* 168 and *E. coli* K-12 1655 strains at the biosurfactant content of 0.01 mg/ml, which significantly differs from the control sample in the absence of a biosurfactant. At a concentration of the biosurfactant in the range of 0.0002 to 0.01 mg/ml, were found no statistically significant differences between them, whereas the increase of their content to 0,04 mg/ml was accompanied by the inhibition of sporulation in the structure of mixed biofilms (Table 1 and 2).

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# Step-by-step formation of humic-like biologically active substances in the technological process of Lignohumate® synthesis

O. GLADKOV [(b)], O. YAKIMENKO [(a)*], O. OSIPOVA [(b)], R. POLOSKIN [(b)], A. STEPANOV [(a)]

(a) Department of Soil Science, Moscow State University, 119992 Moscow, Russia
(b) LTD RET, St-Petersburg, Russia
* E-mail: iakim@soil.msu.ru

**Keywords:** Lignohumate, humic-like substances, elemental content, FTIR, $^{13}$C-NMR, bioassay, PCA

**Abstract.** Composition, structure, and biological activity of humic-like substances (HLS) synthesized in the process of lignosulfonate conversion for the production of humic product Lignohumate® (LH) were examined. It is shown, that in the course of the hydrolytic-oxidative process the transformation of raw material and accumulation of HLS take place. Data on chemical parameters (elemental content, functional groups, carbohydrate content, MW, FTIR, $^{13}$C-NMR) and biological activity (in photo-test) combined with PCA show that LH-samples can be divided into three groups, depending on the duration of synthesis: initial raw material (0-time sample); "young" HLS (15-30 min) and "mature" HLS at 45-120 min of treatment.

## Introduction

Conversion of lignin-containing organic matter into qualitative organic fertilizers is an important task of the present. LTD RET company has developed and patented an industrial technology for the conversion of technical lignosulfonate with the following synthesis of a number of humic-like biologically active substances (HLS) [1], which finally compose the commercial humic product Lignohumate® (LH). Depending on the technological regimes used (alkali content, changes in temperature, pressure, oxidation rate and time), composition and properties of the obtained product are different. Yield, composition and biological activity of the synthesized HLS change with the course of the technological process.

This process can be conditionally considered as an imitation of organic matter (OM) evolution in diagenesis, taking into account only chemical (abiotic) factors of transformation.

The objectives of the study are: (1) to characterise chemical composition and biological activity of the product in a number of LH samples taken at different times during the technological process; (2) to reveal qualitative differences in LH composition as a function of time and to determine the optimum duration of the process for obtaining a high-quality product.

## Materials and methods

Samples of LH were taken from the heated working mixture at certain time intervals from the start of the oxidation process: at 0, 15, 30, 45, 60 and 120 min and after a forced two-hour exposure without oxygen supply (conservation; >120 min). Samples were analysed for content of (OM) as loss of ignition, the humic-like fraction (HLF) as dry weight of precipitate after acidification, and fulvic-like fraction (FLF) by a difference. Elemental (CHNS) composition, the content of ash, acid functional groups, carbohydrates and amino acids were determined in dried LH-samples. Molecular weight distribution was determined by SEC; FTIR and $^{13}$C-NMR spectra were recorded.

Biological activity was evaluated in laboratory photosets as an increment of the oven-dry biomass of shoots and roots. Radish seeds were treated with 0.02% solution of LH-samples with distilled water as the control and germinated at 23°C; oven-dry biomass was determined for 5-days seedlings. All bioassays were done in triplicate, ANOVA was carried out to determine statistical differences; the means were compared by the post hoc Fisher's least significance test (LSD).

**Statistics.** Principal component analysis (PCA) was carried out considering variables: pH; DM, g/l; OM, % DM; HLF, % OM; S,%; atomic ratios, functional groups, and root biomass.

## Results and Discussion

### Composition of HLS

In the course of oxidation process, gradual synthesis of HLS takes place (Fig. 1). At the beginning (15 min) content of HLF is less than 20% of OM, later it grows up and reaches a maximum at 60 min with the corresponding decrease in the proportion of FLF.

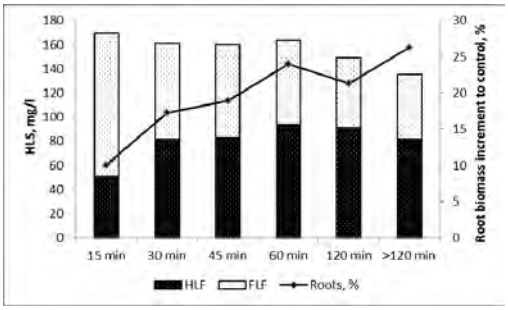



**Figure 1.** Dynamics of humic-like (HLF) and fulvic-like (FLF) fractions content and biological activity of LH depending on the sampling time.

All the LH-samples exhibit certain biological activity on plants (Fig. 1). Dry shoots biomass showed insignificant differences ($p<0.05$), whether root biomass significantly ($p>0.01$) increased and after 30 min

provided 17-26% increment. The highest activity was recorded for 60min-sample with the highest content of HLF.

From the beginning of the synthesis, a permanent transformation of OM takes place. Raw material (0-time) differs significantly from LH-samples. Elemental content changes drastically (Fig. 2); certain changes are also observed for the content of functional groups, carbohydrates and amino acids.

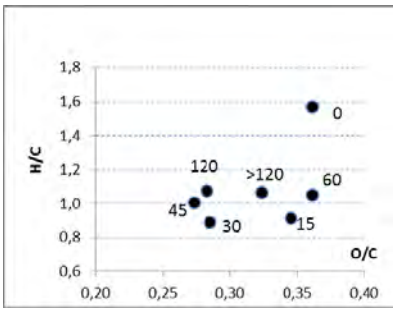



**Figure 2.** Atomic ratio diagram for LH sampled at different treatment time.

From 15 min MW-distribution begins to change: the share of high molecular weight fraction decreases and medium-molecular-weight fractions accumulate. After 60 minutes proportion of HMF increases again, and by 120 minutes it reaches 27% of the sum of fractions.

IR spectra differ from each other and from typical patterns for HA. Spectra contain both characteristic absorption bands of HS and other ones, especially in the range 1000-500 $cm^{-1}$ (Fig. 3) [3, 4].

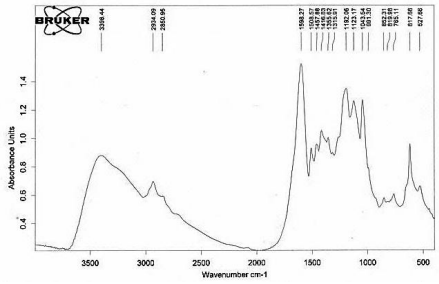



**Figure 3.** IR-spectra of LH sampled at 120 min.

The quantitative $^{13}$C-NMR spectra of LH-samples showed a low content of aliphatic carbons, the presence of a sharp signal of lignin O-$CH_3$ groups at a shift of 57 ppm, and signals of the phenolic carbons near 145 ppm. Besides, there are signals that indicate the presence of specific compounds, the nature of which is not clear at the moment.

### Identification of differences in LH-originated HLS using principal component analysis

PCA yielded two principal components (PC) with eigenvalues more than one. PC1 explains 60% of total variance and shows strong loadings for pH; DM, OM, H/C, O/C and root biomass. PC2 explains 28% of the total variances and shows strong loading for HLF and C/N. PCA plot (Fig. 4) shows that LH-samples can be divided into three groups, depending on the duration of the technological process of HLS synthesis: initial raw material (0-time sample); HLS of 1st 30 min and HLS at 45-120 min of treatment.

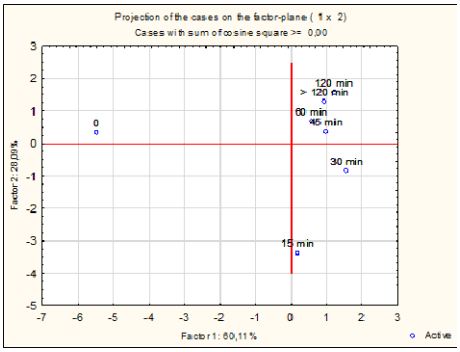



**Figure 4.** Factor score plot for LH sampled at different treatment time.

### Overall views

Already from the beginning of synthesis process an accumulation and transformation of HLS take place. Starting from 15 min, the content of HLF, elemental composition, the content of functional groups, molecular weight distribution, the character of IR and $^{13}$C-NMR spectra change. After 45-60 minutes the composition stabilizes, the share of high molecular weight fraction increases, and biological activity reaches the maximum. At that stage, the further synthesis process can be stopped.

Biological activity, one of the main quality indicators of the final product, reaches a maximum in samples of 45-60 minutes, before the maximum for HLF (120 min). This can indicate that other active compounds, probably low-molecular non-humic substances presenting in the LH, also affect the manifestation of biological activity.

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# Molecular and structural changes in dissolved organic matter in the Critical Zone

G. GLEIXNER [(a)*]

(a) Max Planck Institute for Biogeochemistry, Hans-Knöll-Straße 10, 07745 Jena, Germany
* E-mail: ggleix@bgc-jena.mpg.de

**Keywords:** DOM, FT ICR MS, ecosystem, isotope, NMR

**Abstract.** Dissolved organic matter (DOM) fundamentally impacts biogeochemical processes in the Critical Zone and ecosystem services such as organic matter storage, groundwater cleaning and nutrition of marine ecosystems are facilitated. During transport through the Critical Zone and with ongoing decomposition, it is assumed that DOM converges to recalcitrant molecules of low molecular weight and isomeric structures. Here we show a loss of small molecules (m/z = 154 - 370) with increasing depth, leading to an increase in the mean molecular size of DOM. Plant-derived molecules were preferentially consumed by microorganisms and transformed into larger microbial molecules lacking plant-related ligno-cellulosic structures. Our results suggests that DOM is not recalcitrant but instead mirrors the properties of the ecosystem it is formed in.

## Introduction

Dissolved organic matter (DOM) fluxes are an important component of the global carbon cycle [1, 2] that enable cycling and distribution of carbon and nutrients within and between ecosystems [3]. Therefore, DOM enables the functioning of the critical zone [4, 5]. However, the mechanisms of DOM formation, transformation and fate in soils and its transport into aquatic systems are still unclear. Here we focus on structural changes of DOM with soil depth.

## Materials and methods

**Field site and sampling.** Soil water samples were collected on semi-natural grassland with different grassland communities. The site, located in Jena, Germany (50°55' N, 11°35' E, altitude 130 m) close to the Saale River. All samples were collected in 10, 20, 30 amd 60 cm depth using glass suction plates.

**Sample Preparation.** On subsamples pH and the concentration of DOC (highTOC, sum parameter analyzer, Elementar Analysensysteme GmbH, Hanau, Germany) and dissolved organic nitrogen (DON) were mesured. The remaining samples were acidified to pH 2 and DOM was concentrated and desalted by solid phase extraction (SPE) [6].

**FT-ICR-MS.** We used the Bruker Solarix FT-ICR-MS (15 Tesla) at the University of Oldenburg (Germany) for FT-ICR-MS measurements. 500 single scans with an ion accumulation time of 0.2 s were recorded over a mass range of m/z 150 to 2000 and added to one spectrum. An in-house mass reference list was used for internal calibration. For comparison, we also analyzed a deep-sea sample from the North Pacific that was extracted from seawater following the same procedure as our samples, for details see [7].

## Results and Discussion

### Change of bulk and molecular characteristics with depth

We observerd a loss of small molecules (m/z = 154 - 370) with increasing soil depth (Figure 1). This led to an increase in the mean molecular size of DOM. Plant-derived molecules were preferentially consumed by microorganisms and transformed into larger microbial molecules lacking plant-related ligno-cellulosic structures (Figure 1). The shift to higher mean molecular weights with degradation also continued towards the riverine and marine ecosystems.

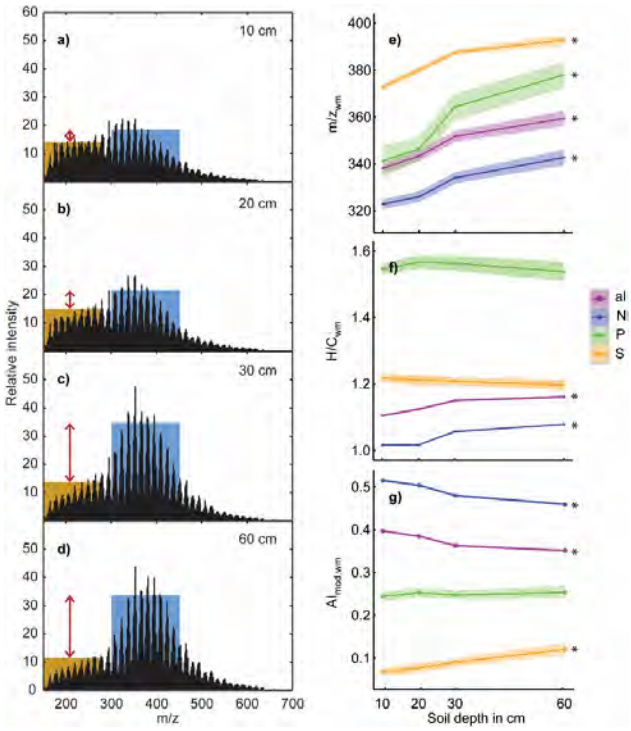



**Figure 1.** Molecular changes in soil DOM.

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# The effect of humic preparation on the microbiological activity of the soil in the case of herbicide application

A. GOROVTSOV [a,b], E. POLIENKO [b], O. BEZUGLOVA [a,b], V. LYHMAN [b]*, A. DEMIDOV [a]

(a) Academy of biology and biotechnology. Southern federal university. 344006. Rostov-on-Don. Russia
(b) FRANC. 346735. Rostov-on-Don. Russia
* E-mail: polienkoe468@gmail.com

**Keywords:** humic preparation. herbicide-induced stress. winter wheat. rhizosphere microbial community

**Abstract.** The effect of humic preparation on the yield of winter wheat and the rhizosphere microbial communities has been studied at the background of the herbicide of the sulfonylurea group (GranstarPro) use. It has been shown that the use of herbicides in combination with humic preparation can lead to a significant increase in winter wheat yield. It has been shown. that the microbial community of winter wheat rhizosphere reacted to all types of treatment but the species composition at the plot. treated with both humic preparation and herbicide was altered to a lesser extent. Soil fungi have shown the most strong reaction to the treatment with humic preparation.

## Introduction

In the practice of winter wheat cultivation, it is common to use herbicides during the spring vegetation renewal in order to preserve moisture for agricultural crops. As a rule dicotyledons prevail among the autumn weeds and therefore the optimal solution for the protection of winter cereals in the early stages is the use of herbicides from the class of sulfonylureas. This group of herbicides has a high risk of a toxic effect [1], which primarily affects the microorganisms of the rhizosphere and indirectly affects the productivity of winter wheat. Humic preparations promote the best development of plants, especially their root systems; intensify the root exudation and as a result increase the biological activity in the rhizosphere zone which promotes a complete assimilation of nutrients both from soil stocks and from fertilizers [2]. Thus the efficiency of mineral fertilizers is increased which makes the treatment of plants with humic preparation an expedient and economically profitable method that contributes to obtaining a higher yield. The aim of the research was to study the effect of humic preparation on soil and plants and to assess its adaptogenic effect when combined with the herbicide of sulfonylurea group.

## Materials and methods

The research site is an experimental field of the Don Zonal Research Institute of Agriculture. Soil type - ordinary carbonate chernozem. The humic preparation was used in a dose of 1 l/ha for treating plants in the tillering phase and in the phase of entering the tube (the concentration of the active substance was 0.2% in terms of carbon content). The herbicide of the sulfonylurea group (GranstarPro) was used at an average recommended dose of 15 g/ha.

The content of mobile phosphorus in the soil was determined according to GOST 26205 [3], the number of ecological-trophic groups of microorganisms determined by the standard method of sowing soil suspension dilutions onto the dense nutrient media was used to assess the microbiological activity of the soil. The dominant species of bacteria were isolated from experimental plots and species were identified with the use of Bruker Daltonics Biotyper based on proteomic fingerprinting using MALDI-TOF mass spectrometry.

## Results and Discussion

The studies have shown (Table 1), that carrying out herbicide treatment in combination with a humic preparation can reduce the toxic effect and as a result, increase the productivity of winter wheat up to 15%.

**Table 1.** The productivity of winter wheat in the complex use of humic preparation and herbicide

| Variant | Yield c/ha | Increase c/ha |
|---|---|---|
| 1. Background ($N_{34}$) | 73.6 | - |
| 2. Background + herbicide | 79.7 | +6.1 |
| 3. Background + humic preparation | 70.6 | - |
| 4. Background + herbicide + humic preparation | 84.4 | +13.8 |
| $LSD_{05}$ | | 6.58 |

In the course of the experiment it was found that the use of the humic preparation BIO-Don had a positive effect on the mobility of phosphates. The use of the sulfonylurea herbicide resulted in the chemical stress of winter wheat crops but treatment with the humic preparation during the growing season helped to substantially reduce this effect and increase the assimilability of available phosphorus compounds (Table 2).

Further experiment led to the mitigation of the differences between the variants treated with the humic preparation BIO-Don and without treatment. However, there is a manifestation of soil stress resistance to the herbicide, which indirectly indicates the formation of a stable rhizosphere microbiological community in the arable layer.

The analysis of dominant species composition in the rhizosphere of winter wheat has shown, that herbicide treatment caused a significant shift in the microbial communities.

In the rhizosphere of the control plot the dominant species were *Micrococcus luteu, Arthrobacter sulfonivorans, Bacillus jeotgali, Bacillus oleronius Pseudomonas chlororaphis.*

**Table 2.** Dynamics of mobile phosphorus (mg/kg) in the soil under winter wheat with complex use of humic drug BIO-Don and herbicide Granstar Pro at a dose of 15 g/ha

| Variant | Before treatment | After treatment |
|---|---|---|
| 1. Background ($N_{34}$) | 21.45 | 13.3 |
| 2. Background + herbicide | 20.29 | 12.1 |
| 3. Background + humic preparation | 18.18 | 14.1 |
| 4. Background + herbicide + humic preparation | 17.31 | 12.6 |
| $LSD_{05}$ | 2.64 | 2.42 |

The use of humic preparation caused a significant change in species composition with an increase of Gram-negative bacteria presence. The dominant species were *Micrococcus luteus Ps. chlororaphis* and *Ps. kilonengesis*, Among the other species identified were *Acinetobacter schindleri* and *Rhizobium radiobacter*.

The use of herbicide caused a major change of species composition and a decline in numbers of bacteria which led to a reduced biodiversity. The characteristic colonies of *Bacillus sp* were absent, the number of *Micrococcus luteus* colonies was strongly reduced, and the most abundant colonies were identified as *Enterobacter cloacae, Exiguobacterum sp.* and *Staphylococcus condiment.*

The species composition at the plot treated with both humic preparation and herbicide was altered but to a lesser extent. The dominant species at this plot were *Micrococcus luteus Arthrobacter stackebrandii* and *Kocuria rosea.*

It can be concluded, that the bacterial communities of the rhizosphere are sensitive to the herbicide-induced stress and it can lead to decline in the numbers of particularly sensitive species. The vacant ecological niches are then quickly occupied by more tolerant strains.

In addition to the species composition, the numbers of microorganisms in the rhizosphere was also studied. Under the conditions of the production experiment there was a significant variation between the experimental field sections, the range of which was 114.7-422.4 million CFU/g for fast growing and 30.2-147.5 for slowly growing forms of copiotrophic bacteria. The latter demonstrated a more sharp reaction to the different treatment schemes indicative of a higher sensitivity of slow-growing copiotrophic bacteria to local soil conditions. The distribution pattern of copiotrophic bacteria in the rhizosphere soil of experimental plots was quite ambiguous. Soil fungi on the contrary, demonstrated a very clear pattern (Fig.1).

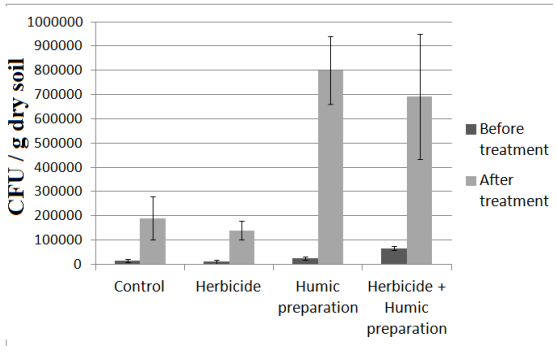



**Figure 1.** The numbers of soil fungi in the rhizosphere of winter wheat at the experimental plots.

Soil micromycetes react actively to the treatment of crops with humic substances. There is even a slight increase in the number of soil fungi after the herbicide treatment which can be attributed to the inhibition of the bacterial component of microbiocenosis. The seasonal dynamics of this group of microorganisms was also very prominent: by the time of treatment with humic preparation the numbers of soil fungi in the control plot was 13 times higher which is due to intensive development of plant root systems in spring. However, after the treatment of crops with humic preparation, the number of fungi in the rhizosphere was more than 4 times higher in comparison to the control plot. The use of humic preparation on the herbicide-treated plot had the same stimulating effect which indicates the role of humic substances in mitigating the herbicide-infuced stress in winter wheat.

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# Application of activated persulfate for improving alum coagulation performance to remove natural organic matter

E. N. HIDAYAH [(a)]*, O. H. CAHYONUGROHO [(b)]

(a) Department of Environmental Engineering,
University of Pembangunan Nasional Veteran Jawa Timur, Indonesia
*E-mail: euisnh@gmail.com

**Keywords:** activated persulfate, coagulation, FTIR, NOM surrogates

**Abstract. N**atural organic matter (NOM) in source water and treated water from coagulation preceded by persulfate preoxidation with activation, were examined by using NOM surrogates parameters and Fourier-transform infrared (FTIR). The results show that coagulation preceded by activated persulfate could remove bulk parameters, in term of dissolved organic carbon (DOC) and the ultraviolet light absorbance at 254 nm ($UV_{254}$), and the infrared absorption showed a relatively lower intensity. It is conjectured that effect of the oxidation by Fe(II)/persulfate, which generated in situ-formed ferric ions, and alum coagulation contributed to the improved rejection of the organic compounds. In addition, either formation of sulfate radicals or in situ-formed ferric ions in activated persulfate with coagulation has a good performance to remove organic compound at 1200 $cm^{-1}$ and 3440 $cm^{-1}$.

## Introduction

Preoxidation has been known as an effective way of improving coagulation efficiency for the subsequent removal of organic matter and algal cells [1, 2], and its widely used in drinking water treatment. Recently, the application of short-lived radical species as an oxidant in disinfection and in remediation technology for soil and groundwater has gained attention due to their high redox potential and high reactivity. Persulfate is one of the strongest oxidants and has the high oxidation potential (2.12 V), has received increasing attention [3]. The reaction of persulfate with NOM and micropollutants are generally slow at ambient temperature, and it can be activated to generate sulfate free radical ($SO_4^-$), a stronger oxidant with a redox potential of 2.6 V, by thermal, chemical and photochemical technique [4]. Persulfate as a novel oxidant has many advantages over the other well-known oxidants, especially the activated persulfate with the ferrous ion. It has been observed the effectiveness of $Fe^{2+}$-activated persulfate oxidation process to degrade toluene in aqueous solutions [5] and increase membrane permeability [6].

In this study, persulfate was applied for preoxidation to enhance coagulation in water treatment processes. In our recent study [2], coagulation preceded by permanganate oxidation was found to cause a greater reduction in all organic fractions as specified based on their molecular weight, than with coagulation alone. Since sulfate radicals have the higher standard redox potential [7] than permanganate, therefore sulfate radicals could be considered as alternative preoxidant for coagulation process in order to remove organic matter. NOM in source water and the treated water, from coagulation preceded by persulfate preoxidation with activation using ferrous ion ($Fe^{2+}$), were examined by Fourier transform infrared (FTIR) besides bulk parameters analysis, such as dissolved organic carbon (DOC), $UV_{254}$, and specific UV absorbance (SUVA) value.

## Materials and methods

**Experimental design.** The experimental was set up through a six-paddle gang stirrer jar test apparatus (Phipps & Bird, Richmond, Virginia, USA). A specific aliquot from stock solution 100 mM $Na_2S_2O_8$ and $FeSO_4.7H_2O$ (Merck, Germany) was diluted in the batch reaction to achieve an initial concentration 0.5; 1; 5; 10 mM and 0; 1; 2; 4 mM, respectively, and added into the water-filled jar. The contact time was 5; 10; 20; 30; 40; 50; 60 minutes, under slow mixing at 35 rpm. Further, under 100 rpm rapid mixing, the stock solution of alum ($Al_2(SO_4)_3.18H_2O$, Merck, Germany) was added with dosage 20 mg/L as Al. The supernatants were collected, and filtered through 0.45 µm filter (cellulose acetate, Toyo Roshi, Japan) before further analysis.

**NOM surrogates and FTIR analysis.** The source water was analyzed for non-purgeable dissolved organic carbon (NPDOC) by the combustion-infrared method using total organic carbon analyzer (Model TOC-500, Shimadzu, Kyoto, Japan), ultraviolet absorbance at 254 nm ($UV_{254}$) was measured using UV/Vis spectrophotometer (Model U-2001, Hitachi, Japan) [8], SUVA values is obtained by dividing the $UV_{254}$ absorbance value by the concentration of NPDOC. FTIR spectroscopy (Thermo Nicolet NEXUS 670) was used to identify the functional groups, scanning from 4000 $cm^{-1}$ to 400 $cm^{-1}$.

## Results and Discussion

### NOM surrogates removal by alum coagulation preceded by persulfate oxidation

Figure 1 shows the performance of activated persulfate pretreatment followed by coagulation in the removal of DOC (Figure 1a), removal of $UV_{254}$ (Figure 1b) and SUVA value (Figure 1c) during water treatment with reaction time 30 minutes. First, the results suggested that the removal of DOC and $UV_{254}$ increased with increasing Fe(II) activation dosage and persulfate dosage. The highest removal by activated persulfate/coagulation

indicated that persulfate could be improved coagulation performance. It has been studied that DOC removal by coagulation about 20-40% [9], while $KMnO_4$ preoxidation followed by coagulation could remove DOC about 45-50% [2]. Second, it seems that activated persulfate showed the best performance in $UV_{254}$ removal, which was probably caused by strong degradation by sulfate radical. Third, Figure 2c shows SUVA value of the source water was 3.84 L/mg.m, which indicated of mixture organic matter properties. Activated persulfate followed by coagulation process has insignificantly performance in SUVA value, it shows decreasing SUVA value. However, the organic properties are still between 2 – 4 L/mg.m, which still indicated the mixture of hydrophilic and hydrophobic matter. Decreasing SUVA value probably represented the removal of hydrophobic matter and or humic compound.

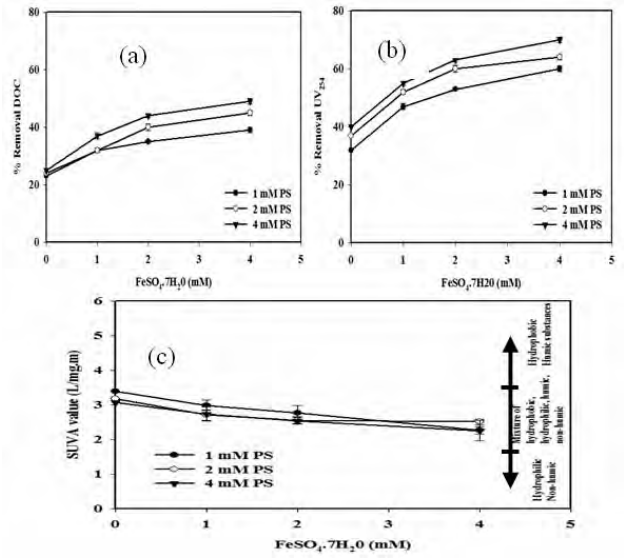



**Figure 1.** Performance of coagulation preceded by various activated PS oxidation with reaction time of 30 minutes for removing organic matter as represented by (a) DOC removal, (b) UV254 removal, (c) SUVA value.

### Fourier-transform infrared (FTIR) spectroscopic analysis

The spectrum from the FTIR analysis of the raw water and treated water DOC sample is shown in Figure 2. Major spectra bands or dominant functional groups were assigned as follows: 3440 $cm^{-1}$ (aromatic N-H stretching; alcohols and carboxylic acid O-H stretching), 2430 $cm^{-1}$ and 2340 $cm^{-1}$ (nitrile C≡N stretching), 1650 $cm^{-1}$ (aromatic C=C and conjugated carbonyl C=O), 1200 $cm^{-1}$ and 1120 $cm^{-1}$ (alcohols and carboxylic C-O stretching; aliphatic amine C-N stretching). Diagnostic bands at 620 $cm^{-1}$ are indicated as inorganic functional group $PO_4^{-3}$ bending and sulfate bending [10]. Therefore, the absorption spectrum clearly illustrates that the dominant functional groups' alcohol, carboxylic acid and amino acids are the main functional group present in the raw water. Activation persulfate followed by coagulation expectedly displayed a relatively low infrared absorption intensity than source water, which is consistent with the high removal of TOC and $UV_{254}$. The absorption intensity at 1120 $cm^{-1}$ showed insignificantly removal under activated persulfate with coagulation process. It seems that combination of hydrolysed species of alum and in situ-formed ferric ions or sulfate radical have a good capacity in removing functional groups of organic matter.

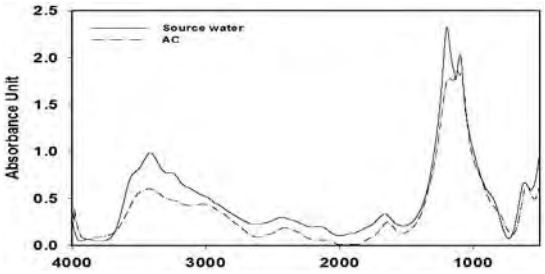



**Figure 2.** Organic fraction distribution and its percentage area during the operation of the batch biofilter.

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# Integrity of the use of humic substances (HS) as tracers of biological transformation during storage of treated sludge

A. KHAKBAZ [(a,c)*], C. BRAVO [(b,c)], M. CONTIN [(c)], D. GOI [(a)], M. DE NOBILI [(c)]

(a) Dipartimento Politecnico di Ingegneria e Architettura,
University of Udine, via delle Scienze 206, 33100 Udine, Italy.
(b) Department of Life Sciences, University of Trieste, Via Licio Giorgieri 5, 34128 Trieste, Italy.
(c) Dipartimento di Scienze Agroalimentari, Ambientali e Animali,
University of Udine, via delle Scienze 208, 33100 Udine, Italy.
* E-mail: khakbaz.ali@spes.uniud.it

**Keywords** Emerging contaminants; transformations; tracers; sewage thickening; humification.

**Abstract**. This work examines the possibility to use spectroscopic characteristics of humic substances as tracers of the degree of biological transformation occurred during storage of sewage sludge in thickening beds. Chlorinated compounds, linear alkylbenzensulphonates (LAS) and emerging contaminants may undergo significant degradation during sludge storage before application to agricultural land.

HS were extracted from substrates at different stages of decomposition and from sludge samples taken from different municipal wastewater treatment plants. HS were extracted and fractionated according to the classic solubility scheme and to an SPE procedure. Fractions and whole extracts were quantified and characterized by UV-vis and fluorescence spectroscopy.

## Introduction

Emerging contaminants, among which are plasticizers, antibiotics, chlorinated compounds, linear alkylbenzenesulfonate (LAS) are a major reason of concern for the application of treated sewage sludge to agricultural soils. The concentration of these substances, many of which can undergo decomposition under anoxic conditions, it may decrease during sludge storage in thickening beds. Monitoring of these contaminants is time-consuming and expensive.

HS, namely humic (HA) and fulvic (FA) acids, are refractory natural compounds produced during decomposition of organic matter in soil and natural waters. Their quantification has been widely used in the evaluation of the quality of sewage sludge for their use in agriculture. Changes in their spectral properties (UV and fluorescence) could represent a convenient way to assess the degree of biological transformation that occurs in thickening beds. A recent paper published in Nature [2] has challenged the traditional views on humic substances suggesting that they might be artefacts produced during extraction because of the harsh alkaline conditions employed.

The integrity of their use as markers of biological transformations in sewage sludge must, therefore, be ascertained.

## Materials and methods

Sludge samples were collected manually at the end of the sludge treatment (SLa) and from thickening beds (SLb) from very small to medium size municipal wastewater treatment plants located in Friuli Venezia Giulia.

Extracts were fractionated according to the classic solubility scheme and to an SPE procedure. Fractions were quantified (TOC) and characterized by UV-vis and fluorescence spectroscopy. Three-dimensional spectra of FA were performed over an excitation range of 240-400 nm and emission range of 250-600 nm with a slit size of 5 nm.

To eliminate inner-filter effects samples were diluted to achieve absorbance values less than 0.1 $cm^{-1}$ at 254 nm. Sludge samples were analyzed for their EOX and LAS content.

## Results and Discussion

Humic substances extracted from peat display the typical featureless UV-vis spectra which do not substantially differ from those extracted under neutral conditions. On the contrary, alkaline extracts of sphagnum display a well pronounced absorption peak at 290 nm. Artefactual HA from sphagnum could not be redissolved completely and none could be redissolved from the acid precipitated materials extracted with neutral pyrophosphate. This was not observed for sludge HA, showing that humic substances from sludge did not have an artefactual nature. UV spectra of sludge FA display the same type of change, after the thickening stage. This indicates that extensive biological transformation of organic materials has gone on after the end of the treatment, affecting even the most recalcitrant types of molecules such as humic substances.

Fluorescence spectra confirm the difference among FA extracted from the different substrate and show that their characteristic spectral features are conserved when FA are extracted by a neutral buffered solution or distilled water. They also clearly showed that although the fluorescence spectra of treated sewage sludge greatly differ from that of well humified materials, this difference is greatly reduced during permanence in the thickening beds (Figure 1).

Analysis of LAS and EOX performed on the same samples confirmed that these changes in humic substances can be used as tracers of biological transformation that lead to the decomposition of contaminants.

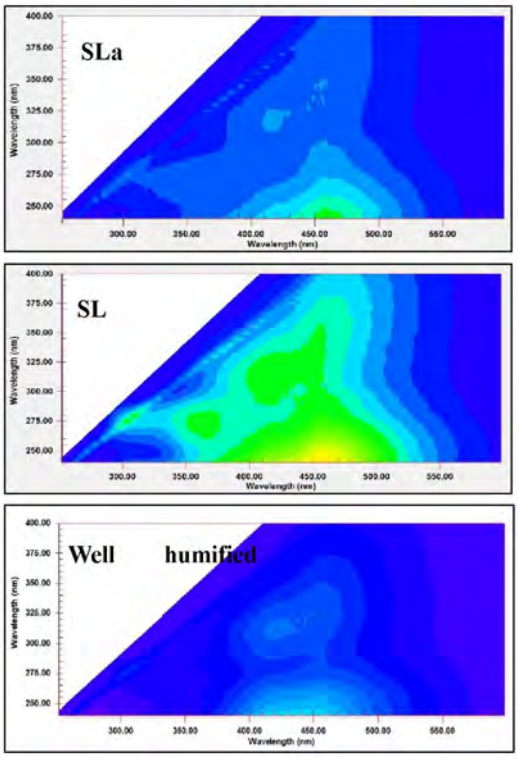



**Figure 1.** Contour plots of FA extracted from a sample of well-humified peat and from two treated sewage sludge samples taken before (Sla) and after (SLb) storage in thickening beds.

## Conclusions

Spectroscopic characteristics of FA and HA clearly show that these empirically defined fractions can be usefully employed to monitor the intensity of biological transformation of sewage sludge during thickening.

Conditions and length of the thickening stage may, therefore, be important in determining a further reduction of the concentration of biodegradable emerging pollutants.

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# Both (root ABA-Lpr)- and (root IAA-PM H+ATPase)- signalling pathways are crucial in the plant growth promoting action of rhizospheric humic acids

M. OLAETXEA [(a)], R. BAIGORRI [(b)], M. FUENTES [(a)], V. MORA[(d)], M. GARNICA [(a)], L. SPICHAL [(c)], A. M. ZAMARREÑO [(a)], JM GARCIA-MINA [(a)*]

(a) Department of Environmental Biology, University of Navarra, 38008, Pamplona, Spain
(b) Department of Technical Development, TIMAC AGRO INT, Lodosa, Spain
(c) Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Olomouc, Czech Republic.
(d) Plant Physiology and Plant-Microorganism Laboratory, University of Rio Cuarto, Campus Universitario, CP 5800, Río Cuarto, Cordoba, Argentina
* E-mail: jgmina@unav.es

**Keywords:** Humic substancest root growth, shoot growth, plant hormones, ATPase

**Abstract.** A number of studies have demonstrated the beneficial action of humic substances (HS) on plant growth. Different studies have shown that besides the action of HS improving root nutrient uptake, other actions affecting specific signalling pathways in the root and shoot seem to play relevant roles. In this study we show, using cucumber as plant model, that a sedimentary humic acid (SHA) able to stimulate both the shoot and the root through the simultaneous activation of ABA- and IAA signalling pathways involving root hydraulic conductivity and root PM H+-ATPase activity respectively.

## Introduction

Humic substances (HS) are principal components of both soil organic matter (SOM) and dissolve organic matter (DOM) [1]. Although the structure of HS is still a matter of open debate, it includes the distribution of aromatic and aliphatic domains with O, N, and S – functional groups such as carboxylic, phenolic, amine and sulfhydryl with high biological and chemical activity [1, 2].

Many studies have demonstrated that the presence of transformed (humified) SOM is a major factor influencing soil fertility [3, 4]. This action seems to result from the ability of some of their components to improve plant nutrient availability in soil and plant growth [1, 3]. The effects of HS on plant growth seem also to involve, at least in some ecosystems, an action on plant metabolism and physiology derived from the interaction of HS with plant roots in the rhizosphere [3, 4]. However, the knowledge about the mechanisms responsible for these HS actions remains rather fragmentary [4, 5, 6].

With regard to HS-mediated action on root growth and architecture, a number of studies suggest that it is mediated by auxin signalling pathways, also linked to NO and ethylene, affecting root PM H+-ATPase activity [7-10]. However, the ability of HS to increase root dry matter remains unexplained as well as the causal relevance of the HS-mediated increase in root PM H+-ATPase activity in the plant root growth promoting action of HS.

As for the shoot-promoting action of HS, recent studies indicated that it is regulated by the action of HS on IAA-NO signalling [11], as well as the HS-mediated increase in root ABA linked to aquaporin activity and an Lpr increase [12]. Other study suggested that this HS action may also involve cytokinin activity in the shoot and PM H+-ATPase activity in the root [13]. However, the real causal role of both cytokinin activity and root PM H+-ATPase activity in the shoot promoting action of HS; as well as their relationships with the events controlled by IAA and ABA, remains unknown.

In this context, the aim of our study is to investigate the causal role of root PM H+-ATPase activity and Cks signaing in the effect of HS on root and shoot growths, as well as their functional links with the effects regulated by root ABA affecting Lpr.

## Materials and Methods

Seeds of cucumber (Cucumis sativus L. cv "Ashley") were germinated in water with 1 mM of CaSO4, in darkness, on perlite and moistened filter paper in a seed germination chamber. One week after germination, plants were transferred to 8 L receptacles in hydroponic solution [12]. The pH of the nutrient solutions was held at 6.0 and did not change significantly during the experiment. All experiments were performed in a growth chamber at 25/21 ºC, 70-75% relative humidity and with 15/9 h day/night photoperiod (irradiance: 250 µmol m-2 s-1) [12].

Four different experiments were undertaken:

1. The study of the involvement of root PM H+-ATPase and/or Lpr (and their upstream hormonal signals, IAA and ABA respectively) in the SHA-mediated effects on plant development.

1.1. Influence of SHA on activation of root PM H+-ATPase and its significance on plant development upon the application of the PM H+-ATPase inhibitor (N, N' dicyclohexyl-carbodiimide, DCC).

1.2. Effects of SHA-mediated ABA-Lpr activation on plant development by using a well-known ABA biosynthesis inhibitor, fluridone [12]. Study of the effects on root PM H+-ATPase activity.

2. Involvement of the SHA-mediated increase in cytokinin activity in leaves, in the shoot growth

promoting action of SHA by applying a cytokinin perception blocker, PI-55 [14].

All treatments involved a minimum of five replications. The main analytical methods are described in [11-13].

**Results and Discussion**

The main results showed that:

The shoot and root growth promoting action of SHA in cucumber involves significant increases in both Lpr and root PM H+-ATPase associated with a previous increase in root ABA and root IAA respectively.

PM H+-ATPase is crucial for SHA shoot and root growth promoting action. Nevertheless, SHA action on PM H+-ATPase is independent on the SHA-mediated ABA-Lpr activation.

SHA-mediated ABA-Lpr increase is essential for the shoot and root growth promoting action of SHA, with this effect being independent on the SHA-mediated increase in root IAA-H+-ATPase.

The SHA-mediated increase in shoot cytokinin concentration and cytokinin activity is crucial for the shoot growth promoting action of SHA and is linked to the SHA-mediated increase in root H+-ATPase activity.

**Conclusion**

The plant growth -promoting action of SHA requires both the increase in Lpr mediated by ABA and the increase in root PM H+-ATPase activity mediated by IAA**.**

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# Effects of microbial activity and cations on association of selenium with humic substances in deep groundwater

M. TERASHIMA[(a)*], Y. AMANO [(a)]

(a) Japan Atomic Energy Agency (JAEA), Toakai-Mura, Ibaraki, 319-1194, Japan
* E-mail: terashima.motoki@jaea.go.jp

**Keywords:** humic substances, selenium, association, microbial activity, cations

**Abstract.** Effects of microbial activity and cations on association of selenium (Se) with humic substances (HS) in deep groundwater were investigated by means of incubation experiments, DAX-8 extraction, and size-exclusion chromatography combined with UV-Vis and ICP-MS detections (SEC-ICP-MS). Selenite added into the groundwater was effectively reduced to elemental Se(0) by microbial activity stimulated by a nutrient. The DAX-8 extraction showed the existence of Se fraction that was extracted with HS. The SEC-ICP-MS analysis for alkaline extracts of the DAX-8 showed that the chromatograms of Se, HS and Fe were consistent, and the consistency was observed regardless of the presence of nutrient and/or microorganisms. These data indicate that Se can associate with HS in deep groundwater via the interaction with Fe.

## Introduction

Selenium-79 ($^{79}$Se) has a half-life for 2.95 x $10^5$ years [1] and is a concern in the performance assessment for a system of geological disposal of high-level radioactive waste. In the natural environment, Se can occur in an organic form (***e.g***. methylated Se [2], amino Se [3] and humic Se [4, 5]) as well as an inorganic form (***i.e***. selenate Se(VI), selenite Se(IV), elemental Se(0), or selenide Se(-II)). Performance assessment has identified potential differences in the migration behaviour of organic Se compared to inorganic Se. These differences need to be considered in the development of Se migration models in deep groundwater relevant to geological disposal. The microbially mediated formation of organic Se in deep groundwater environments, however, has not yet been confirmed. In the present study, to clarify the formation of organic Se, an association of Se with HS in raw, deep groundwater was investigated in terms of microbial activity and cations by incubation experiments and speciation analysis.

## Materials and methods

**Groundwater sample.** The deep groundwater sample was collected from the borehole (07-V140-M03) in the Horonobe Underground Research Center, Hokkaido, Japan, using a packer-type sampling system. The groundwater chemistry was analyzed: pH 7.5, dissolved organic carbon (DOC) 25 mg C $L^{-1}$, Na 2900 mg.$L^{-1}$, Ca 68 mg.$L^{-1}$, Mg 46 mg.$L^{-1}$, Mn < 0.01 mg.$L^{-1}$, Al < 0.01 mg.$L^{-1}$, Fe (total) 0.51 mg.$L^{-1}$ [6].

**Incubation experiments.** Sterilization of the groundwater was conducted by filtration (pore size = 0.22 μm). A 0.1 mL aliquot of 0.5 M $Na_2SeO_3$ was added into the 50 mL groundwater samples (total Se concertation = 1.0 mM). Nutrient (R2A: yeast extract, protease peptone, casamino acids, glucose, soluble starch, $K_2HPO_4$, $MgSO_4$, sodium pyruvate, agar) was added into the sample (0.09 g R2A to 50 mL sample) to stimulate microbial activity. The mixture was incubated under an Ar atmosphere at room temperature and kept in the dark for 3 years. The conditions of samples in the incubation experiments are summarized in Table 1.

**Table 1.** Samples in the incubation experiments

| No. | Conditions |
|---|---|
| 1 | Groundwater |
| 2 | Groundwater + Se |
| 3 | Groundwater + microorganisms |
| 4 | Groundwater + Se + microorganisms |
| 5 | Groundwater + R2A |
| 6 | Groundwater + R2A + Se |
| 7 | Groundwater + R2A + microorganisms |
| 8 | Groundwater + R2A +Se + microorganisms |

**DAX-8 extraction.** DAX-8 extraction technique [7] was used to determine the concentrations of HS and Se that may associate with HS. Water samples were filtered (pore size = 0.22 μm). The filtrate was acidified using 2.5 M $H_2SO_4$, and was mixed with 0.2 g of DAX-8 resin at 5ºC in the dark for 24 hours. After the equilibration, the mixture was passed through a glass fibre filter (pore size = 0.3 μm). Concentrations of HS and Se in the filtrate were then measured by a TOC analyzer and ICP-MS, respectively.

**SEC-ICP-MS measurement.** The 0.01 M NaOH extracts of the DAX-8 resins used for the HS extraction were subjected to SEC-ICP-MS analysis. A 100 μL aliquot of the extract was injected into a LC pumping system. A Hitachi GL-W530 column was used as a solid phase. A 0.01 M Tris buffer containing 0.01 M NaCl (pH = 8.0) was used as a mobile phase. Detection wavelength of UV-Vis detector was set at 280 nm. Isotopes of $^{82}$Se, $^{40}$Ca, $^{24}$Mg, $^{55}$Mn, $^{27}$Al and $^{56}$Fe were detected by an ICP-MS.

## Results and Discussion

### Effect of microbial activity.

After the 3 years incubation, red and black coloured precipitates were observed in samples No.6 and No.8 (Table 1), respectively. XRD patterns for the precipitate

in No.8 showed the typical pattern of Se(0) (Fig.1). This showed that Se(IV) can be reduced to Se(0) by the microbial activity stimulated by the R2A. A comparable result was also observed in No.6, while No.6 was sterilized by the filtration in the same manner as No.2. This might be due to the microorganisms potentially having passed through the filter. Further study is required to clarify this issue.

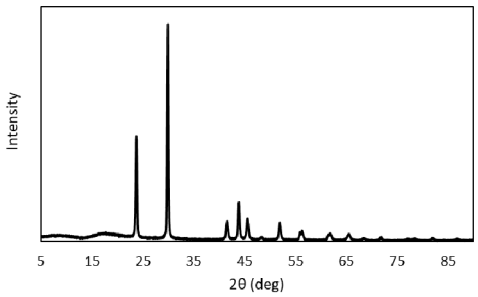



**Figure 1.** XRD spectrum of the precipitate in No. 8.

Table 2 shows the results of the DAX-8 extractions. Before the extraction, the concentrations of Se in No.2~8 were reduced from $10^{-3}$ M to $10^{-6}$ M, corresponding to the formation of Se(0). Differences between before and after the extractions showed that the Se in No.4, 6 and 8 were extracted from the DAX-8 resin, especially in No.8 where up to 51% of Se was extracted. Conversely, the HSs were extracted to the DAX-8 resin by up to 60% of the total DOC in No.4 and 17% of total DOC in No.8. These results showed the existence of Se fraction that was extracted to the DAX-8 together with HS from the groundwater.

**Table 2.** The concentrations (M) of Se in the groundwater before and after the DAX-8 extractions.

| Sample No. | Before the DAX-8 extraction | After the DAX-8 extraction |
|---|---|---|
| 2 | $1.02 (\pm 0.01) \times 10^{-3}$ | $0.98 (\pm 0.03) \times 10^{-3}$ |
| 4 | $1.01 (\pm 0.03) \times 10^{-3}$ | $0.96 (\pm 0.01) \times 10^{-3}$ |
| 6 | $2.97 (\pm 0.08) \times 10^{-4}$ | $2.69 (\pm 0.02) \times 10^{-4}$ |
| 8 | $3.56 (\pm 0.04) \times 10^{-6}$ | $1.75 (\pm 0.04) \times 10^{-6}$ |

To confirm the chemical form of Se extracted, SEC-ICP-MS analysis was applied for a 0.01 M NaOH extract of the DAX-8. Figure 2 shows the SEC chromatograms of Se and HS in the NaOH extracts. Chromatograms of Se and HS in No.8 were found to be consistent with each other. Similarly consistent chromatograms were also observed in No.2, 4 and 6, regardless of the presence of R2A and/or microorganisms. The $Na_2SeO_3$ added was detected at a retention time of 10.5 minutes, which is later than that for HS. Se could therefore be associated with HS in the raw groundwater without relation to the microbial activity. Incorporation of Se into HS might be a minor mechanism because organically-bound Se can be formed via microbial metabolism [3].

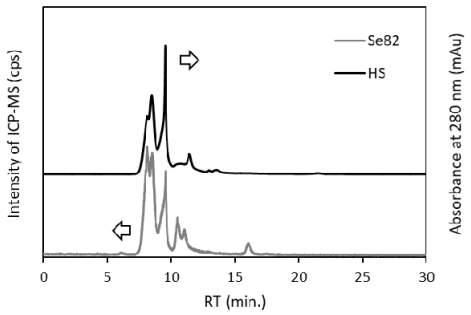



**Figure 2.** SEC chromatograms of Se and HS in the 0.01 M NaOH extract of the DAX-8 in No.8.

### Effect of coexisting cations

Figure 3 shows the SEC chromatograms of Se, Mn and Fe in No.8. The dominant peak of Mn was not observed and so Mn provides a minor contribution for the association. Comparable results were also obtained for Ca and Mg. Conversely, it was found that the SEC chromatogram of Fe was consistent with that of Se, and was also observed in No.2, 4 and 6. In previous studies, it is known that Se can be associated with Fe oxides to form colloidal Se [8]. Moreover, multivalent cations such as $Fe^{3+}$ can act as a binder between HS and Se [4]. This indicates that Fe can play an important role in the association of Se with HS in the groundwater.

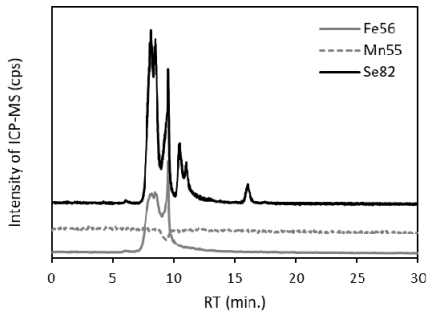



**Figure 3.** SEC chromatograms of Se, Mn and Fe in the 0.01M NaOH extract of the DAX-8 in No.8.

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# Modification of iron oxide nanoparticles bioactivity by humic acids

V. TEREKHOVA [a,b], N.CHISTYAKOVA [c], L. KULIABKO [d]*, P. UCHANOV [b], K.KYDRALIEVA [d]

(a) Faculty of Soil Science, Moscow State University, 119991 Moscow, Russia
(b) Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, 119071 Moscow, Russia
(c) Department of Physics, Moscow State University, 119991 Moscow, Russia
(d) Moscow Aviation Institute, 125993 Moscow, Russia
* E-mail: l.kyluabko@gmail.com

**Keywords:** iron oxides nanoparticles, humic acids, bioactivity

**Abstract.** In this study, mitigating effect of humic acids (HA, passivation agent) on bioactivity of two types of iron oxide nanoparticles of $Fe_3O_4$ and $\gamma$-$Fe_2O_3$ to green algae and a higher plant (white mustard) were investigated, with a focus on the effect of oxidation state. Results show that the growth inhibition of iron oxides nanoparticles (NPs) to both test-species increased with oxidation of the NPs with an order $Fe_3O_4$ < $Fe_3O_4$/HA < $\gamma$-$Fe_2O_3$/HA. The main toxic mechanism, which could explain the difference in toxicity of the NPs is probably $Fe^{2+}$ and $Fe^{3+}$ ions release. These findings will be helpful for the understanding of HA role in the fate and toxicity of iron-based NPs in the environment.

## Introduction

The increasing application of iron-based nanoparticles has raised concerns regarding their environmental behaviour and potential ecological effects. After release into the environment, iron-based NPs undergo various transformations via the interactions with different geochemical and biological components, which ultimately influence their behaviour and potential toxicity [1]. The iron-based nanoparticles are known to be transformed into each other. The oxidation process of magnetite to maghemite is difficult to monitor as both iron oxide polymorphs possess connatural chemical properties [2], but NPs have various biological activity. Most studies emphasize the importance of the interactions between natural organic matter, in particularly humic acids (HA) and NPs in the environment, which have a profound effect on the properties and behaviours of NPs [3]. Owing to its macromolecular nature, HA are expected to contribute to the stability of iron-based NPs thanks to polyelectrolyte nature by providing electrosteric stabilization. HA can also alter the structure of aggregate and plays an important role in colloid stability of NPs, including dispersion, aggregation, disaggregation etc. Here, the two types of iron oxide nanomaterials − magnetite and maghemite were used to investigate cytotoxic behaviour of magnetite and its oxidized species. Humic acids were used as a passivation agent to prevent aggregation, enhance dispersion stability as well as modify bioactivity of iron oxide NPs.

## Materials and methods

**Preparation of $Fe_3O_4$ and $Fe_2O_3$ nanomaterials.** Magnetite NPs $Fe_3O_4$ synthesis was carried out by the co-precipitation of $Fe^{2+}$ and $Fe^{3+}$ aqueous salt solutions in an alkaline environment with ($Fe_3O_4$/HA) and without HA ($Fe_3O_4$) as previously reported [4]. Preparation of $Fe_2O_3$/HA was performed by mechanical treatment of $Fe_3O_4$/HA in air in grinder at 1400 rpm in 30 min.

**Structure analysis.** X-ray diffraction (XRD) was performed in transmission geometry with a Phillips X-pert, CuKα source. Mossbauer investigations of NPs were carried out at temperature range from room temperature to 5 K to avoid the relaxation processes and to identify the phases more precisely. The isomer shift was given by relative *α*-Fe [5].

**Biotesting.** The higher plant - white mustard *Sinapis alba* L. and green algae *Scenedesmus quadricauda* (Turb.) Brev. were used as test organisms for bioactivity evaluation. *S. alba* is recommended for humus substances assessment by Russian Federal Register of bioassay standard methods as described in [6, 7]. Biotest with algae *S. quadricauda* was used according to ISO 8692:2012(en) with modification [8]. The effect of each concentration of nanocomposite preparation in both biotests was expressed as the percentage of difference with the control.

## Results and Discussion

The XRD patterns confirm preservation of the spinel structure during synthesis and oxidation processes (Fig. 1). The crystal phase for both samples according to XRD analysis is magnetite.

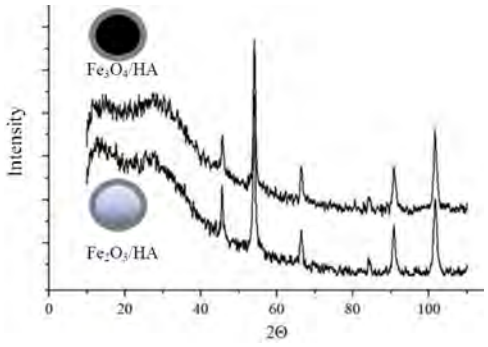



**Figure 1.** XRD patterns of NPs.

The synthesized magnetite particles exhibit a median particle diameter of ~6 nm for both samples. XRD technique suffers from serious limitations, peak broadening due to the nanometer crystal domain size make conventional XRD rather ineffective in discriminating magnetite (a=8.397 Å) and maghemite (a=8.346 Å16) [9].

Mössbauer spectroscopy is a powerful tool for the identification of the chemical state and crystal environment of iron species [9] since the distinction between the relatively similar iron oxides maghemite and magnetite is possible.

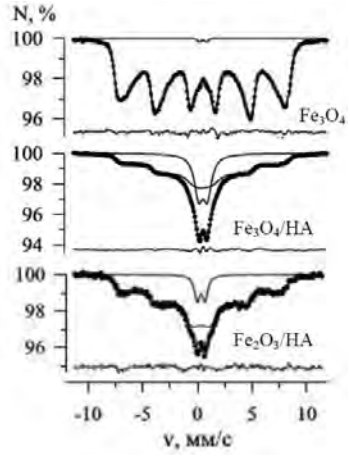



**Figure 2.** Mossbauer spectra for NPs [5].

The analysis of the spectrum of the γ-$Fe_2O_3$/HA (prepared in oxidation conditions) (Fig. 2) has shown that sample of maghemite rather than pure magnetite.

Bioassay experiments showed that bare $Fe_3O_4$ has a toxic effect on the seedings of *S. alba* (Fig. 3) and inhibits the growth of *S. quadricauda* cells as well as chlorophyll fluorescence (Fig. 4).

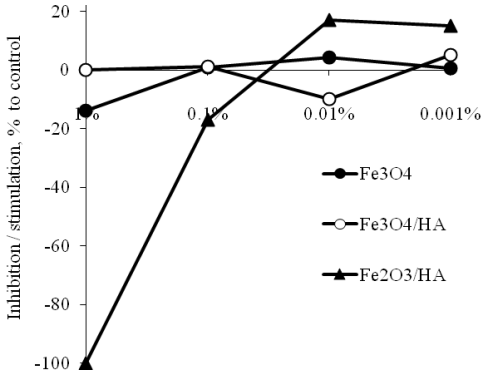



**Figure 3.** Bioactivity of NPs in biotest with higher plant *S. alba.*

Iron oxides covered by HA reduces toxicity for both $Fe_3O_4$ and $Fe_2O_3$ in the test with the seedings (Fig. 3) and *S. quadricauda* cells (Fig. 4). Results of biotests with *S. alba* and *S. quadricauda* show that the growth inhibition of iron oxides nanoparticles (NPs) to both test-species increased with oxidation of the NPs with an order $Fe_3O_4$ < $Fe_3O_4$/HA < $Fe_2O_3$/HA.

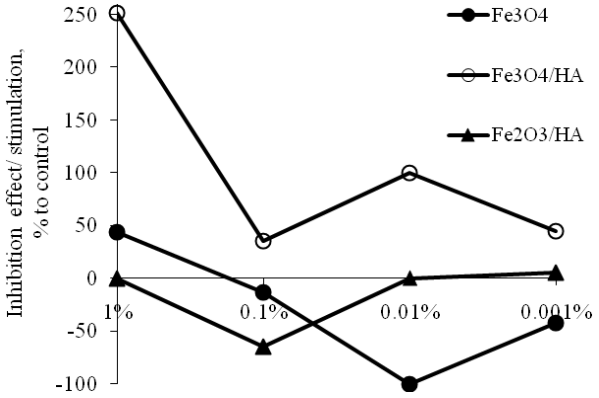



**Figure 4.** Bioactivity of NPs in biotest with algae *S. quadricauda.*

Probably the maghemite γ-$Fe_2O_3$ could cause the more pronounced negative effect in compare with bare $Fe_3O_4$ and $Fe_3O_4$/HA, suggesting that its toxicity originates from the released ferrous ions during mechanical treatment of magnetite. Especially ferrous ions occurring in iron oxides are far more toxic than the inherent toxicity of the NPs [10].

Experiments with two biotests showed that, in controlled chemical conditions, water suspensions of the preparation, can be used safely for biota only up to a certain concentration limit. Probably, in natural conditions the presence of NOM, especially of humic substances, may contribute to an increase in the permissible concentration limit and reduce the harmful effect of the NPs.

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# The influence of three polyphenols at different pH on the abiotic formation of humic polycondensations

J. ZOU [(a)], D. YUE [(a)]*

(a) School of Environment, Tsinghua University, Key Laboratory for Solid Waste Management and Environment Safety (Tsinghua University), Ministry of Education of China, Beijing 100084, China
* E-mail: zjm16@mails.tsinghua.edu.cn yuedb@tsinghua.edu.cn

**Keywords:** Humic polycondensation, pH, abiotic

**Abstract.** The effect of three different polyphenols (catechol, pyrogallol and tea polyphenols) to glycine (polyphenol pathway) on humification reaction products at different pH was described, as catalyzed by $MnO_2$. The experimental data indicated that alkaline conditions are more conducive to humification productivities, even without catalyst. Furthermore, the element composition analysis showed that the polycondensation degree of simple polyphenols is more like the IHSS humic-like acid (HLA). 2D-NMR spectra proved that the construction of HLA are not destroyed by the existing extract method. FTIR indicated similarity of construction of HLA with HA standard.

## Introduction

Biomass waste disposal is drawing people's attention to a great extent with the depletion of fossil fuels and the increase in the greenhouse effect. Compared with mineralization treatment such as biomass diesel, pyrolysis gasification and anaerobic digestion technology, humification serves as carbon sink is one of the ideal biomass treatment for the ecosystem environment. The biochemistry of formation of humic substances (HSs) is one of the least understood aspects of humus chemistry, and an understanding of pathways of humus synthesis would result in greater comprehension of C cycle and control greenhouse emission.

Humic substances are formed through both biotic (enzymatic) and abiotic (mineral) catalytic mechanism [1-3]. Abundant research evidence at the molecular level shows that mineral colloids can enhance the abiotic polymerization of biomolecules such as amino acid, sugars and polyphenol [4]. The polyphenol pathway of humification (polymerization reactions between polyphenols and amino acids) has been intensely studied using various mineral colloids, such as Al, Fe, and Mn oxides as catalysts [5-8], and polyphenols had been elucidated as building blocks of HS in the "polyphenol theory" of HS formation. Therefore, the diphenols have received serious attention in the past decades, but the concentration of such simple phenols in real biomass waste is typically low. Therefore, the ultimate goal in my research was to elucidate the relation and difference between real sources of phenols from biomass waste like tea polyphenols and simple polyphenols such as catechol and pyrogallol in influencing the abiotic mineral – catalyzed humification pathway with different pH condition, in order to as a fundamental theory for humification used in biomass waste treatment.

## Materials and Methods

Solutions for humification experiments contained 0.2 M phosphate buffer at pH 6, 7, 8 respectively, and two grams of $MnO_2$ were suspended in 200 ml of each reaction solution system: (i) 0.02 mole catechol (C) and 0.01 mole glycine (G) under pH 6, 7, 8 respectively (C+G+M-6, C+G+M-7, C+G+M-8), (ii) 0.02 mole pyrogallol (P) and 0.01 mole glycine (G) under different pH (P+G+M-6, P+G+M-7, P+G+M-8) and (iii) 0.02 mole tea polyphenols (TP) and 0.01 mole glycine (G) under different pH respectively (TP+G+M-6, TP+G+M-7, TP+G+M-8), and the control treatment in this system without catalyst (TP+G -6, TP+G -7, TP+G -8). 0.02% (w/v) thimerosal was added as a preservative. All systems were shaken at 35ºC in a rotatory shaker at 170 rpm for 166 h.

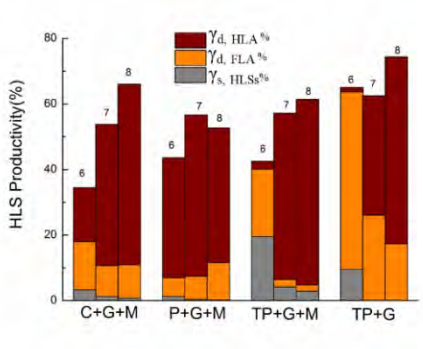



**Figure 1**. Comparison of humification productivity with three polyphenols to glycine in the supernatant and solid residues at pH 6, 7, 8, respectively.

$$\gamma_{HLS} = \gamma_{s,\,HLSs} + \gamma_{d,\,HLA} + \gamma_{d,\,FLA}$$

$$\gamma_{d,\,FLA} = \frac{DOC_{FLA} \times V}{OC_{T,0}} \times 100\%$$

$$\gamma_{s,\,HLSs} = \frac{(m_{s,t} - m_{s,0}) \times C_s\%}{OC_{T,0}} \times 100\%$$

$$\gamma_{d,\,HLA} = \frac{DOC_{HLA} \times V}{OC_{T,0}} \times 100\%$$

γHLS is the productivity of HLSs, and γs, HLSs is that in solid residues; ms, t and ms, 0 is the mass of solid residues at the terminal and the original moment, g; Cs% is the mass fraction of carbon element in the terminal solid residues; OCT,0 is the quality of original TOC, g;

γd, HLA and γd, FLA are the productivity of HLA and FLA in the supernatant respectively; DOCHLA and DOCFLA are the dissolved organic carbon of extractable HLA and FLA from the supernatant respectively, $g.L^{-1}$; V is reaction volume, L.

**Results and Discussion**

In all reaction systems (Figure 1) illustrated that the alkaline condition plays a significant role in promoting the productivity of humification among the three polyphenols, especially in the systems of tea polyphenols with pH=8 buffer, even without the catalyst-$MnO_2$. Therefore the pH is very significant in the processing of the humification.

Table1 provides a summary of C/N mass ratio (m/m) of HLSs in the supernatant and solid residues, which suggested that all HLSs in solid residues have the higher degree of polycondensation than that in supernatant, and is increasing with the rising of pH simultaneously. The degree of polymerization of HLSs with simple polyphenols precursor such as are more like that of IHSS HA, and the relatively low polymerization degree of HLSs produced by tea polyphenols system are more similar to the commercial HA extracted from lignite.

**Table1.** Comparison of the mass ratio of C/N in solid residues and supernatant HLSs at the different pH

| | Solid residues | Supernatant |
|---|---|---|
| C+G+M-6 | 22.87 | --- |
| C+G+M-7 | 15.405 | 17.385 |
| C+G+M-8 | 5.67 | --- |
| P+G+M-6 | 22.455 | --- |
| P+G+M-7 | 13.095 | 21.52 |
| P+G+M-8 | 1.535 | --- |
| TP+G+M-6 | 43.115 | 79.07 |
| TP+G+M-7 | 35.545 | 51.31 |
| TP+G+M-8 | 9.8205 | 46.805 |
| TP+G-6 | 50.47 | 119.4 |
| TP+G-7 | --- | 101.845 |
| TP+G-8 | --- | 73.905 |
| HA standard | 15.36 | |
| C-HA | 48.92 | |

Note: HA standard is purchased from International Humic Substances Society (IHSS), and C-HA is a commercial HA extracted from lignite purchased from Sigma Aldrich.

In order to prove the extracted method do not affect the structure of HLA, 2D-NMR was used to described the extractable HLA molecular fingerprint. From the HSQC spectra (Figure 2) showed that an exact match between the situ suspension biopolymers and extracted HLA. So, there is little impact with the existing extract method for HLA. FTIR spectra (Figure 3) showed that compared with the production of HLA from the three different polyphenols systems, literally, the structure almost the same.

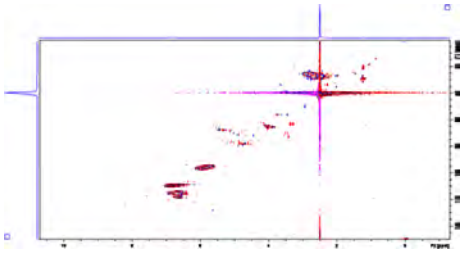


**Figure 2.** Compare the two biopolymers of HSQC spectra in sit suspension biopolymer and extracted HLA.

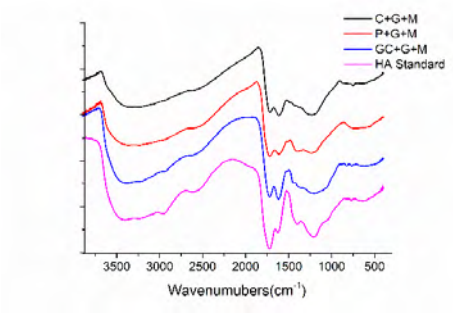



**Figure 3.** Compare the FTIR of the three polyphenols production HLA with standard HA.

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# TOPIC 3.
# “WATER QUALITY - DRINKING WATER - SURFACE WATERS”

# The application of natural organic matter characterization techniques to an Australian drinking water catchment

A. AGOSTINO [(a,b)], S. MORADI [(a,b)], H. BUSTAMANTE [(c)], R.K HENDERSON [(a)], G. LESLIE [(b)*]

(a) Microbial Advanced Separation Systems Laboratory (bioMASS Lab), School of Chemical Engineering, The University of New South Wales, Sydney, NSW, 2052, Australia
(b) UNESCO Centre for Membrane Science and Technology, School of Chemical Engineering, The University of New South Wales, Sydney, NSW, 2052, Australia
(c) Sydney Water Corporation, Parramatta, Sydney, NSW, 2150, Australia
* E-mail: G.Leslie@unsw.edu.au

**Keywords:** Natural Organic Matter, Characterization, Surface Water

**Abstract.** Research into the elucidation of NOM character in surface waters and the link to NOM removal is concentrated to Europe and North America, with limited research conducted on the vastly differing climatic and ecological conditions of Australia. This study aims to characterize NOM from a riverine, transitional and lacustrine site of an Australian drinking water catchment and compare to similar American, Canadian and French drinking water sources. It is concluded that NOM characterization should be conducted more globally, such that conclusions on NOM treatability can be more widely applicable.

## Introduction

The link between Australian and Norwegian NOM character of both surface water and treated water demonstrated NOM with a higher humic basis and higher molecular weight distribution in Norwegian surface water compared with Australian raw water [1]. After treatment both water sources demonstrated similar NOM characteristics, indicating recalcitrant NOM as determined by bulk parameters is attributed to treatment processes rather than source variations. However, disinfection chlorine demand was 55% higher for Norwegian treated water yet yielded a 54% decrease in the Trihalomethane Formation Potential compared to Australian samples. It is evident that bulk parameters such as Total Organic Carbon (TOC) and specific ultraviolet absorbance failed to elucidate the difference in NOM treatability differing spatial domains.

A similar comparison between Australian and American surface waters highlighted total organic nitrogen was more effectively removed in Australian raw water samples than American. It follows that Australian NOM is derived from more allochthonous sources than American samples [2], hence effecting the response of NOM loading to water treatment processes. Again, this exposes the lack of resolution bulk parameters display regarding NOM characterization and the link with NOM treatability.

Studies that link NOM character to water treatability, disinfection by products formation, membrane fouling and taste and odour compound formation are concentrated on NOM from vastly differing continental climates of Europe and North America. Previous comparative studies measure similar bulk parameters for surface waters yet conclude with differing NOM treatment outcomes. Consequently, the application of increasingly sophisticated NOM characterization techniques is required to elucidate differences in treatability of surface waters of differing spatial domains and the relation to differences in NOM character.

## Materials and methods

**Catchment Sampling.** Samples were collected from riverine, transitional and lacustrine sites of a water catchment located on the New South Wales Central Coast, Australia. Samples were collected in duplicate in 1.25L polyethylene terephthalate bottles at consistent depth profiles. Samples were kept at 4$^{o}$C in cold storage and unexposed to light for delivery and storage. Analysis was conducted within 72 hours.

**Dissolved Organic Carbon.** DOC was measured using a Total Organic Carbon (TOC-LCSH) analyser (Shimadzu, Japan). Samples were filtered through a 0.45 μm PES filter (Merck Millipore, Ireland) and DOC was quantified utilizing the difference method.

**Rapid Resin Fractionation (RRF).** The RRF method used in this analysis follows the methods described by Chow *et al.* (2004), which were modified from fractionation schemes developed by Bolto *et al.* (1999) [3, 4]. Briefly, samples (400 mL) are filtered through a 0.45 μm PES filter (Merck Millipore, Ireland) and acidified with HCl (1M) to a pH of 2, passed through a column of bed height 10 cm of Supelite DAX-8 resin (Sigma-Aldrich) with a flowrate of 2 mL per minute. A sample (50 mL) of the completed column effluent is taken for DOC analysis, with the remaining effluent passed through a second and third column containing Amberlite XAD-4 resin and Amberlite IRA958 resin (Sigma-Aldrich), at identical bed height and flowrate.

**Liquid Chromatography – Organic Carbon Detection (LC-OCD).** NOM was quantified by molecular weight distribution into five distinct fractions, as denoted by Huber *et al.* (2011) [5]. Samples were filtered through a 0.45 μm PES filter (Merck Millipore, Ireland) and loaded into a proprietary LC-OCD instrument (DOC-LABOR, Germany), with integration of chromatographs performed on corresponding ChromCALC software.

**Zeta Potential.** Triplicate measurements were made with folded capillary cells (DTS1070, Malvern, UK) in a Zetasizer Nano instrument (Malvern, UK).

## Results and Discussion

**Dissolved Organic Carbon & Zeta Potential.** DOC increased from riverine to lacustrine sites, as indicated by Figure 1, consistent with observations on American surface waters made by Rosario-Ortiz *et al.* (2007) and Kraus *et al.* (2010). The effect of water retention time on increasing NOM concentration in surface water reservoirs is further emphasised in the Australian context. Zeta potential decreased from -12.1mV in the riverine site to -24.9 mV in the lacustrine site. Since the maximum NOM removal by coagulation occurs from +5 – -10 mV [6], the decline in zeta potential from riverine to lacustrine would lead to an increasingly recalcitrant NOM.

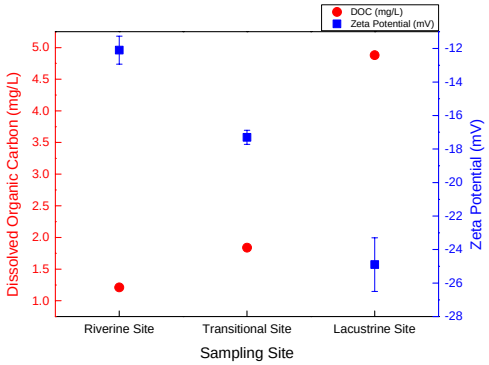



**Figure 1.** Dissolved Organic Carbon and Zeta Potential results from catchment analysis.

**Rapid Resin Fractionation.** An increase in SHA and VHA fractions is evident from Transitional to Lacustrine sites, as indicated in Figure 2. Low initial DOC for the riverine site meant RRF could not be accurately applied. Comparing the Australian lacustrine site to a Canadian lacustrine site analysed by Sarathy *et al.* (2010), demonstrates similar NOM character as measured by RRF [7]. The Canadian site had slightly higher % VHA (66% compared with 58% at the Australian Lacustrine site) and a lower %SHA (17% compared with 23%).

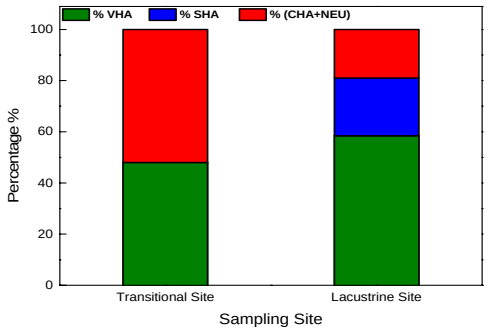



**Figure 2.** Rapid Resin Fractionation indicating percent Very Hydrophobic Acids (VHA), Slightly Hydrophobic Acids (SHA), Hydrophilic Charged (CHA) and Hydrophilic Neutral (NEU).

**Liquid Chromatography – Organic Carbon Detection (LC-OCD).** The biopolymer (BP), Humic Substances (HS), Building Blocks (BB) and Low Molecular Weight Neutral (LMWN) constituents of NOM increased in concentration from riverine to lacustrine sites (Figure 3**)**. The riverine site displayed no BP fraction, whilst an increase was observed from transitional and lacustrine sites. The HS fraction was the most dominant fraction in all sites, but only accounted for 35% and 43% of DOC in transitional and riverine sites respectively, yet represented 66% of DOC in the lacustrine site. Water retention time in reservoir systems is seen as contributing towards the concentration of humic substances. BB also increased in concentration from riverine to lacustrine sites but was most heavily represented at the transitional site.

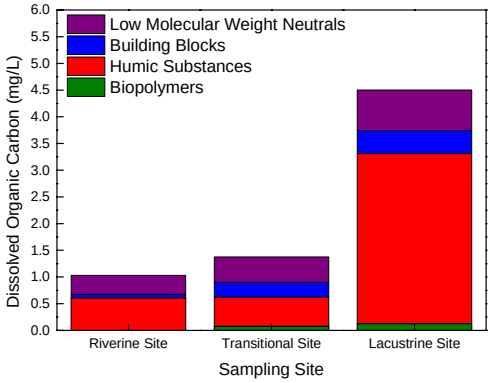



**Figure 3.** LC-OCD results from catchment analysis.

French lacustrine surface waters evaluated by Baghoth *et al.*, had a higher proportion of BP (10% of DOC compared with 3% for the Australian lacustrine site), slightly lower HS values (59% compared to 65%), higher BB values (16% compared to 9%) and equal LMWN values (16%) [8]. Hence, Australian NOM is indicatively less degraded through a higher percentage of HS and a contrastingly lower percentage of BB and BP.

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# Antioxidant properties of humic acids in Aquatic Environments - a Thermodynamic study

E. BLETSA [(a)], Y. DELIGIANNAKIS [(a)*]

(a) Laboratory of Physical Chemistry of Materials and Environment, Department of Physics
University of Ioannina, 45110 Ioannina, Greece
* E-mail: ideligia@cc.uoi.gr

**Keywords:** antioxidants, HALP, Leonardite HA, Soil HA, pH effect, thermodynamics, activation energy

**Abstract.** The antioxidant capacity of several humic acids (HALP, Leonardite and Soil HA) have been investigated in aquatic conditions, under a range of pHs. According to our previous work [1], humic acids are shown to perform successfulyl H-Atom Transfer (HAT) i.e. one $H^+$ and one $e^-$ being transferred *in tandem* (H-atom=$H^+e^-$) from the -OH of the polyphenol towards the target radical [1]. The radical-scavenging capacity (RSC) of humic acids was quantified based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical method. At the present study –for the first time- we investigate the effect of solvent and pH on the antioxidant capacity of HAs. In addition, herein, we present a detailed thermodynamic study on Hydrogen Atom Transfer dynamics (activation energy(Ea), entropy ($\Delta S^{\ddagger}$), enthalpy ($\Delta H^{\ddagger}$), Gibbs free energy ($\Delta G^{\ddagger}$)) of humic acids towards a reference stable radical DPPH·, in natural and synthetic HAs. The reveal a significant effect of pH and the structure of HAs on the antioxidant radical scavenging capacity of humics.

## Introduction

The antioxidant properties of humic acids have been investigated by several methods due to their high content of polyphenols. Antioxidants are one of primary importance in human health, biosphere function and – subsequently- materials' industry since they can scavenge harmful reactive oxygen species [2-5].

Recently, we first demonstrated that a model humic acid-HALP (Humic Acid Like Polycondensates) synthesized from copolymerization of Gallic and Protocatechuic acid (clear from impurities), performs fast/efficient antioxidant activity against ROS, which is achieved only through the Hydrogen Atom Transfer [HAT] mechanism i.e. one H+ and one e- being transferred in tandem (H-atom=$H^+e^-$) from the -OH of the polyphenol towards the target radical.

At the present study – for the first time – we investigate

(i) the antioxidant capacity of HALP, as well as of other natural HAs (standard Leonaridite HA from IHSS and a soil HA) **in real biological systems**,

(ii) the effect of pH on radical-scavenging capacity (RSC) of humic acids

(iii) a detailed thermodynamic study on antioxidant capacity (activation energy(Ea), entropy ($\Delta S$), enthalpy ($\Delta H$), Gibbs free energy ($\Delta G$)) of humic acids towards a reference stable radical DPPH· in these natural systems.

## Materials and methods

***Synthesis of HALP.*** Synthesis of HALP has been performed by oxidative copolymerization of gallic acid (GA) (3,4,5-trihydroxybenzoic acid) and protocatechuic acid (PA) (3,4-dihydroxybenzoic acid) with no use of a catalytic material, according to Giannakopoulos et al [1].

**Evaluation of antioxidant properties of Humic Acids.** The evaluation of antioxidant capacity of humic acids was achieved based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical method. The solution of humic acids were prepared in natural waters and the pH adjusted with NaOH. The solution of the DPPH•radicals was in Methanol, as the DPPH is insoluble in water. The reaction of humic acids with DPPH• radicals carried out in natural waters at the pH range between 6-12, in comparison to a polar solvent (methanol).

The antioxidant capacity of HAs were determined by monitoring the decay of the DPPH radical at 515 nm by using a UV-Vis spectrometer (Hitachi U-2900) equipped with a Temperature-controlled cryostat (Unisoku model CoolSpek UV USP-203B). Our set-up allows recording of UV-Vis spectra kinetics (10ms per point) in temperature range $+100^0$C to $-100^0$C

***Thermodynamic analysis.*** In all pH values, we obtained experimental kinetics data at different temperatures, in order to evaluate the thermodynamic parameters including enthalpy ($\Delta H^{\ddagger}$), activation energy (Ea) and entropy ($\Delta S^{\ddagger}$), which are basic principles in thermodynamics. The data-kinetics in each temperature was fitted with the **Arrhenius** and **Eyring-Polanyi** models to calculate the activation energy (Ea), and the enthalpy and entropy of reaction.

## Results

### Radical Scavenging Capacity of HAs (pH, solvent and structure effect)

Figure 1 displays the kinetic traces at 515nm of HALP interacting with the DPPH radicals at 26°C recorded for different pH values.

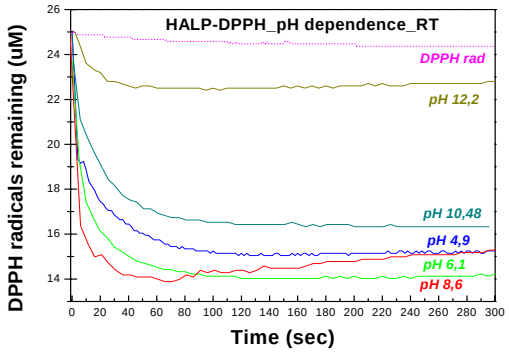



**Figure 1.** Kinetics of decay of absorbance at 515 nm for DPPH radicals ([DPPH]$_0$ = 30.0±0.1μM) reacting in the pH range from between 5 and 12, at 26°C.

Upon addition of the humic acid's solution to the DPPH radical solution in MeOH, the absorbance at 515nm immediately decreased (for reaction time<20 sec), as shown in Figure 1. These spectral changes suggest that the hydrogen atom transfer from the phenolic OH group of humic acids to the DPPH radical, take place to scavenge the radicals, according the folowing mechanism:

$$ArOH+DPPH\bullet \rightarrow ArO\bullet+DPPH-H \quad (1)$$

$$ArO\bullet+DPPH\bullet \rightarrow -ArO\text{-quinone} \quad (2)$$

Similar behaviour appears all HAs when interacting with the DPPH radicals.

From the total kinetic traces of HAs, we observed that:

(i) In natural systems, the rate of the reactions are hunderd of times faster of as compared to the reaction in organic solvents (Methanol).

(ii) The antioxidant capacity of humic acids are highly affected by the pH value, as in pH 8is observed the greater reactivity of HAs. Some of these results are summarized in table 1.

(iii) the structure of HAs (hydrophilicy/ hydrophobicity) determines the radical scavenging capacity of these compounds.

**Table 1**

| Humic acid/ $H_2O$ pH 8 | n (μM) scavenged DPPH/ g of HA | OH (μM) per g of HA | Amount of -OH which performs HAT (%) | Number of spins (μmol/g HA) |
|---|---|---|---|---|
| HALP | 269 | 5390 | 5% | 4.1 |
| Leonardite | 51 | 3300 | 1.5% | 1.8 |
| Soil | 40 | 1000 | 4% | 3.8 |

**Thermodynamic parameters**

After we recorded the DPPH· quenching kinetic, from the rate (*k*) we used Arhhenius and Eyring-Polanyi equations to identify the thermodynamic parameters of antioxidant mechanism.

In Figure 2, we compare the values of activation energy (Ea) in all pH range for each material, as we investigated from Arrhenius plots (ln*k* vs 1/T) of HAT reaction rates (***k***)

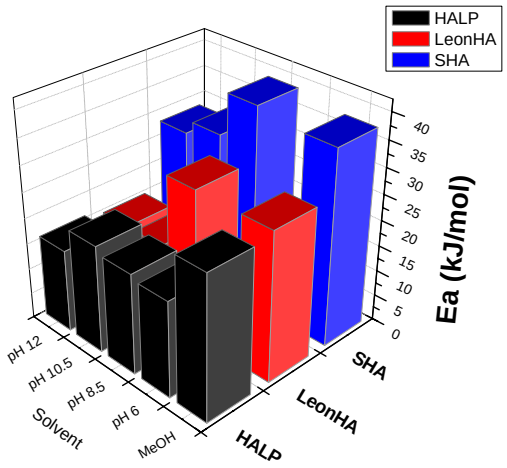



**Figure 2.**

According to the corresponding Ea values we observed:

(i) A decrease (~8 kJ/mol) at the activation energy of HAT mechanism when we used water as solvent vs. aprotic methanol.

(ii) At higher pH values, the energy barrier of the reaction is decreased [7].

Thus when extrapolated to real systems, this work provides a quantitative evidence that HALP or other natural humic acids exhibit high antioxidant activity, which is severely influenced by (i) pH, (ii) solvent and (iii) structure of HAs.

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# Vermicomposted tannery wastes in the organic cultivation of sweet pepper: growth, nutritive value and production

R. M. BONTEMPI [(a)], T. S. OLIVEIRA [(a)], M. O. O. REZENDE [(a)], R. R. NUNES [(b)*]

(a) São Carlos Institute of Chemistry, University of São Paulo, Brazil
(b) Academic Unity of Serra Talhada, Federal Rural University of Pernambuco, Brazil
* E-mail: ramom.rachide@ufrpe.br

**Keywords:** Waste management, Tannery wastes, Vermicomposting, Sweet peppers

**Abstract.** In this study, vermicomposted tannery wastes were applied in the organic cultivation of sweet pepper in a greenhouse. The effects of this organic matter addition on plant development was assessed. In a greenhouse, plants were cultivated adding vermicomposted tannery to the substrate (sample VRC) and the results were compared with the samples control (dYL; only soil) and reference (NPK; adding mineral fertilizer). The growth, nutritive value and fruit production were assessed to evaluate the plant development in different types of substrate. The vermicompost addition was biostimulating to the plants, influencing development positively, in addition to increasing fruit production. When the vermicompost were applied, up to 3 harvested sweet peppers were obtained per plant, compared with only 1 fruit for the reference treatment with NPK addition.

## Introduction

Environmental toxins and demand for safe disposal of industrial wastes are currently topics of concern. The leather industry is associated with the generation of huge amounts of solid wastes, and waste disposal is a serious problem. Untreated solid waste and wastewater generated by the tanning industry can cause serious environmental damage, particularly to surface water and groundwater. To avoid such pollution, specific chemical, physical and biological treatments have been adopted to reduce waste toxicity and enhance tannery waste use/recycling systems [1, 2].

In this context, vermicomposting is an effective technology that can reduce the toxic effects of tannery wastes, based on earthworm activity of *Eisenia fetida* L., which also improves nutrient availability of OM. Moreover, earthworms can accumulate heavy metals in their chloragogen cells, resulting in detoxification of polluted biomass [2].

Sweet pepper (*Capsicum annuum* L.) is a highly consumed vegetable that is better adjusted to and therefore most frequently produced under greenhouse conditions. Vermicompost fertilization is well known for its effects on plant nourishment, which result in reduced requirements for (or the elimination of) chemical pesticides and inorganic fertilizers.

The aim of this work was to determine the effects of application of vermicomposted tannery wastes on organic sweet pepper cultivation in the greenhouse. In particular, the effects of this organic matter addition on plant development, i.e., growth, nutritive value and fruit production, were assessed.

## Materials and methods

This study was conducted using vermicomposted tannery wastes in the form of both sludge and tanned chips mixed with cattle manure and sawdust. The proportion of the residues was determined by a combination of their C:N ratios. Each barrel received 20 L of the mixture of wastes as follows, based on fresh volume: i. Sludge mix (S); ii. Tanned Chips mix (C); and iii. Sludge + Tanned Chips mix (SC). For comparison, a vermicompost with no leather wastes was also prepared (standard treatment, STD). For vermicompost production, 500 newly born earthworms (*Eisenia fetida* L.) were added to each vermicomposter. Values of vermicomposts MO, TOC, CEC are available in Scaglia et. al. (2014) [1].

**Sweet pepper organic cultivation.** Samples of a dystrophic yellow latosol (dYL) were collected on Campus II of the University of São Paulo (21°59'51" S, 47°56'20" W).

The attributes of the latosol were typical of a dYL soil, i.e., acidic with a pH of 5.74, poor OM content of 1.52%, TOC of 0.25%, CEC of 64.91 $cmol_c\ kg^{-1}$, and small base saturation of BS 27.99%.

Sweet pepper seedlings (biologically certified) was grown in a 25.0 L vessel containing 20.0 L of substrate. According to the different types of substrates, the following treatments were studied: dYL: Soil, without previous treatment (control treatment); NPK: Soil and 3.0% (V/V) of mineral NPK fertilizer at 12:5:14 (reference treatment); VRC: Soil and vermicompost (S, C or SC) at concentrations of 1.5, 3.0 and 6.0% (V/V).

Sweet pepper growth was monitored. The height (stalk size) of the plants was measured from the base (substrate) to the culm apex. The number of fixed leaves (below the apex) was counted and recorded every two weeks for each plant. The width (w) and length (h) of the 5 largest leaves of each plant were measured every fifteen days. From these data, the average leaf area (A) was estimated.

Four periods related to flowering and the formation of green and mature fruits were assessed with daily monitoring. The average flowering time was based on initiation of flowering. Fruit formation was measured from shortly after petal fall. Formation of stable green fruits was determined by size and appearance. The average maturation time was determined by formation of stable, full-red fruits.

## Results

The development of sweet pepper plants in dYL and NPK was similar; however, fruits were only produced on plants in the NPK treatment.

Plant growth in treatments dYL and NPK was not significantly different at 12.7 and 19.0 cm, respectively. Therefore, the addition of NPK fertilizer did not significantly affect plant growth. Plant growth in the control and reference treatments was significantly different from all treatments in the VRC group at all studied concentrations.

Plants cultivated with the control vermicompost (STD) added at 1.5%, 3.0% and 6.0% did not show significant differences in growth (ANOVA bootstrap, $p < 0.05$), after 120 days of the experiment. The difference in plant growth was ~6–28%, with mean growth between 32.0 and 41.0 cm.

Data related to the leaves (e.g., number, area, and nutritive value) were important to monitor and to qualify in the development of sweet pepper plants in different types of substrate. For the leaf area (A), after 60 days, the area of leaves in dYL was significantly smaller than that in the other treatments (8.33 $cm^2$). In NPK and 1.5% - VRC treatments, the areas of leaves were not significantly different, with values varying between 23.82 and 31.27 $cm^2$ (ANOVA bootstrap, $p < 0.05$).

Concerning the micronutrients, the highest concentrations were in plants from the 6.0% - STD treatment, with high levels of B (164.12 $mg.kg^{-1}$), Zn (145.84 $mg.kg^{-1}$) and Fe (1316.88 $mg.kg^{-1}$); and in the plants from SC treatments, with high levels of Cu (29.64 $mg\ kg^{-1}$ in 3.0% - SC) and Mn (492.57 $mg.kg^{-1}$ in 3.0% – SC).

Fruit production, in addition to fruit characteristics (Table 1), are important attributes for evaluating the effects of vermicompost addition on sweet pepper cultivation. Regarding production, plants subjected to the control treatment (dYL) did not produce any fruit, whereas those exposed to the reference treatment (NPK) produced only 1 cropped fruit per plant. The optimum results were obtained for the plants exposed to the 3.0% - SC and 6.0% - SC treatments, which produced 3 fruits per plant. Plants subjected to the 6.0% - S, 3.0% - C, and 6.0% - C treatments produced 2 fruits. For the other treatments, the plants only produced 1 fruit.

**Table 1.** Red sweet pepper production on different substrates (6.0% of STD, S, C and SC), and samples reference (NPK) and control (dYL) (mean, ANOVA bootstrap, $p < 0.05$, $n = 3$).

| | Fruit production | Time formation | Fruit characteristics | | |
|---|---|---|---|---|---|
| | | | Size | Circumference | Weight |
| | fruit $plant^{-1}$ | days | cm | cm | g |
| dYL | 0a | - | - | - | - |
| NPK | 1bcd | 94a | 11.3abc | 19.1bcd | 96.8abc |
| 6.0% - STD | 1cd | 87a | 10.9abc | 18.8abcd | 134.5c |
| 6.0% - S | 1cd | 91ab | 12.4c | 23.0d | 107.0bc |
| 6.0% - C | 2de | 100abc | 12.5c | 19.8dd | 113.3c |
| 6.0% - SC | 3e | 87a | 10.5abc | 19.0abc | 100.8cc |

Values in the same column followed by the same letter are not significantly different at $P < 0.05$ according to Duncan's test.

## Conclusion

The present study confirmed the expectation that the vermicomposted tannery wastes can be applied in the organic cultivation of sweet pepper. The vermicompost addition was biostimulating to the plants, influencing development positively, in addition to increasing fruit production. Although the fruits obtained in the NPK (reference treatment) were statistically similar in characteristics to those cultivated with vermicomposted tannery wastes, the production in this treatment was limited to 1 fruit per plant, whereas in other treatments, up to 3 red sweet peppers were harvested per plant.

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# Measurement of CDOM distribution in two northern ecoregions by satellite imagery

P. L. BREZONIK [(a)]*, R. M. HOZALSKI [(a)], L. G. OLMANSON [(b)], J. C. FINLAY [(c)], C. G. GRIFFIN [(c)], M. E. BAUER [(b)]

(a) Dept. of Civil, Environmental, Geo-Engineering., Univ. of Minnesota, Minneapolis, MN 55455, USA
(b) Dept. of Forest resources, University of Minnesota, St. Paul, MN 55108, USA.
(c) Dept. of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN 55108, USA
* E-mail: brezonik@umn.edu

**Keywords:** CDOM, DOC, Landsat, remote sensing, lakes

**Abstract.** CDOM levels in lakes of two large north-temperate ecoregions were determined using Landsat 8 imagery calibrated with ground-collected data. The Northern Lakes and Forests and the North Central Hardwood Forest ecoregions span northern Minnesota, Wisconsin, and Michigan in the U.S. Upper Great Lakes region. Factors controlling distributions of CDOM in the ecoregions and differences between the regions are described. The usefulness of satellite-derived CDOM to estimate lake DOC is discussed.

## Introduction

CDOM (colored dissolved organic matter) is the major component of dissolved organic matter in many natural waters, and its presence has major effects on the ecology, chemistry, and suitability of water for human use. Many studies have reported close correlations between CDOM and dissolved organic carbon (DOC) concentrations [e.g., 1-2]; its use to estimate DOC is of interest to quantify the role of freshwater DOC in carbon cycles. In spite of its importance, information is limited on the spatial distribution and temporal variability of CDOM (also called "organic color" or simply "color" in earlier literature) in U.S. waters, including the Upper Great Lakes states (UGLS), because few routine monitoring programs measure it. Satellite imagery is an attractive choice for measuring CDOM because it can acquire data comprehensively over broad regional scales. Several studies [e.g., 3-5] have retrieved CDOM levels from satellite reflectance data, but they generally have used calibration data from relatively few lakes or small regions. The reliability of these methods to retrieve CDOM on thousands of diverse lakes across large geographic regions has not been tested.

The limited availability of CDOM data for the U.S. means that some fundamental questions about CDOM behavior remain unanswered. For example, we lack basic information on its geographic distribution and how it varies by ecoregion, land cover, and other terrain features. We also lack quantitative understanding of the factors that control CDOM inputs to lakes. Data on temporal trends are scarce, and we cannot state with confidence whether CDOM levels have been increasing on a regional scale in U.S. waters, as they have in Scandinavia [6]. This paper presents comprehensive data on CDOM levels in two large, lake-rich ecoregions of the north-central U.S. and evaluates its distribution across a range of spatial scales. We also address the usefulness of satellite-based CDOM data to estimate DOC concentrations at regional scales.

## Methods

**Study area:** Our study focused on two large ecoregions that extend across the U.S. Upper Great Lakes states of Minnesota, Wisconsin, and Michigan [Figure 1]: Northern Lakes and Forests (NLF) and North Central Hardwood Forest (NCHF). The NLF is highly forested (49%; 61% for the Minnesota portion), with many wetlands and open water, including many thousands of lakes. In contrast, almost half (48%) of the NCHF is used for agriculture; forests account for 26% (only 11% for the Minnesota portion).

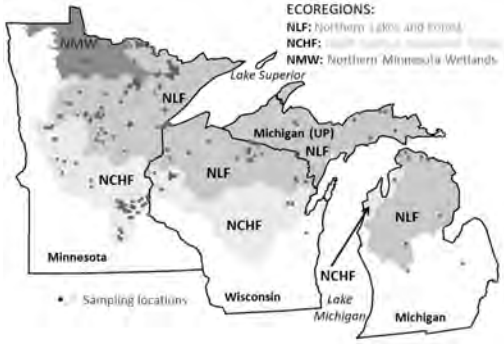



**Figure 1.** Map of study area in north-central U. S. A.

To obtain calibration data for CDOM retrieval from satellite data and to develop a better understanding of the relationships between CDOM and other chemical/ ecological characteristics of the region's surface waters, we sampled ~300 lakes in the three states from 2014 to 2017. Samples were analyzed for CDOM as absorptivity at 440 nm ($a_{440}$), DOC, dissolved Fe, chlorophyll, suspended solids, and other background variables.

Landsat 8 images of the 3-state region for several summer/fall 2016 dates were downloaded from the USGS EROS Center as Collection 1 (atmospherically corrected) surface reflectance data. We applied a two-term band-ratio model to the imagery that uses the Landsat-8 green, red, and NIR bands (OLI 3-5), which we found in previous studies [5] to work well for optically complex lakes. Applied to the 2016 data, the model became:

$a_{440}$ = -5.48*OLI3/OLI4 - 0.63*OLI4/OLI5) + 8.14;
$R^2$ = 0.77; RMSE = 0.624; N = 194

## Results and Discussion

**CDOM distribution:** The NLF and NCHF ecoregions are too large to display maps here of lake CDOM levels across their entirety. Figure 2 shows CDOM distribution in the Minnesota part of the NLF, an especially lake-rich area encompassing 64.277 $km^2$ with ~5.650 lakes. For color maps of the entire ecoregions, readers are referred to https://water.rs.umn.edu.

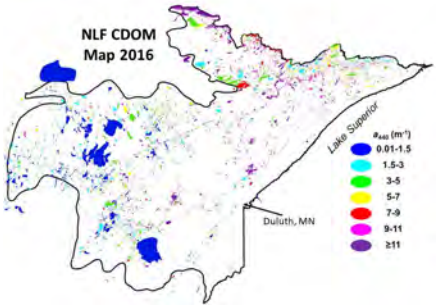



**Figure 2.** CDOM distribution in lakes of the Minnesota portion of the Northern Lakes and Forests ecoregion in 2016.

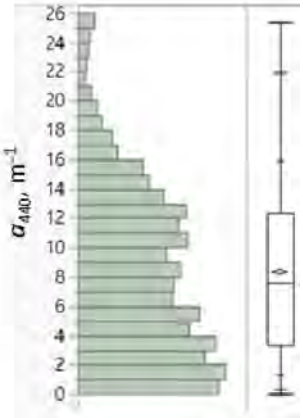
The Minnesota NLF has a wide range of lake CDOM levels ($a_{440}$ < 1 to > 25 $m^{-1}$; Table 1; Figure 3) and overall an abundance of CDOM-rich lakes (mean $a_{440}$ = 8.3 $m^{-1}$). Relationships between CDOM levels and catchment land cover and other geo-statistical analyses of CDOM distributions in the NLF and NCHF ecoregions will be presented at the conference.

**CDOM-DOC relationships:** A high $r^2$ (0.89) was found between CDOM and DOC for the 300 sites in our 2014-2016 studies, but a poorer relationship with a higher slope was apparent for low-CDOM waters ($a_{440}$ < 3.0 $m^{-1}$; Figure 4). Based on these findings and a review of relationships in the literature [*7*], we conclude that satellite-based CDOM can predict DOC from for waters with moderate to high CDOM levels ($a_{440}$ > 3 $m^{-1}$).

**Table 1.** Distributional statistics for CDOM in Minnesota NLF

| Quantiles | |
|---|---|
| 100% | 25.40 |
| 97.5% | 21.90 |
| 90.0% | 15.92 |
| 75% | 12.38 |
| 50% median | 7.60 |
| 25% | 3.39 |
| 10% | 1.32 |
| 2.5% | 0.30 |
| 0.0% | 0.01 |
| **Summary statistics** | |
| Mean | 8.33 |
| Std Dev | 5.79 |
| Std Err Mean | 0.08 |
| N | 5648 |

**Figure 3.** Histogram of MN NLF CDOM.



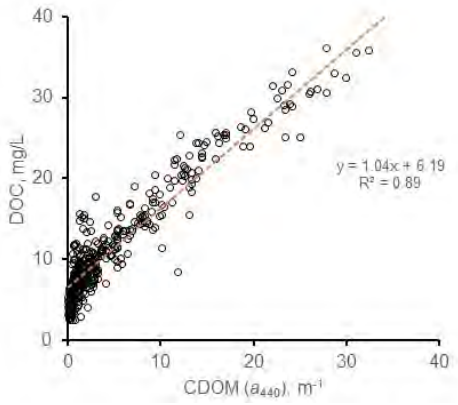




**Figure 4.** DOC vs. CDOM ($a_{440}$) for all 2014-2016 ground-based measurements in the three-state region.

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# Characteristics and effect of the photo−dissolved organic matter from suspended soil organic matter

H.S. LEE [(a)], J. HUR [(b)], H.S. SHIN [(a)*]

(a) Department of Environment Engineering, Seoul National University of Science & Technology, 01811 Seoul, South Korea
(b) Department of Environment & Energy, Sejong University, 05006 Seoul, South Korea
* E-mail: hyuns@seoultech.ac.kr

**Keywords:** particulate organic matter, photo−dissolution, EEM−PARAFAC, XAD fractionation, THMFP

**Abstract.** In this study, we investigated the characteristics and effect of the photochemical release of dissolved organic matter (DOM) from suspended particulate organic matter (POM) in the soil. Photo−dissolved organic matter from the suspended POM was quantified and characterized using fluorescence exitation−emission matrix combined with parallel factor analysis (EEM−PARAFAC) and XAD fractionation, and its specific trihalomethane formation potential (THMFP) was evaluated. The results confirmed that terrestrial humic components in the DOM were increased by UV irradiation and highly contribute to the formation of THM.

## Introduction

High amounts of soil organic matter (SOM) flows into the river from rainfall of high intensity [1]. The inflowed soil POM is transformed to DOM by physical and photochemical effects during flotation [2]. By many of earlier literature, POM is known to produce much DOM into water system by the photo−dissolution, but only a few studies have been reported on the characteristics and effect of the photo−dissolved organic matter [3].

EEM−PARAFAC has been widely used to decompose DOM by the fluorescence characteristics for past of decade, and XAD fractionation also used to divide the DOM into hydrophobic and/or hydrophilic fractions [3] found that the DOM produced during photo dissolution of river sediments included primarily humic-like PARAFAC components [2], reported that more than half of the water extractable DOM from soil was a hydrophobic acid fraction. In this study, the characteristics of DOM generated from the resuspended soil particles by UV irradiation were quantified and identified by using EEM−PARAFAC and XAD fractionation. The effect of the photo−dissolved organic matter on THM formation was also evaluated.

## Materials and methods

**UV irradiation experiment** Soil was taken from the surface horizon within 0−5 cm depth near Han River, South Korea, and sieved less than 200 μm in size. The particle resuspension solution was prepared by mixing the soil with distilled water at a ratio of 20:1 (g/mL). The suspension samples were divided into two as the control and experiment groups. The UV irradiation was conducted in a closed cabinet with twelve 8W−UVA lamps (F8T5BL, Sankyo Denki). An aliquot of the solution was taken at appropriate time intervals during UV irradiation and immediately filtered using 0.45 μm membrane filter.

**DOC and EEM-PARAFAC** DOC was measured by a TOC analyzer (Vario TOC select, Elmentar). The EEMs were measured with spectrofluorometer (F−7000, Hitachi) and PARAFAC was performed on EEMs of 30 samples in MARLAB 7.0.4 (Mathworks, Natick).

**XAD fractionation and THMFP test** XAD resin fractionation was conducted by Imai's method [4]. THMFP (trihalomethane formation potential) was analyzed by adding sodium hypochlorite to the diluted solution, in which the concentration was 1 mg−C/L, at a sufficient chlorine dosage of 5 mg−$Cl_2$/mg−C. The chlorinated solution was incubated for 24 hr and analyzed by GC-MS (7890B−5977A, Agilent) with purge and trap.

## Results and Discussion

### Characterization of photo-released DOM

As shown in Fig. 1, DOC values increased with increasing UV irradiation time compared with dark conditions. DOC in the absence of UV was generated about 20% compared with the initial value for 48 hrs, while increase rate of DOC with UV was about 80%. It was nearly 4 times higher than without UV.

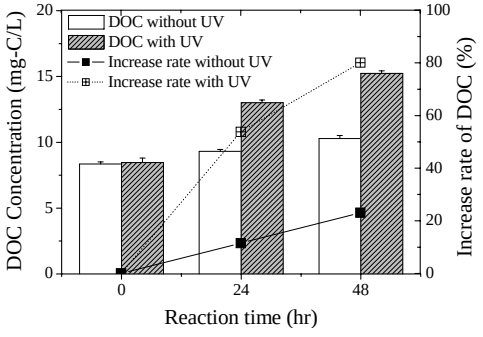



**Figure 1.** Changes in DOC in the presence and absence of UV irradiation time.

Considering the total organic carbon in the soil sample, only 2−4% of the carbon content was present in the dissolved state regardless of the presence or absence of UV.

The 4 PARAFAC components were assigned as three humic substance-like (C1−C3) and one protein−like

(C4) based on the comparison of spectral characteristics reported in the previous studies [1, 3, 4]. Among the humic substance-like components, C1 was mainly attribute to the terrestrial humic−like and C2 and C3 were assigned to microbial humic-like and fulvic−like characteristics, respectively.

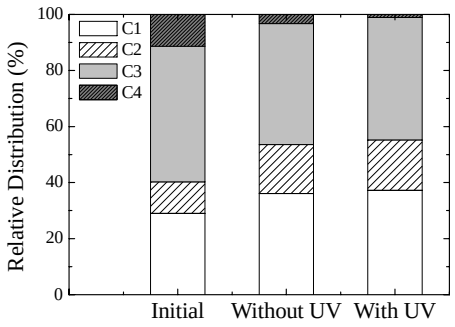



**Figure 2.** Comparison of relative distribution(%) of fluorescence PARAFAC components (C1−C4) in the presence and absence of UV irradiation.

Fig. 2 illustrated that the relative contents of C1 and C2, which correspond to humic components with aromatic structure, increased after 48 hrs of incubation, while C3 and C4 known as fulvic−like and protein−like respectively, decreased. On the other hand, there was no significant difference between the presence and absence of UV irradiation, which may suggest with the results of DOC changes in Fig. 1 that the increase of the photo-released organic matter under the UV irradiation is mainly due to desorption rather than the photo-decomposition of SOM.

### Changes in XAD fractions and THMFP of photo−dissolved organic matter

The XAD fraction of photo-dissolved organic matter with respect to UV irradiation time was presented in Fig. 3. The DOM was divided into three fractions, which are hydrophobic acid (HoA), hydrophobic neutral (HoN) and hydrophilic (Hi). The HoA fraction increased significantly under the UV irradiation in accordance with reduction of HoN fractions. The reduction of HoN might be associated with the decrease in C3 and C4 as shown in Fig. 2, and increase in HoA was closely correlated to the increases in C1 and C2.

Table 1 showed that the specific THMFP value of the photo−dissolved DOM with respect to UV irradiation time. The specific THMFP highly increased from the initial values of 73.0 μg/L and reached the maximum of 114.6 μg/L at 24 hr.

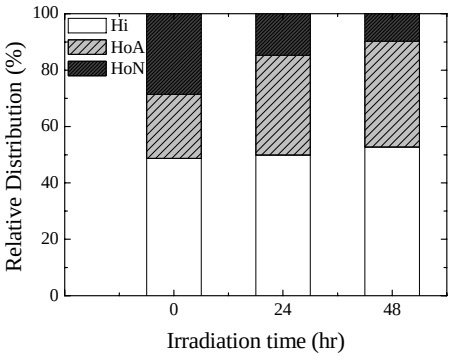



**Figure 3.** Changes in relative distribution (%) of XRD resin fractions of DOM leached from suspended SOM with respect ot UV irradiation time.

**Table 1.** THMFP value of DOM released from suspended SOM with respect to UV irradiation time.

| Irradiation time (hr) | CF[a] (μg/L) | DBCM[b] (μg/L) | Total (μg/L) |
|---|---|---|---|
| 0 | 72.2 | 0.78 | 73.0 |
| 24 | 113.5 | 1.10 | 114.6 |
| 48 | 81.1 | 0.55 | 81.7 |

[a]Chloroform
[b]Dibromochloromethane

In this study, characterization of DOM released from the resuspended soil particles in the presence and absence of UV light was compared each other and confirmed that terrestrial humic-like components in the DOM(i.e., C1 and C2, HoA) were increased by UV irradiation. The DOM may significanlty contributed to the formation potential of THM in water.

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# Removal of metals from seawater using a magnetic hybrid matrix synthesized with natural organic matter (NOM)

J. R. A. LIMA [(a)], I. A. A. SILVA [(a)], G. C. DA CUNHA [(a)], L. P. C. ROMÃO [(a)*]

(a) Federal University of Sergipe (UFS), 49100-000 São Cristóvão, SE, Brazil
*E-mail: luciane@ufs.br

**Keywords:** NOM, magnetic hybrid materials, remediation, metals.

**Abstract.** The discharge of wastewater containing metals risks the contamination of water resources. This work investigates the removal of Cu and Pb present together in solutions prepared with deionized water and seawater, using a magnetic hybrid adsorbent synthesized according to a modified sol-gel route employing water with a high content of natural organic matter (NOM) to form the organic phase. XRD analyses confirmed formation of the cobalt ferrite phase. FTIR spectra showed the presence of NOM in the structure of the hybrid with a surface area of 108.2 $m^2$ $g^{-1}$. In adsorption tests using mixed solutions of Cu and Pb, the removal rates ranged from 70.5 to 93.2% (deionized water) and from 84.5 to 92.0% (seawater), respectively, at pH 5 and an equilibrium time of 90 min.

## Introduction

Metals are widely used in many industrial sectors, including the manufacture of batteries, metal alloys, and pesticides. The effluents from these industries can contain high levels of metals that, when discharged without prior treatment, can contaminate aquatic environments and soils [1, 2].

The most toxic metals with the highest concentrations in wastewater are nickel, arsenic, chromium, zinc, copper, cadmium, cobalt, and lead [2]. Consequently, there is a constant search for effective treatments capable of removing these different metal species present in industrial effluents.

Adsorption using hybrid materials has emerged as a new option for the remediation of aquatic environments contaminated by metals. When such materials possess magnetic properties, they can be readily separated from the aqueous medium after the adsorption, by the application of an external magnetic field. In addition, hybrid materials may present functional groups that act as active sites for interaction with the metal ions present in the aqueous medium.

The purpose of this work was to synthesize a magnetic hybrid material by means of a sol-gel route modified by the use of natural organic matter (NOM) to compose the organic phase. Evaluation was then made of the effectiveness of this material for the removal of Cu and Pb from deionized water and seawater.

## Materials and Methods

### Synthesis of HMGS

The synthesis of the hybrid material was adapted from the methodology proposed by Culita *et al*. [3]. The inorganic precursors used were $CoCl_2.6H_2O$ and $Fe_2(SO_4).6H_2O$, in a proportion of 1:2, which were dissolved in 200 mL of water with a high content of natural organic matter (NOM), under constant mechanical stirring. The pH of the medium was then gradually increased to pH 9 with aqueous NaOH, with the mixture remaining under agitation for 30 min.

After gel formation, the system was heated at 100°C for 6 h to remove water and obtain a xerogel. This was then washed with deionized water to remove the excess sodium chloride generated, followed by drying in an oven at 60°C and homogenization. The resulting material, denoted HMGS, was characterized by X-ray diffraction (XRD), infrared absorption spectroscopy (FTIR), and surface area analysis.

### Adsorption assays

Firstly, the effect of the pH of the mixed metals solution was evaluated, varying the pH from 3.0 to 6.0. The tests were carried out with 100 mg of HMGS and 10.0 mL of the mixed solution of copper and lead prepared in deionized water, with initial metal concentrations of 50 mg $L^{-1}$, at 25°C and with agitation at 150 rpm for 60 min. Subsequently, the adsorbent was separated from the solution by application of an external magnetic field and the metal concentrations in the solution were determined by flame atomic absorption spectrometry (FAAS). Kinetic assays were performed as described above, at pH 5, with samples being removed at pre-established times between 5 and 240 min. In order to simulate a real sample, the adsorptive capacity of HMGS was evaluated using a mixed solution of Cu and Pb prepared in seawater, with initial metal concentrations of 50 mg $L^{-1}$. The tests were performed under the best conditions, at pH 5.25°C, and agitation at 150 rpm for 90 min.

## Results and Discussion

### Characterization

The most intense diffraction peaks of the hybrid material (Fig. 1) were in agreement with the diffraction data for the $CoFe_2O_4$ standard available in the Inorganic Crystal Structure Database (ICSD #192031). This demonstrated the success of the proposed synthesis route for the $CoFe_2O_4$/NOM hybrid, in which the solvents traditionally used were replaced by NOM, a natural polymer.

The FTIR spectrum of HMGS (Fig. 2) presented broad and intense bands in the region of 3430 $cm^{-1}$, attributed to the stretching of O-H groups of water, alcohols, phenols, and/or carboxylic acids. Bands characteristic of NOM were present at 1626 $cm^{-1}$, corresponding to stretching of C=C bonds of aromatic groups, and at 1366 $cm^{-1}$, attributed to symmetric stretching of $COO^-$. A band at 596 $cm^{-1}$ could be assigned to the Fe-O bond of ferrite [4]. A band at around 1100 $cm^{-1}$, attributed to the stretching of S=O bonds, was indicative of the presence of sulfate derived from the precursor used in the synthesis.

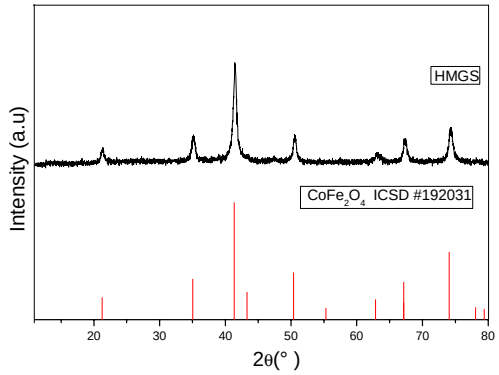



**Figure 1.** X-ray diffractogram of HMGS, compared to the ICSD diffraction pattern for cobalt ferrite.

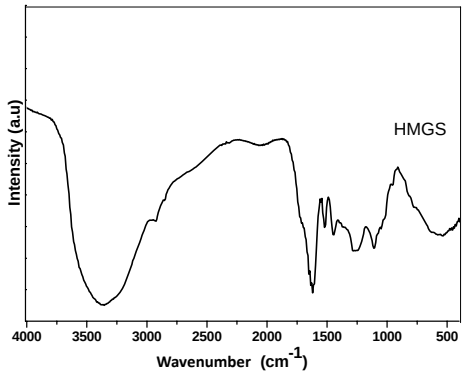



**Figure 2.** IR spectrum of the HMGS hybrid.

The hybrid material showed a high surface area of 108.2 $m^2\ g^{-1}$, which was higher than values reported in the literature, indicating the technological potential of the eco-friendly synthesis developed in this work. In contrast to other methods, the HMGS hybrid was obtained without heat treatment and using water with high NOM to compose the organic phase.

### Adsorption assays

Fig. 3 shows the influence of solution pH on the adsorption by HMGS of the metals present in deionized water. The highest removals were obtained at pH 5, with values of 60.1 and 100% for Cu and Pb, respectively. At this pH, the metals were in predominantly cationic forms and the HMGS surface groups were unprotected, allowing the adsorption to occur by ion exchange and/or electrostatic attraction [1, 2]. Both Cu and Pb showed fast adsorption kinetics (Fig. 4), with equilibrium times of 90 min and removal percentages of 93.2 and 70.5% for Pb and Cu, respectively. The kinetic data could be fitted using a pseudo-second order model ($r > 0.99$).

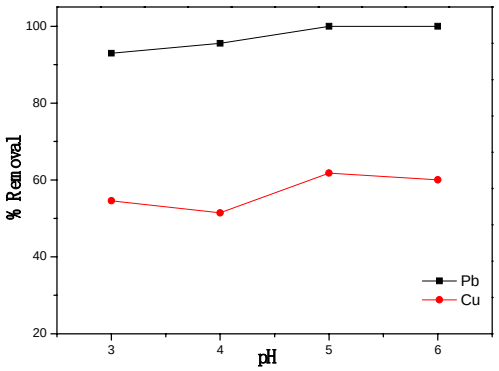



**Figure 3.** Effect of pH on the adsorption of Cu and Pb in mixed solutions. Conditions: $C_i$ = 50.0 mg $L^{-1}$, $m_{ads}$ = 100.0 mg; T = 25°C; agitation speed = 150 rpm.

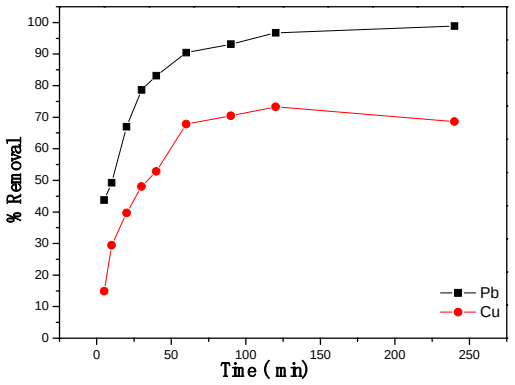



**Figure 4.** Kinetic study of adsorption of Cu and Pb in mixed solutions by HMGS. Conditions: $C_i$ = 50 mg $L^{-1}$, $m_{ads}$ = 100.0 mg; T = 25°C; agitation speed = 150 rpm.

The HMGS hybrid showed high adsorption capacities for the metals present together in seawater with removal percentages of 84.5% (Cu) and 92.0% (Pb). Hence, even in the presence of the high salt concentrations found in seawater, the HMGS hybrid was selective for the metals studied. Therefore this hybrid material can be used as an adsorbent for the treatment of contaminated aquatic environments.

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# Thermodynamic study of antioxidant properties of humic acids-nano hybrids in Aquatic Environments

M. LOULOUDI [(a)*], E. BLETSA [(b)] AND Y. DELIGIANNAKIS [(b)]

(a) Laboratory of Biomimetic Catalysis and Hybrid Materials, Department of Chemistry, University of Ioannina, GR45110, Ioannina, Greece.
(b) Laboratory of Physical Chmistry of Materias and Environment, Department of Physics, University of Ioannina, 45110 Ioannina, Greece
* E-mail: mlouloud@uoi.gr

**Keywords:** antioxidants, HALP, $SiO_2$ nanoparticles, nanohybrids, pH effect, thermodynamics, activation energy

**Abstract.** Antioxidant capacity of Humic Acid nanohybrids as model systems for [HA-soil] composite structures, have been investigated in aquatic conditions, under a range of pHs. Recently we have demonstrated that a Humic Acid Like Polycondensate [HALP][1] can be used for the synthesis of HALP-$SiO_2$ nanohybrids that perform efficient Hydrogen Atom Transfer [2, 3]. The radical-scavenging capacity (RSC) of HA-nanohybrids was quantified based on DPPH• radical method. At the present study –for the first time- we investigate the effect of solvent, pH and NP-size on antioxidant Hydrogen Atom Transfer capacity by HA-$SiO_2$ nanohybrids.

## Introduction

Humic substances (HS) are heterogeneous redox-active organic macromolecules, widely distributed in the environment, including waters, soils, and sediments. Among various functional groups in HAs, phenolic moieties show strong antioxidants reactivity against reactive oxygen species (ROS), in natural and engineered systems. In general, the antioxidant properties of these compounds are related with the presence of hydroxyl groups in their aromatic rings [4].

In the present study, well characterized HALP was used [1, 2] that can be used as a model to estimate the antioxidant capacity of the derived $SiO_2$-HALP nanohybrids **in real biological systems**. $SiO_2$-HALP nanohybrids combine the advantages of well-defined/ metal-free HALP and their grafting on $SiO_2$. $SiO_2$ nano-hybrids may be viewed as model systems for [HA-soil] composite structures.

The aim was to establish – for the first time:

(i) the antioxidant capacity of HALP nanohybrids **in real biological systems**,

(ii) a detailed thermodynamic study on Hydrogen Atom Transfer dynamics (activation energy (Ea), entropy ($\Delta S^{\ddagger}$), enthalpy ($\Delta H^{\ddagger}$), Gibbs free energy ($\Delta G^{\ddagger}$)) of humic acids towards a reference stable radical DPPH·, in HA-nanohybrids. These reveal a significant effect of pH and the structure of HAs on the antioxidant radical scavenging capacity of humics.

## Materials and methods

**Synthesis of HALP-SiO2 nanohybrids.** Synthesis of HALP has been performed by oxidative copolymerization of gallic acid (GA) (3,4,5-trihydroxybenzoic acid) and protocatechuic acid (PA) (3,4-dihydroxybenzoic acid) with no use of a catalytic material, according to Giannakopoulos et al. [1]. The preparation of HA- nanohybrids includes the immobilization of HA on the surface of $SiO_2$ NPs [2]. Covalent immobilization of HALP on the formed aminopropyl- $SiO_2$ NPs was achieved via the carboxylate groups. Two well-characterized commercially available, hydrophilic fumed $SiO_2$ nanoparticles with different specific surface area [Aerosil 300 and less-aggregated Aerosil OX50] were used as received [2].

**Evaluation of antioxidant properties of Humic Acids-nanohybrids.** The evaluation of antioxidant capacity of humic acids was achieved based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical method. The solution of humic acids were prepared in natural waters and the pH adjusted with NaOH. The solution of the DPPH•radicals was in Methanol, as the DPPH is insoluble in water. The reaction of humic acids with DPPH• radicals carried out in natural waters at the pH range between 6-12, in comparison to a polar solvent (methanol) [5].

The antioxidant capacity of HAs were determined by monitoring the decay of the DPPH radical at 515 nm by using a UV-Vis spectrometer (Hitachi U-2900) equipped with a Temperature-controlled cryostat (Unisoku model CoolSpek UV USP-203B). Our set-up allows recording of UV-Vis spectra kinetics (10ms per point) in temperature range $+100^0$C to $-100^0$C.

**Thermodynamic analysis.** In all pH values, we obtained experimental kinetics data at different temperatures, in order to evaluate the thermodynamic parameters including enthalpy ($\Delta H^{\ddagger}$), activation energy (Ea) and entropy ($\Delta S^{\ddagger}$), which are basic principles in thermodynamics. The data-kinetics in each temperature was fitted with the **Arrhenius** and **Eyring-Polanyi** models to calculate the activation energy (Ea), and the enthalpy and entropy of reaction.

## Results and Discussion

### Radical Scavenging Capacity of HA-nanohybrids (pH, solvent and particle size effect)

Figure 1 displays the kinetic traces at 515nm of HALP@$SiO_2$ NPs interacting with the DPPH radicals at 26°C recorded for different pH values.

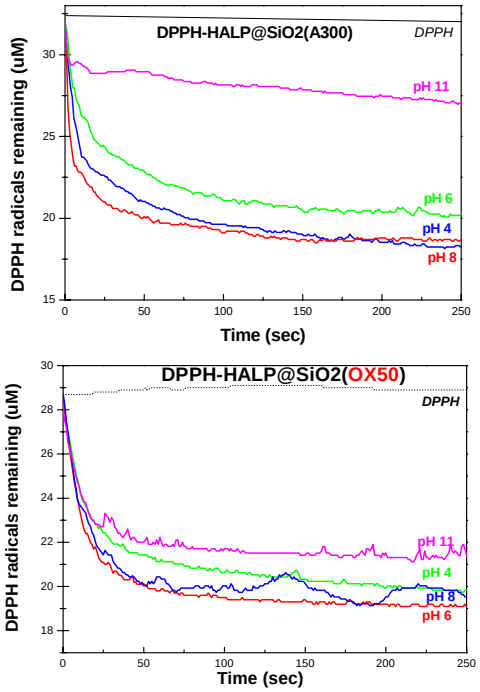



**Figure 1.** Kinetics of decay of absorbance at 515 nm for DPPH radicals ($[DPPH]_0$ = 30.0±0.1μM) reacting with (i) HALP@$SiO_2$ (A300) and (ii) HALP@$SiO_2$ (A300) NPs in the pH range from between 5 and 12, at 26°C.

Upon addition of HALP@$SiO_2$ NPs to the DPPH radical solution in MeOH, the absorbance at 515nm immediately decreased (for reaction time<20 sec), as shown in Figure 1. These spectral changes suggest that the hydrogen atom transfer from the phenolic OH group of humic acids to the DPPH radical, take place to scavenge the radicals, according to HAT mechansim.

Figure 2 shows an indicative Arrhenius plots (ln*k* vs 1/T) of the HAT reaction rate (***k***) for HALP (OXA300)-$SiO_2$ (Ea~14kJ/mole).

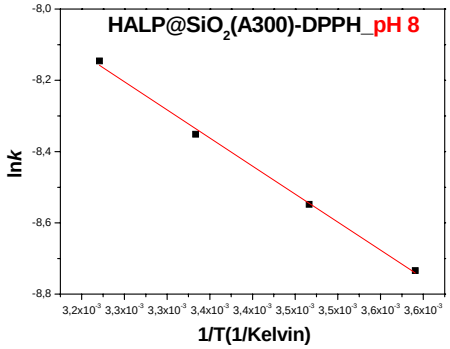



**Figure 2.** *Arrhenius* plot for HAT mechanism upon reaction of HALP@$SiO_2$-A300 NPs with the DPPH radical, at pH 8.

Similar behavior appear all HALP nanohybrids upon interacting with the DPPH radicals. From the total kinetic traces of HALP-$SiO_2$ nanohybrids we observed that:

(i) In natural systems, the rate of the reactions are hunderd of times faster of as compared to the reaction in organic solvents (Methanol).

(ii) The antioxidant capacity of humics acids are higly affected by the pH value, as in pH 8 is observed the greater reactivity of HAs.

(iii) the particle size of HAs has an strong effect at the radical scavenging capacity of these compounds.

According to the corresponding Ea values we observed:

(i) A decrease (~8 kJ/mol) at the activation energy of HAT mechanism when we used water as solvent vs. aprotic methanol.

(ii) At higher pH values, the energy barrier of the reaction is decreased.

Thus when extrapolated to real systems, this work provides a quantitative evidence that humic acids exhibit high antioxidant activity, which is severely influenced by (i) pH, (ii) solvent and (iii) particle size of NPs.

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# Molecular components related electron donating capacities of humic substances

J. T. LV [(a)], S. Z. ZHANG [(a)*]

(a) State Key Laboratory of Environmental Chemistry and Ecotoxicology,
Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
* E-mail: szzhang@rcees.ac.cn

**Keywords:** redox, FT-ICR-MS, molecular components, electron donating capacities

**Abstract.** The present study investigated the relationships between electron donating capacities (EDCs) of humic substances (HSs) and their molecular components detected by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). In order to decrease the molecular heterogeneity of bulk HSs, HSs were firstly separated into three fractions according to their polarities. The results demonstrated that compounds in HSs fractions with moderate polarity possessed a high content of total phenols and consistently had high EDCs. A strong linear correlation existed between EDCs and the total phenols content, which confirmed that phenols contributed to the EDCs of HSs. Further analysis of molecular components confirmed polyphenol-like compounds with medial oxygen were the major moieties acting as electron donors of HSs. This study provides a linkage between molecular components of HSs and their EDCs, which will help us to understand the molecular-dependent reducing properties of HSs or other dissolved organic matters under oxic conditions.

## Introduction

Because of their chemical diversity, humic substances (HSs) have reversible redox capacities within a wide range of redox potentials, and therefore are able to act as redox buffers and play very important roles in numerous biogeochemical processes [1]. Depending on how many redox-active functional groups in HSs are reduced or oxidized, HSs possess oxidation capacities to take up electrons by the oxidized moieties (electron accepting capacities, EACs) or reducing capacities to release electrons from reduced moieties to oxidant with a more positive redox potential (electron donating capacities, EDCs) [2]. However, the inherent mechanism by which the molecular compositions of HSs determine their electron transfer properties is still poorly understood, largely because of the compositional heterogeneity and chemical diversity of HSs [3]. At present only Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) can offer the possibility to detect the thousands of individual molecular formulae in HSs, which provides the opportunity to interpret the redox properties of HSs at the molecular level [4].

The present study selected four kinds of HSs and separated each bulk HS into three fractions according to their polarity, by a facile solid-phase extraction-stepwise elution (SPE-SE) method [5]. The molecular components, total phenolic contents and EDCs of all the HS fractions were analyzed and a direct linkage was established between the molecular characteristics and EDCs of HSs. The results of this study will enhance our understanding of the molecular-dependent electron donating capacity of HSs under oxidizing conditions.

## Materials and methods

**Fractionation of Humic Substances.** The bulk HSs (SRFA, SRHA, NAFA and PFA) were subjected to component fractionation into three fractions by solid phase extraction-stepwise elution (SPE-SE) processes reported previously [5].

**FT-ICR-MS analysis.** Ultrahigh resolution mass spectra were acquired using a Bruker SolariX FT-ICR-MS equipped with a 15.0 T superconducting magnet and an ESI ion source, as our previous study described [5].

**Folin-Ciocalteu assay.** Poly-phenolic substances in HS fractions were assessed according to the Folin−Ciocalteu method [6], with a minor modification.

**Mediated electrochemical oxidation (MEO) analysis**. Electrochemical experiments were controlled with a CHI630D electrochemical workstation. The EDCs of HSs were evaluated using the MEO method developed by Aeschbacher et al. [2] and conducted in a glovebox at ambient temperature.

## Results and Discussion

The molecular compositions of twelve obtained HSs fractions were analyzed using ESI-FT-ICR-MS. The identified $C_cH_hO_oN_{0-3}$ molecular formulae in each fraction are presented in van Krevelen diagrams (Figure 1).

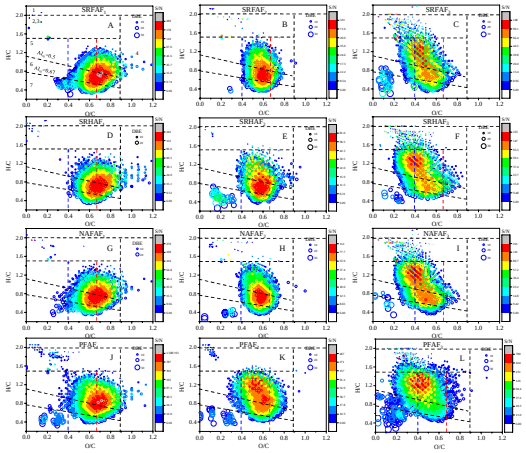



**Figure 1.** van Krevelen diagrams for $C_cH_hO_oN_{0-3}$ formulas obtained in HS fractions (F1, F2, F3) isolated from SRFA (A, B, C), SRHA (D, E, F), NAFA (G, H, I) and PFA (J, K, L) by SPE-SE. Color bars represent the S/N of peaks, bubbles represent DBE values.

The results clearly demonstrated that fractionation increased the chemical differences between different fractions but decreased the molecular heterogeneity within a fraction. Such fractionation is beneficial to investigation of the relationship between molecular components and specific chemical properties, such as the reducing capacities of HSs. Molecular formulae such as aliphatics, aromatics and polyphenols, etc., are distinctly located in different regions in the van Krevelen diagram. The formulae were further categorized into seven groups based on their AImod and H/C cutoffs. Considering the fact that many chemical properties, such as the EDCs of compounds, are dependent on their oxidation degree, the formulae in groups 5-7 were further divided into poor-, mid- and rich-oxygen subgroups according to their O/C values.

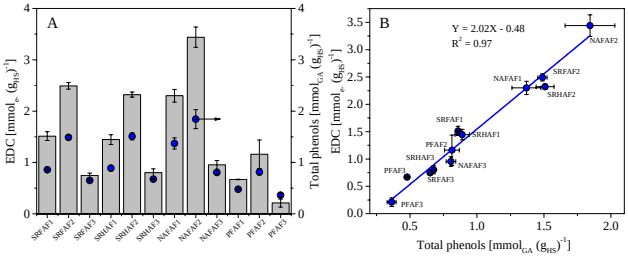



**Figure 2.** Electron donating capacities (EDCs) of HSs fractions of SRFA, SRHA, NAFA and PFA at an applied potential of Eh = 0.725 V at pH 7 (A), and plot of their EDCs versus total phenols measured by the Folin−Ciocalteu method, blue band is the 95% confidence interval for linear fitting (blue solid line).

The total phenolic concentrations determined are shown in Figure 2, a strong linear correlation was found between the total phenolic concentrations and the EDCs of all the HS fractions ($R^2$ = 0.97) in this study, which indicated that phenolic moieties acted as the major electron-donating moieties in HS fractions, which was consistent with a previous study [2].

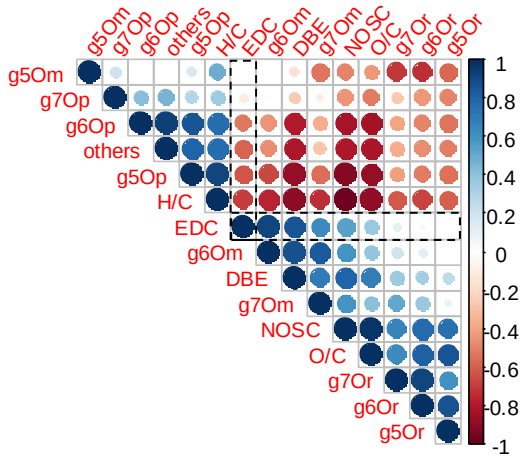



**Figure 3.** Pearson correlation matrix of the electron-donating capacities (EDCs) with the molecular characteristics of HS fractions (F1, F2, F3) of SRFA, SRHA, NAFA and PFA, $p < 0.05$.

Figure 3 displays the Pearson correlation matrix of the EDCs with the molecular characteristics of the fractions of SRFA, SRHA, NAFA and PFA with $p < 0.05$. The relative contributions of formulae distributed in the different molecular collections to their EDCs could be estimated intuitively from the van Krevelen diagrams.

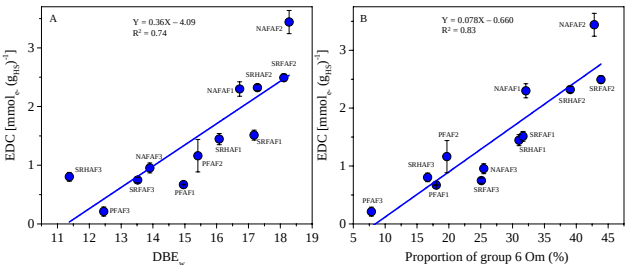



**Figure 4**. Plot of electron-donating capacities (EDCs) versus double-bond equivalence (DBE) (A), and EDCs versus the proportions of polyphenol-like molecules with mid-oxygen in HS fractions (F1, F2, F3) of SRFA, SRHA, NAFA, PFA.

Only DBE and the proportion of g6 Om were linearly correlated with the EDCs ($R^2$ = 0.74 and 0.83, respectively). As mentioned above, compounds in group 6 with medium oxygen content (g6 Om) made major contributions to the total contents of phenols in HSs, and a strongly linear correlation between the proportion of molecules in g6 Om and EDCs further indicated that mid-oxygen–containing polyphenol like compounds made more contribution to EDCs than other phenols in HSs. Understanding the relationship between formulae in HSs identified by ESI-FT-ICR-MS and their electron-donating capacity will help us to understand changes in the reducing capacities of HSs or other DOM in dynamic environmental processes.

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# Molecular fractionation of dissolved organic matter induced by adsorption on soil mineral

J. LV [(a)], S. ZHANG [(a)*]

(a) State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
* E-mail: szzhang@rcees.ac.cn

**Keywords:** DOM, molecular fractionation, ESI-FT-ICR-MS, adsorption, iron Oxyhydroxides

**Abstract.** This study investigated the molecular scale fractionation of liable DOM by adsorption on iron oxyhydroxides using ESI FT-ICR MS. The results demonstrated that poorly crystalline ferrihydrite exhibited higher affinity to DOM and induced more pronounced molecular fractionation of DOM than did goethite or lepidocrocite. High molecular weight compounds and compounds rich in oxygen or high unsaturation including polycyclic aromatic, polyphenols and carboxylic rich compounds had higher affinity to ferrihydrite. While low molecular weight compounds and compounds with low unsaturation and low oxygen were preferential maintain in water.

## Introduction

Dissolved organic matter (DOM) is ubiquitous in the environment and many chemical reactions such as complexation, redox reactions and photodegradation occur between contaminants and DOM. Interaction between DOM and minerals is widespread in both aquatic and terrestrial environments and acts as a key factor in the long-term storage of organic carbon, thus contributing to the global cycles of carbon [1, 2]. According to the supramolecular association concept, DOM is composed of numerous small molecular components which are self-assembled by hydrophobic interactions and hydrogen bonds. We therefore hypothesize that such associations may be destroyed when external forces from mineral surfaces to some small molecules are greater than the intermolecular forces between the molecular components of DOM. Molecules having higher affinity to mineral surfaces will therefore be sequestered while other molecule associations remain in water, leading to the molecular fractionation of DOM at the mineral-water interface. We therefore selected three common Fe oxyhydroxides, namely poorly crystalline ferrihydrite, crystalline goethite and lepidocrocite, to investigate the molecular fractionation of DOM at Fe oxyhydroxide and water interfaces using ESI-FT-ICR-MS.

## Materials and methods

The iron oxyhydroxides ferrihydrite, goethite, and lepidocrocite were synthesized according to methods described in the literature. DOM of peat leachate (PL) was extracted from Danish peat. Batch experiments for the sorption of DOM (0−100 mg $L^{-1}$) onto iron oxyhydroxides (0.82 g $L^{-1}$ferrihydrite, 1.66 g $L^{-1}$, goethite, 1.65 g $L^{-1}$ lepidocrocite) without background electrolyte were conducted in glass centrifuge tubes. Filtered supernatants of the sorption samples and control samples were analyzed by ESI-FT-ICR-MS. Solid phase extraction (SPE) was performed on all the samples to desalinate and concentrate. All possible formulas were calculated with Formula Calculator software based on the requirement that the mass error for a given chemical formula between measured mass and calculated mass was < 0.5 ppm.

## Results and Discussion

### Adsorption of DOM on Iron hxyhydroxides

Typical adsorption isotherms of DOM on iron oxide surfaces showed an initial steep slope and reached a plateau at elevated equilibrium C concentrations (Fig. 1). Goethite and lepidocrocite exhibited similar adsorption capacity to PL, but much lower than that of ferrihydrite.

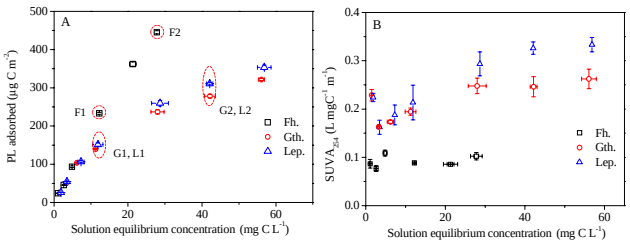



**Figure 1.** Adsorption of DOM on ferrihydrite (Fh.), goethite (Gth.), lepidocrocite (Lep.) (A); $SUVA_{254}$ values (B) of residual DOM suspension after adsorption on Fh., Gth., and Lep., dash circles F1, F2, G1, G2 and L1, L2 in (A) are samples selected to analysis by ESI-FTICR-MS.

### Molecular Characterization of DOM

The original DOM and DOM supernatants extracted from the adsorption isotherm experiments (as shown in Fig. 2A) for ferrihydrite (F1, F2), goethite (G1, G2) and lepidocrocite (L1, L2) were analysed by ESI-FT-ICR-MS. Totally 1923 molecular formulas were identified in the original PL. Molecular weight fractionation of DOM was evidenced comparing the ESI-FT-ICR-MS spectrum between the original DOM and DOM supernatant after adsorption on the iron oxyhydroxides with the most pronounced for ferrihydrite (Fig. 2B). The molecular weight (MW) above 500 decreased from 39.8% for the original PL to 5.7% (F1) and 13.7% (F2), while MW below 400 increased from 24.2% (original DOM) to 61.5% (F1) and 44.3% (F2).

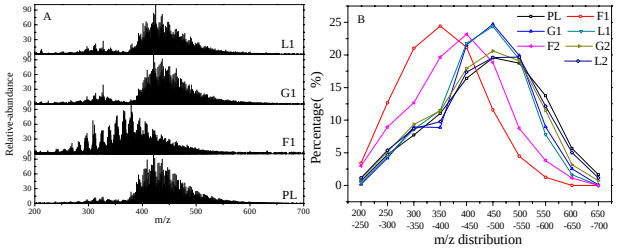



**Figure 2.** Broadband ESI-FTICR-MS spectrum (A) and molecular weight distribution (B) of original DOM and residual DOM suspension after adsorption on ferrihydrite, goethite, and lepidocrocite, F1, F2, G1, G2 and L1, L2 are samples labeled in Figure 1.

### Molecular Component Fractionation of DOM

The van Krevelen diagram is a useful tool which can provide a visual graphic display of compound distribution [3]. Compounds such as aromatics, polyphenols and aliphatics are located in different regions of the van Krevelen diagram. Aromaticity Index (AI) has been widely used to evaluate the aromaticity of a given molecular formula [4]. By using AI and element ratios of H/C, all the detected molecules displayed in the van Krevelen diagram were divided into four groups according to their molecular components as described in the method (Figure 3A-E). For the original DOM the majority (~ 85%) of the compounds were polyphenols (18%, group 2) and highly unsaturated organic acids and phenolics (67%, group 3). After adsorption on ferrihydrite, the chemical constituents of DOM supernatant changed, and about 62 and 43% of the total compounds in the original PL did not further exist in the DOM supernatant (F1 and F2). Specifically, about 100 and 96% of the compounds in group 1, 88 and 77 % of the compounds in group 2, 57 and 35% of the compounds in group 3, and 33 and 8% of the compounds in group 4 in the original PL disappeared in the DOM supernatant (F1 and F2), indicating that the affinities of the compounds in these groups to ferrihydrite were in the order: group 1 > group 2 > group 3 > group 4, whereas the differences in compounds between the original PL and PL supernatant in each group decreased in the inverse order. This further indicates the preferential adsorption of aromatics and then polyphenols, while aliphatic compounds were preferentially retained in the aqueous phase.

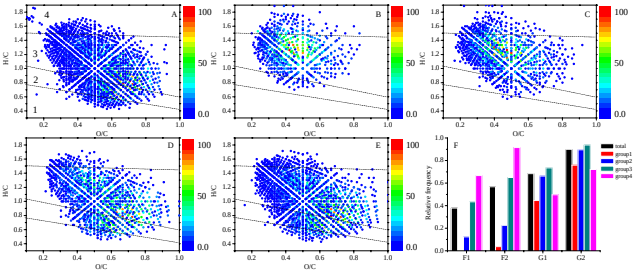



**Figure 3.** The molecular composition of DOM before and after adsorption on iron oxyhydroxides. A, B, C, D and E are van Krevelen diagrams of PL, F1, F2, G1 and G2, respectively.

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# Analysis of the parameters of agricultural viability and environmental impacts in an aquaponic system at farm São João – São Carlos, SP

A. A. MALHEIROS[(a)], G. RIBEIRO, [(a)], M. O. O. REZENDE [(b)*]

(a) São Carlos School of Engineering, University of São Paulo, 13566-590 São Carlos - SP, Brazil
(b) São Carlos Institute of Chemistry, University of São Paulo, 13566-590 São Carlos - SP, Brazil
* E-mail: mrezende@iqsc.usp.br

**Keywords:** aquaponics; integrated system of agriculture production; environmental education; education for sustainable development

**Abstract.** Aquaponics is the combination of aquaculture with hydroponics, in a symbiotic environment that allows the mitigation of the environmental problems caused by the fish breeding and present better social-environmental alternatives to conventional agriculture. This project aims the implementation of an aquaponics system at the farm São João in São Carlos-SP, Brazil, for the purpose of comparison with the conventional agriculture already running at the place and developing scientific literature regarding aquaponics. The system will be composed of nine grow boxes with tomatoes and one fish tank with tilapia.

## Introduction

Aquaponics is the production of food that uses fish and plants in a symbiotic environment, with less consumption of water and high reduction of organic waste. The term comes from the junction of two words, "aquaculture" and "hydroponics". Research on aquaponics started to show good results over the last years [1]. Some advantages of aquaponics are the low use of water, the possibility of food production in cities, the exploitation of fish waste, control of fungi and algae proliferation, diversification of the production and continuous generation of income and the minimization of environmental risks regarding contamination. Moreover, this system suggests the total reuse of the water, which avoids wasting and reduces the liberation of effluents in the environment, being more efficient at the water use and effluent generation than the hydroponics [2]. However, the operationalization of the aquaponics system is not easy, given its environmental, social and economic complexity. It is a non-organic system, since it uses industrial processed nutrients, and the low offer of natural fertilizers for the hydroponics causes difficulties in the production. If compared to traditional agriculture, aquaponics presents some disadvantages, like the continuous need for electric energy, restrictions on the use of pesticides and antibiotics, low technology in Brazil and the need for basic knowledge in certain areas, like engineering, hydraulic, biology, plant and fish breeding. From the educational point of view, there is the necessity of capacitation of the small producer for the right and sustainable operation of the system. As well as it is necessary to increase the channels of communication and awareness with the consumers, local governors, watershed committees and agencies of regulation and licensing related to the waters. Thus, this project aims to contribute in the generation of local knowledge to implementation of aquaponics systems in the region of São Carlos – SP. To understand its potential and possible social and environmental benefits, there is a case study in a local farm.

## Materials and methods

The present project was divided in two main methodological stages:

assembly of the infrastructure and data analysis. a) Assembly of the infrastructure.The structure of the system is composed of fish tank, pump, water tank of 20 $m^3$, greenhouse containing 9 boxes for planting and piping between the components. The tank has about 600 $m^3$ of water and 2500 fish and is waterproofed by PVC blanket; the bomb has 3 horses of power and supplies the water tank with 20 $m^3$/h; the greenhouse is 8 meters wide and 25 m and the boxes are 2x2 meters in area and 0.3 meters deep and are made with eucalyptus boards and screws and also coated with PVC blanket. Are filled with expanded clay, is emptied by means of a "bell" type siphon and filled with tubing, from the force of gravity (Figure 1). b) Analysis of the data

Twenty tomatoes were planted per box and the analyzes are done in two points: water tank and of the boxes. The collection is performed weekly and in the morning. The analyzes are done by spectrophotometry, using Hanna and Hach equipment. In addition, physical-chemical characteristics of the water are made in the water of the fish tank with the same frequency and at the same points. The samples are prepared and determined according to Standard Methods for the Examination of Water and Wastewater to $NH_3$, $NO^{2-}$, $NO^{3-}$, $PO^{4-}$, $Cl^-$, alcalinity, $Ca^{2+}$, Mg, Zn, K, Fe, pH, dissolved oxygen, electrical conductivity, Total Dissolved Solids, redox potential, pressure, temperature and salinity.

## Results and Discussion

Evaluation of the water quality was performed to the nutrients Fe, P, Mn, Zn, Cu and K. Boxes of 2x2x0.3 m3 were filled with expanded clay for the tomato cultivation, performing 180 plants, distributed in nine boxes. In the system, the water from the fish tank is pumped until a big water tank, which stays in a level above the level of the boxes. So, the water runs by gravity until the boxes to fill them. When it reaches the level of the siphon (about 4 centimeters below the level of the box), it empties

completely by the siphon system, and the water returns to the fish tank. This dynamic ensures that the system box-clay-microorganisms-plants remains uniformly oxygenated (Carneiro et al.), because that cycle happens twice an hour.



**Figure 1:** Assembly of boxes with expanded clay, inside the greenhouse.

The plants will absorb the nutrients which accumulate during the life of the fishes, contributing, along with the bacteria, to the biological filtering of water, ensuring a proper condition for the fish development. The roots, in turn, are a favorable environment for nitrifying bacteria development, further contributing to the formation of biologic filters in the boxes.

So far, the boxes work very well, guaranteeing two cycles per hour (the boxes will empty in about 6 minutes). For the next few months, the proper functioning of the boxes will be evaluated, taking into account the formation of biological filters in the system, to achieve the expected performance at the end of the process, without undesirable interventions.

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# Using water hyacinth (*Eichhornia crassipes*) biochar to remove chromium ions from tannery effluent

T. H. S. MENEZES[(a)], I. A. A. SILVA[(a)], J. R. A. LIMA[(a)], G. C. DA CUNHA[(a)], L. P. C. ROMÃO[(a)*]

[(a)]Federal University of Sergipe (UFS), 49100-000 São Cristóvão, SE, Brazil
* E-mail: luciane@ufs.br

**Keywords:** Water hyacinth (*Eichhornia crassipes*), biochar, remediation, leather industry, chromium.

**Abstract.** Increasing concentrations of toxic metals in water bodies require a constant search for effective treatments to remove industrial waste. This work describes the removal of chromium ions from tannery effluent employing water hyacinth (*Eichhornia crassipes*) biochar. FTIR analysis of the *in natur*e hyacinth (AGIN) and its biochar (BIO) revealed that the thermal treatment did not eliminate the principal chemical groups present in the *in natur*e biomass. However, the treatment increased the surface area, with values of 93.48 $m^2$ $g^{-1}$ for AGIN and 135.84 $m^2$ $g^{-1}$ for BIO. The biochar exhibited fast adsorption kinetics, with 55% removal of chromium ions from tannery effluent in only 20 min. The adsorption processes reached equilibrium after around 40 min, with a final removal percentage higher than 81% (6.8 mg $g^{-1}$).

## Introduction

Invasive aquatic species threaten socio-economic and ecological systems, by invading freshwater ecosystems and influencing their functionality and productivity, as well as disturbing key hydrological processes. Field surveys and reports for aquatic systems indicate that water hyacinth (*Eichhornia crassipes*) is one of the most aggressive invasive plant species [1].

It is necessary to develop new technologies for the sustainable use of many waste materials. One example is their use as adsorbents in applications that are economically and environmentally viable. Various wastes have been studied as adsorbents for the removal of organic and inorganic contaminants [2].

Leather manufacturing industries worldwide transform raw skins into resistant products by means of a sequence of mechanical and chemical treatments, with chromium being the main tanning agent used, due to the superior quality of the final product, compared to the use of other tanning agents [3]. In leather production, only 60-70% of the chromium applied in the form of basic chromium sulfate reacts with the skins, so about 30-40% of the chromium is discharged as solid and liquid wastes [4].

The chromium species Cr(III) and Cr(VI) are major environmental contaminants that are increasingly dispersed in hydric resources [5]. The biological effects of Cr(VI) include carcinogenicity and mutagenicity, while Cr(III) can become harmful at high concentrations, reducing immunological activity and, like Cr(VI), causing structural damage in human erythrocyte membranes and DNA [5,6]. Studies by Uluozlu et al. [6] and Lindsay et al. [7] found that under suitable oxidizing conditions, including the chlorination of water to make it potable, Cr(III) can be oxidized to Cr(VI).

For these reasons, it is extremely important to develop methods capable of removing chromium, independent of its oxidation state. The aim of this work was to produce biochar from water hyacinth and to evaluate the effectiveness of this material for the removal of chromium ions from tannery effluent.

## Materials and Methods

**Biochar.** The biochar was produced by pyrolysis of the dry biomass in a furnace (FRO 1100, FORTELAB), with heating at 400°C for 60 min, using a heating rate of 5°C $min^{-1}$ and a nitrogen flow rate of 4 mL $min^{-1}$. The resulting material was denoted BIO. The in natural water hyacinth was denoted AGIN. The two materials were characterized using infrared absorption spectroscopy (FTIR) and surface area measurements.

**Adsorption assays.** Firstly, the assessment was made of the influence of the pH of the effluent, which was adjusted in the range from 4 to 7. Experiments were performed using 10 mL of effluent with a total chromium concentration of approximately 80 μg $L^{-1}$. After addition of 100 mg of BIO, the mixtures were agitated at 150 rpm (MA 832/1 shaker, Marconi) for 60 min at 25 ºC. The samples were then filtered and the residual concentrations of total chromium in the filtrates were determined by FAAS. Control solutions were prepared without the adsorbent material, in order to determine the initial concentrations of total chromium in the effluent at different pH values. Adsorption kinetics experiments were then carried out using the most favourable pH for chromium removal, with aliquots being removed for analysis at pre-determined times between 5 and 60 min. The experiments were performed in duplicate.

## Results and Discussion

**Characterization.** The FTIR spectra of BIO and AGIN (Figure 1) showed broad and intense bands at around 3430 $cm^{-1}$, attributed to the stretching of O-H groups of water, alcohols, phenols, and/or carboxylic acids. A band at 1626 $cm^{-1}$ corresponded to stretching of C=C bonds of aromatic groups present in the structure of the lignin, while a band at 1506 $cm^{-1}$ was attributed to symmetric stretching of $COO^-$. A band at 929 $cm^{-1}$ could be attributed to the unfolding of C-H bonds of lignin, hemicellulose, and cellulose present in the composition of the water hyacinth lignocellulosic biomass.

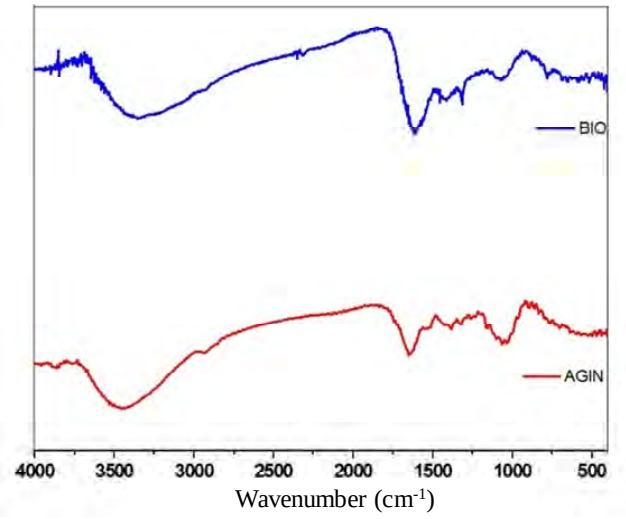



**Figure 1.** Infrared spectra of BIO and AGIN.

The thermal treatment did not eliminate the main groups present in AGIN, as shown by the FTIR spectra (Figure 1). However, it resulted in an increase of the surface area from 93.48 $m^2$ $g^{-1}$ (AGIN) to 135.84 $m^2$ $g^{-1}$ (BIO).

**Adsorption tests.** Figure 2 shows the chromium adsorption percentages obtained using BIO, together with the initial concentrations of chromium in the effluent, as a function of pH. The results indicated that the adsorption was highly efficient, especially in the pH range between 5 and 7, with chromium removal percentages in the range 71-81%. It can be seen that the concentration of chromium in the effluent was lower at pH 7, due to the association of chromium ions with $OH^-$ and the consequent formation and precipitation of $Cr(OH)_3$ [8].

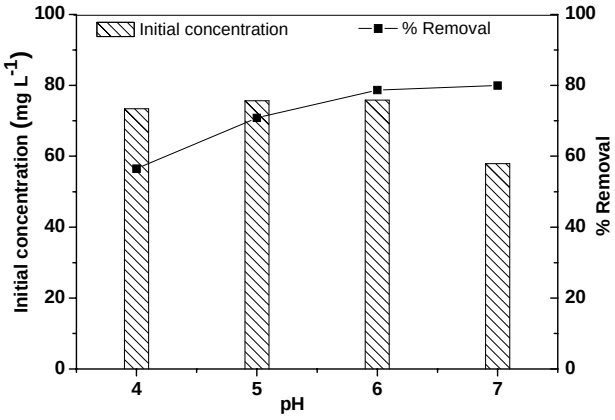



**Figure 2.** Removal percentages obtained using BIO, together with the initial concentrations of chromium, as a function of pH. Conditions: $m_{ads}$ = 100 mg; $v_{effluent}$ = 10.0 mL; t = 60 min; T = 25°C; agitation speed = 150 rpm.

The adsorption kinetics study was carried out using BIO with the effluent pH adjusted to 6 since the highest adsorption capacity was observed for this condition.

Figure 3 illustrates the chromium removal efficiency, given by the adsorption capacity ($q_{ads}$), together with the adsorption percentage, as a function of time. It can be seen that in the first 20 min, the amount of chromium adsorbed by BIO exceeded 55%. The adsorption processes reached equilibrium after around 40 min, with a final removal percentage higher than 81% (6.8 mg $g^{-1}$).

The BIO material showed very fast adsorption kinetics, especially considering that the chromium was removed from a real effluent containing other species that could have competed for the adsorption sites and could, therefore, have decreased the capacity of the material for removal of the metal of interest.

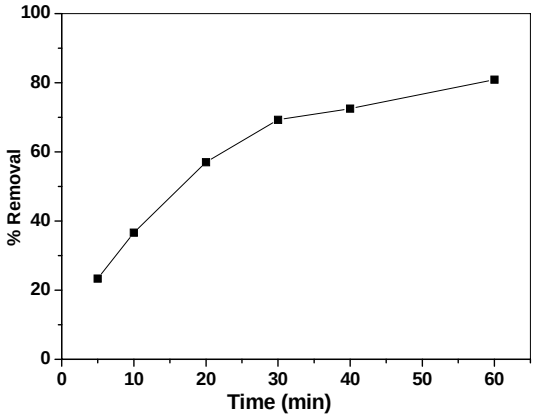



**Figure 3.** Chromium adsorption efficiency *vs.* time for BIO, expressed removal percentage. Conditions: $C_i$ = 76 mg $L^{-1}$; pH = 6; $m_{ads}$ = 100 mg; T = 25°C; agitation speed = 150 rpm.

This study demonstrates the great potential of biochar produced from water hyacinth (*Eichhornia crassipes*) as an adsorbent for removal of chromium ions from leather manufacturing industry effluent.

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# Application of natural organic residues as adsorbents to remove lead from waters

L. C. OLIVEIRA [(a)*], T. S. FARIAS [(b)], W. G. BOTERO [(b)], A. D. M. CAVAGIS[(a)], T. A. CACURO [(a)], W. R. WALDMAN [(a)]

(a) Department of Physics, Chemistry and Mathematics, Federal University of São Carlos, 18.052-780 Sorocaba, SP, Brazil
(b) Department of Chemistry, Federal University of Alagoas, 57.160-000 Maceió, AL, Brazil
* E-mail: lcamargo@ufscar.br

**Keywords:** organic matter residue, adsorption, lead

**Abstract.** After alkaline extraction of humic substances from soils, the supernatant (soluble fraction) is separated from the precipitate (slightly soluble fraction), which is usually discarded. As the soluble fractions are obtained on an industrial scale, finding out an applicability for this waste is an issue of economic and environmental interest. Thus, natural organic residues extracted from soils were applied into natural waters containing $Pb^{2+}$ ions, to verify their potential of adsorption. The results demonstrated that these residues might be promising materials for remediation of environments contaminated by this heavy metal.

## Introduction

Environmental contamination by potentially toxic metals has been widely reported in the literature [1]. Lead is one of these metals, which have stood out due to the environmental impact related to their applicability and toxicity [2]. Since ancient Rome, the use of lead-based compounds has been described: the ancient Romans used to apply lead acetate (also known as "lead sugar" and "salt of saturn") to sweeten wine. Studies of 1959 report that the death of Pope Clement II, in 1047, evidenced lead intoxication. Throughout history, other registers of intoxication by Pb ions have been reported [3]. In the current decade, more specifically in 2014, residents from the city of Flint, Michigan, suffered from water contamination by lead. More than 100,000 residents were exposed to contaminated water, which quality had been compromised due to the uptake of water from a new source: the Flint River, without an adequate control of corrosion [4]. In 2016, a federal emergency state was declared in that city and all residents were instructed to consume only bottled or filtered water, even for personal hygiene.

In this context, substances which interact with metals, decreasing their availability in the environment are of great importance. Humic substances (HS) already fulfil this function in nature and many studies regarding the interaction metals-HS (soluble fraction) have been performed, mainly with the aim of better understanding the metal-HS interactions [5]. In these researches, the alkaline extraction is the most used, after which a series of studies regarding HS and metals, aiming a better understanding of their interactions, have been made. However, there are few studies with the alkaline residue from HS extraction, which, due to its low solubility, could be applied as an adsorbent of potentially toxic metals, thus acting directly on environmental remediation. Considering the need to minimize the waste generated by aggregating value to these residues, besides minimizing environmental impacts, the objective of this work was to use the HS alkaline extraction residue in the adsorption of lead ions present in natural aquatic environments.

## Materials and methods

Natural Organic Residues (NOR). After alkaline extraction of the HS, made in soils with different amount of organic matter, in the proportion of 1:10 (soil: extractor), under 4 hours stirring, the residues were separated from the supernatant, washed thoroughly with distilled water and dried at room temperature, up to constant mass [6]. NOR1 was obtained from a soil containing 4% of organic matter, while NOR2 was obtained from soils with 9% of organic matter.

Characterization of the Natural Organic Residues (NOR) by Fourier Transformed Infrared Spectroscopy (FTIR)

The readings were made in the region from 400 to 4000 $cm^{-1}$, mode of transmission, with a resolution of 4 $cm^{-1}$ and 32 scans, using a spectrophotometer Nicolet IR 200 FT-IR (Thermo Scientific), with KBr pellets in the ratio 1:100 KBr/sample (m/m).

The complexing capacity of the Natural Organic Residues for $Pb^{2+}$ ions.

The complexing capacity of NOR1 and NOR2 for $Pb^{2+}$ ions were obtained by using a tangential flow ultrafiltration system, according to the methodology proposed by Burba et al., 2001 [7]. 100 mg $L^{-1}$ of each NOR were filtered in the system, and increasing concentrations of $Pb^{2+}$ (0.05 – 10.00 mg $L^{-1}$) were added, respecting an equilibrium period of 20 minutes after each addition, followed by filtration.

NOR applications into natural water: 20 mg of each NOR were added into 15 mL of water from Laguna Mundau (Maceió, Brazil), which lead amounts of about 1.87 mg.$L^{-1}$, under a 30 min stirring, followed by a cellulose filtration membrane of 045 μm.

Determination of $Pb^{2+}$. The amounts of $Pb^{2+}$ were determined by redissolution chronopotentiometry, at the Nernst equilibrium and in absence of gradients (AGNES), with a thin film of mercury in vitreous carbon electrode, following the conditions: $Pb^{2+}$ td = 240 s; Ed = - 46 V; Is = 2 μA; the electrode rotation was 1,000 rpm.

## Results and Discussion

### Infrared Characterization

The infrared spectra for NOR1 and NOR2 were very similar (Figure1).

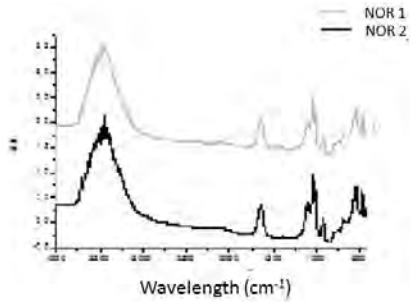



**Figure 1.** Infrared spectra for NOR1 and NOR2.

The main absorption bands observed were: wide band at 3450 $cm^{-1}$, which might be assigned to –OH groups from alcohols, phenols and carboxylic acids; bands at 1600 $cm^{-1}$, attributed to characteristic C=C bonds from organic compounds; bands in the region of 1000 $cm^{-1}$ and 1200 $cm^{-1}$, attributed to C-O stretch from alcohols, phenols and carbohydrates, beyond Si-O bonds [8]. The complexing capacities of NOR for $Pb^{2+}$ ions are presented in Table 1 and the results indicate high affinity of the organic material with $Pb^{2+}$ ions since the literature presents complexing capacities for potentially toxic metals of about 2-17 mg per gram of material [5, 9].

**Table 1.** Complexing capacities of Natural Organic Residues (NOR) for $Pb^{2+}$ ions, in mg per gram of material.

| Material | Complexing capacity (mg $Pb^{2+}$ per gram of material) | Reference |
|---|---|---|
| NOR1 | 2.90 | The present work |
| NOR2 | 4.91 | The present work |

Aiming the applicability of the NOR in real systems, it was evaluated the retention of $Pb^{2+}$ by both residues (NOR1 and NOR2) in natural water samples collected from Laguna Mundau (Maceió, Brazil), as it can be seen in Figure 2. The lead amount in the water samples was about 1.87 mg $L^{-1}$.

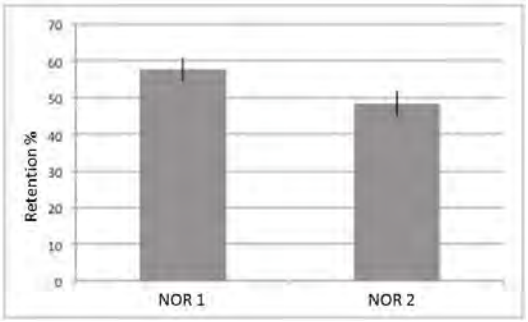



**Figure 2.** Adsorption of lead in waters from Laguna Mundau by the natural organic residues (NOR1 and 2).

In the water from Laguna Mundau, it was observed that the natural organic residues (NOR) retained about 48-55% of the total $Pb^{2+}$ present.

The results from the application of the organic residues in natural waters showed to be very promising in the adsorption of $Pb^{2+}$, highlighting their potential as an environmental remediator for waters contaminated with lead.

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# Protein-like fluorescence of water NOM – the key role of free aromatic amino acids

O. TRUBETSKAYA [(a)*], C. RICHARD [(b)], O. TRUBETSKOJ [(c)]

(a) Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry,
Russian Academy of Sciences, 142290 Pushchino, Moscow region, Russia
(b) Equipe Photochimie, Institut de Chimie de Clermont-Ferrand, Clermont Université, Université Blaise Pascal,
BP 10448, F-63000 Clermont-Ferrand; CNRS, UMR 6296, ICCF, F-63171 Aubiere, France.
(c) Institute of Basic Biological Problems, Russian Academy of Sciences,
142290 Pushchino, Moscow region, Russia
*E-mail: olegi03@yahoo.com

**Keyword:** NOM, protein-like fluorescence, SEC-PAGE, RP-HPLC, multi-wavelength fluorescence detection

**Abstract.** The question about the origin of protein-like fluorescence in the water natural organic matter (NOM) remains still open because this type of fluorescence may be derived from free amino acids, peptides or proteins contained. The reversed-phase high performance liquid chromatography (RP-HPLC) with multi-wavelength fluorescence detection was used for evaluation the origin of protein-like fluorescence in Suwannee River NOM (SRNOM) and its fractions A, B, C+D, obtained by size exclusion chromatography–polyacrylamide gel electrophoresis setup. The electrophoretic mobilities of fractions varied in the order C+D>B>A and the molecular size (MS) in the opposite order. We demonstrated that the protein-like fluorescence was almost exclusively located in high MS fraction A and medium MS fraction B. Retention times and fluorescence emission spectra of authentic free aromatic amino acids tyrosine and tryptophan were identical with the retention times and emission spectra of some RP-HPLC peaks of fractions A and B. The results obtained for the first time showed that the most part (>50%) of protein-like fluorescence of SRNOM were provided by free aromatic amino acids preserved from further degradation by their incorporation in the SRNOM structure by hydrophobic interactions.

## Introduction

As currently accepted, the fluorescence of water NOM is due to two main groups of fluorophores. One group usually has excitation (Ex) maximum less than 305 nm and emission (Em) maximum less than 380 nm, related to aromatic amino acids, and is often referred to as protein-like fluorophores. The other one with Ex/Em 220–360/380–470 nm is attributed to humic substances (HS)-like fluorophores of water NOM. It is well known, that emission maxima around 280–380 nm correspond to aromatic amino acids such as phenylalanine - Phe, tyrosine - Tyr and tryptophan - Trp. Natural protein-like fluorophores in water NOM have been classified by their differences in emission wavelength maximum into tyrosine-like (emission maximum of around 300 nm) and tryptophan-like (around 350 nm) types [1, 2]. However, the question about the origin of protein-like fluorescence remains still open, because this type of fluorescence may be derived from free amino acids, peptides or proteins.

The objectives of the current work were to use reversed-phase high performance liquid chromatography (RP-HPLC) with multi-wavelength fluorescence detection for comparison of free aromatic amino acids fluorescence with protein-like fluorescence of SRNOM and its fractions obtained by coupling size exclusion chromatography/polyacrylamide gel electrophoresis (SEC-PAGE setup). The multi-step scheme is presented on Figure 1.

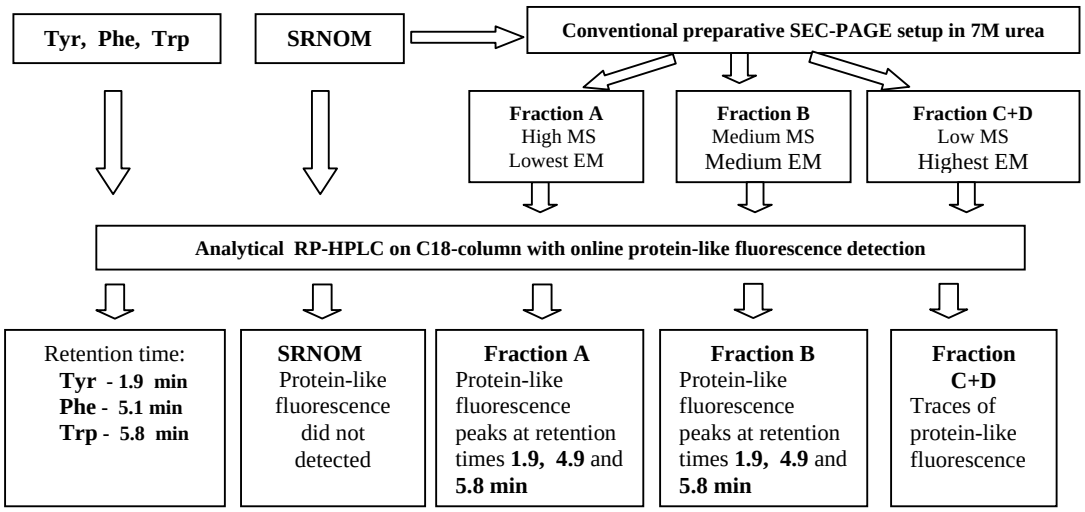



**Figure 1.** Multi-step scheme of SRNOM preparative fractionation and analysis.

**Materials and methods**

The SRNOM (ref. number 1R101N), isolated by reverse osmosis from Suwannee River water, Georgia, USA, was fractionated by SEC-PAGE setup [3, 4]. The SRNOM fractions named A, B and C+D with different electrophoretic mobilities (EM) and M) were obtained (Figure 1). The SRNOM, its fractions and authentic amino acids Tyr, Phe and Trp were analyzed by RP-HPLC on C18-column in 10 mM phosphate buffer (pH 6.5) and stepwise gradient of methanol according to Trubetskaya et al. [5, 6]. A multi-wavelength FLR detector was designed for the excitation wavelength 270 nm and emission range 290-590 nm. To detect peaks with protein-like fluorescence, the RP-HPLC chromatograms were analyzed at excitation–emission wavelength pairs (Ex/Em) 270/330 and 270/290 nm, because of the emission maxima of Phe (278 nm), Tyr (305 nm) and Trp (345 nm) at pH 6.5. The retention times and fluorescence emission spectra of the RP-HPLC peaks of SRNOM, its fractions and authentic aromatic amino acids were compared.

**Results and Discussion**

Setting Ex/Em 270/330 nm to detect the fluorescence related to aromatic amino acids, often referred to as protein-like fluorescence, did not reveal essential signals in the bulk SRNOM chromatogram. However, after SEC-PAGE fractionation several peaks could be detected on chromatograms of high MS fraction A and medium MS fraction B, where the most abundant were a peak 1 occurring at a retention time of 1.9 min and peaks 2 and 3 at the retention times of 4.9 and 5.8 min, respectively (Figure 1). Low MS fraction C+D contained only traces of these peaks.

To understand the origin of protein-like fluorescence, the RP-HPLC analysis of authentic aromatic amino acids Tyr and Trp was done at Ex/Em 270/330 nm, and Phe – at Ex/Em 270/290 nm, because of amino acid Phe did not emitted at 330 nm. Each amino acid gave one peak, the retention times of Tyr (1.9 min) and Trp (5.8 min) were remarkably similar to those of protein-like peaks 1 and 3, respectively, while the retention time of Phe (5.1 min) was different in comparison with any protein-like peaks.

The fluorescence spectra of peaks 1, 2, and 3 of fraction A and authentic aromatic amino acids were extracted from the data of FLR detector and compared. The fluorescence spectrum of Tyr was very similar to that of peak 1 and fluorescence spectrum of Trp to those of peaks 2 and 3 (Fig. 2). Based on the comparison of retention times and emission spectra it could be suggested that peak 1 represents free aromatic amino acid Tyr (retention time 1.9 min, emission maximum about 300 nm) and peak 3 - free aromatic amino acid Trp (retention time 5.8 min, emission maximum about 350 nm). The appearance of Trp-like fluorescence in peak 2 (retention time 4.9 min) could be explained by association of Trp with some hydrophilic compounds of SRNOM, which led to a decrease in the retention time from 5.8 to 4.9 min. It was impossible to detect the peak corresponding to free authentic Phe due to its very low absorption at wavelength 270 nm, which has been used in this study as an excitation wavelength, and because quantum yield of Phe (0.04) is one order of magnitude less than that of Tyr (0.21) or Trp (0.17) in neutral pH. The inspection of RP-HPLC chromato-grams of SRNOM and its fractions at Ex/Em 270/290 nm did not revealed any new peaks. The fluorescence emission spectra of peaks 1, 2, and 3 of fraction B demonstrated similar results, but they were not detected in the bulk SRNOM and very poorly presented in fraction C+D. Based on the relative contribution of peaks 1 (corresponded to Tyr) and 3 (corresponded to Trp) in total area of protein-like fluorescence we could evaluate that fluorescence of free amino acids constitute a major part (more than 50%) of total protein-like fluorescence of SRNOM.

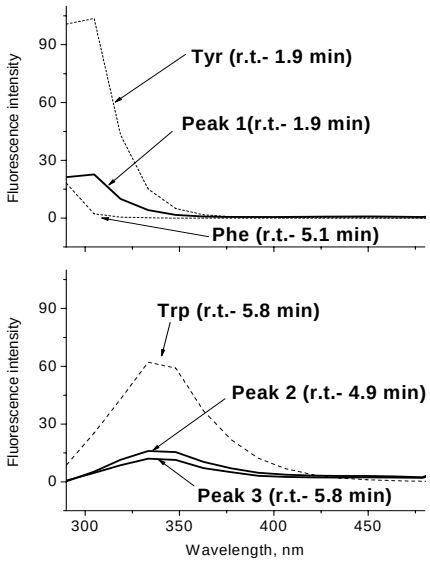



**Figure 2**. Fluorescence spectra of Trp, Tyr, Phe and RP-HPLC peaks 1, 2 and 3 of high MS fraction A , r.t.-retention time.

Actually, incorporated amino acids species seem to survive the SEC procedure (the free amino acids would elute at the end of the SEC chromatogram and lost during dialysis), but obviously do not survive the RP-chromatography (here they seem to elute at similar retention times as the pure free amino acids).

The presence of incorporated free amino acids in water NOM is extremely important for understanding and verification of supramolecular organization of SRNOM sample. We could assume that water NOM may serve as a reservoir for further using of amino acids in the environment.

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# Effects of photo-oxidation on dissolved natural organic matter

R. D. VOGT [(a)*], J. RAJAKUMAR [(a)], E. FÆRGESTAD MOSLETH [(a)]

(a) Department of Chemistry, University of Oslo, 0315 Oslo, Norway
* E-mail: rolf.vogt@kjemi.uio.no

**Keywords:** Dissolved natural organic matter, photo-oxidation, bioavailability

**Abstract.** Physical and chemical properties of boreal freshwater, and thus the ecology and ecosystem services of these ecosystems, are strongly influenced by edaphic export of dissolved natural organic matter (DNOM). Moieties of this allochthonous DNOM is photo-oxidized by sunlight. This alters the characteristics of the DNOM, having effect on its colour, molecular size distribution and bioavailability. These changes to DNOM are studied through laboratory experiments using ultrafiltration, artificial sunlight exposure and a number of photometric proxies for DNOM characteristics.

## Introduction

DNOM affects waters physical properties partly due to its chromatographic moieties that absorb sunlight radiation. Waters containing high concentration of DNOM are thus usually yellow to brown in colour. This characteristic is also associated to a higher concentration of Iron (Fe). DNOM thereby strengthen the thermocline, decreases the depth of photosynthesis as well as visibility of pray, and increases the UV shielding of limnic bacteria. Moreover, DNOM is an important source of nutrients and energy for aquatic ecosystems, although most of the terrestrial DNOM is rather refractory mainly due to high molecular weight. On the other hand, photo-oxidation, by sunlight radiation through the water column, decompose parts of this DNOM into smaller, less aromatic and more readily bioavailable molecules.

The aim of this study is to assess what effect photo-oxidation has on the size distribution and bioavailability of DNOM. Changes to DNOM quality and explanatory factors are assessed by measuring changes in relative absorbencies as well as fluorescence indexes, along with Fe and aluminium (Al) fractions.

## Materials and methods

Presented here are results from the MSc thesis of Rajakunar [1], which is a pilot of an ongoing study.

**Sample material.** Data are presented for reverse osmosis (RO) ultra-filtrated and freeze-dried DNOM samples from a mineral soil dominated watershed (Trehøningen) and a peat (Hellerudmyra) from the NOM-Typing project [2], as well as a fresh sample from the large Päijänne lake in Finland, from the NOMiNOR project [3]. These samples are referred to as PEAT, MINERAL and LAKE, respectively.

**Sample pre-treatment.** Samples are sterilized by filtering through a 0.2 µm filter, and subsequently size fractionated into low molecular weight (LMW) by ultrafiltration through 10 kDa filter (Amicon regenerated cellulose, Millipore). Photo-oxidation is conducted on a Q-sun Xenon test chamber (Q-Panel Lab Products, Cleveland, OH, USA).

**Characterisation of DNOM.** Amount of DNOM is approximated by measuring concentration of total organic carbon (TOC) according to ISO 8245 (1999) using a TOC-VCPH analyser (Shimatzu). Absorbency spectrum is recorded on a Shimadzu UV-1800 UV-Visible Spectrophotometer. A set of readily available proxies (sUVa = $\frac{Abs_{\lambda 254nm}}{TOC}$, sVISa = $\frac{Abs_{\lambda 254nm}}{TOC}$ and SAR = $\frac{Abs_{\lambda 254nm}}{Abs_{\lambda 410nm}}$) are used to describe physiochemical characters of DNOM. Fluorescence Excitation-Emission matrix (EEM) is measured by using Varian Carry Eclipse Fluorescence Spectrophotometer. Fluorescence data are processed using Varian Cary Eclipse software. Al and Fe fractions are determined by complexation and organic extraction, combined with an ion exchange pre-treatment and photometric detection, as described in Sullivan et al. [4].

Biodegradation measurements is conducted by monitoring oxygen levels in sample vials using SensorDish® Reader (SDR). A luminescent sensor spot, exited by the SDR, subsequently emits phosphorescence that is quenched by oxygen. The speed of which the light degreases is thus a measure of the level of oxygen remaining in the sample vial.

## Results and Discussion

Proxies for DNOM characteristics indicate that bog (PEAT) DNOM has darker colour, is more aromatic, and has higher $M_W$ than the DNOM materials from other sources. This is also found in previous studies [5]. DNOM from the large lake (LAKE) is more coloured and aromatic, though has lower $M_W$, than the DNOM from the mineral (MINERAL) soil water.

**Effect of ultrafiltration.** Based on $UV_{\lambda 254}$ absorbency the high molecular weight (HMW) DNOM constitutes between 34 and 15% of the bulk sample (Fig. 1), negatively dependent on the SAR ($r^2$ = 0.69). Moreover, the SAR in the LMW fraction is inherently higher than in the bulk sample. The LMW fraction of the bog (PEAT) sample have sUVa and sVISa that are only 65% and 55 % of the values of the bulk sample, respectively. This is in accordance with that more HMW aromatic moieties, and especially the longer conjugated double bonds, are preferentially removed by the ultrafiltration. The larger reduction in sVISa of bog (PEAT) DNOM reflects thus the larger $M_W$ (lower SAR) with inherently longer conjugated double bonds.

Fluorescence peak ratio (A/B), reflecting the amount of humic acids like (A) relative to fulvic acids

like (B) compounds, is found to best explain the variation in reduction of UV ($\lambda_{254nm}$) absorbency by ultrafiltration of DNOM ($r^2$ = 0.96); The higher the relative amount of humic to fulvic like acids, the more DNOM was removed by ultra-filtration. The concentration of inorganic labile Al (Ali) is also explanatory for the variation in relative decrease in DNOM by ultrafiltration ($r^2$ = 0.82), with highest decrease in DNOM in samples with the highest Ali levels.

**Effect of photo-oxidation.** Photo-oxidation causes an 8 to 26% decrease in DOC concentration and an 23 to 47% reduction in UV absorbency of the bulk sample (<0.2 μm), and between 17 and 32% UV absorbency reduction in the LMW fraction (Fig. 1). The LAKE sample has DNOM that has been a long time in the large lake acting as a natural photo-reactor tank. It thus does not respond strongly to additional photo-oxidation. LMW moieties responds less to photo-oxidation because they have lower content of long conjugated double bonds (low sUVa and sVISa). sUVa and sVISa concurrently decreases while SAR increases in all samples, both bulk and LMW. This photo-bleaching is reflecting that the radiation cracks larger organic compounds into smaller molecules with lower $M_W$ [5]. The blue-shift is larger for the bulk sample (9 – 27%) than for the LMW fraction (2 – 9%). This is likely due to that the HMW DNOM in the bulk sample is more susceptible to photo-oxidation due to more chromophores absorbing the photon energy.

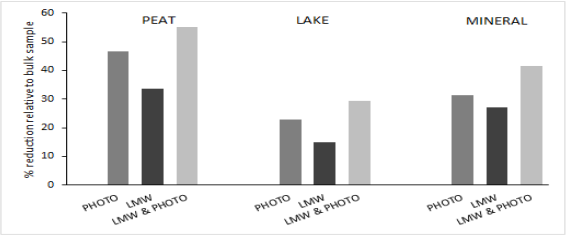



**Figure 1.** Decline in $UV_{\lambda 254nm}$ absorbency relative to bulk DNOM material from a predominantly bog (PEAT) and a mineral (MINERAL) soil dominated catchment, and from a large lake (LAKE), after either photo-oxidation (PHOTO) or 10 kDa filtration (LMW), and both (LMW & PHOTO).

Both the humic- and fulvic like peaks in EEM fluorescence spectra are reduced when DNOM is exposed to sunlight, reflecting that photo-oxidation decomposes fluorescent aromatic compounds.

The explanatory variable found to best explain variation in reduction of $UV_{\lambda 254nm}$ absorbency by photo-oxidation of DNOM is again the A/B ratio ($r^2$ = 0.96): The higher the ratio, the more DNOM is oxidized. Total concentration of Fe ($r^2$ = 0.88) and SAR ($r^2$ = 0.80) are also found to be significantly correlated to the degree of photo-oxidation.

**Biodegradation.** The relatively more aromatic, hydrophobic and HMW DNOM from the peat (PEAT) is less bioavailable than the relatively more aliphatic, hydrophilic and LMW DNOM from the mineral soil dominated watershed (MINERAL). This is conceivably because microorganisms have less ability to degrade HMW aromatic DNOM compounds than the relatively more aliphatic, hydrophilic and LMW DNOM [7]. The biodegradability of the DNOM from the big lake (LAKE) was relatively low. This is likely due to the long residence time in the great lake, allowing for an advanced degradation - rendering mainly non-refractory moieties of the DNOM material.

Biodegradation is as expected stronger of the LMW than the HMW fraction, since LMW compounds are relatively more bioavailable than HMW compounds.

Bioavailability of DNOM increases significantly due to photo-oxidation. This is likely due to that some refractory humic compounds are decomposed to more biodegradable compounds.

Organically complexed iron (Feo) is the only explanatory parameter found to be significantly correlated to the biodegradability of DNOM: The higher iron content on the DNOM the lower is its biodegradability. This may partly be due to that both are co-varied with the influence of bog in the watershed, as DNOM from peat has high content of Fe and has a low biodegradability, while DNOM from mineral soil has low content of Fe and high biodegradability.

This lack of explanatory parameters for the bioavailability of DNOM is due to that HMW hydrophilic compounds, rich in acid functional groups, are less favourable to be assimilated than LMW unsaturated aliphatic compounds. This is antagonistically reflected in our available proxies for DNOM quality.

In our ongoing study this is further addressed by including all NOM-Typing samples and fresh samples.

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# Installation of an injection permeable reaction barrier with the use of silanol derivatives of humic substances for the purification of groundwater.

A. VOLIKOV [(a)*], A. ZHIRKOVA [(a)], S. PONOMARENKO [(b)], I.V. PERMINOVA[(a)]

(a) Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia
(b) Enikolopov Institute of Synthetic Polymeric Materials, Russian Academy of Sciences, Moscow, Russia
* E-mail: ab.volikov@gmail.com

**Keywords:** sorption, prb, humic, cuprum, azo dyes

**Abstract.** The instalation of injectable permeable reaction barrier using silanol derivatives of humic substances is demonstrated. They are polyelectrolyte complexes of humic substances and silsesquioxane oligomers of 3-aminopropyltriethoxysilane (APTES). It is shown that when they are introduced into a column with sand, they form a permeable network, which allows them to be used for the sorption of various toxicants. The possibility of using this technology for the sorption of cuprum ions and also azo dyes from groundwater is demonstrated.

## Introduction

Of particular interest is the production of humic derivatives capable of immobilizing on the surface both fine (silica gel, clay) and coarsely dispersed substrates (sand) to form adsorption layers with high binding activity for various pollutants. The presence of derivatives with these properties opens the possibility of their use for the production of modified sorbents, as well as as an injectable material for in situ installation of permeable reaction barriers (PRB) by means of liquid wells. Such PRBs are high-tech engineering structures that allow the in situ deep-water aquifer to be cleaned without excavating contaminated rocks (Figure 1).

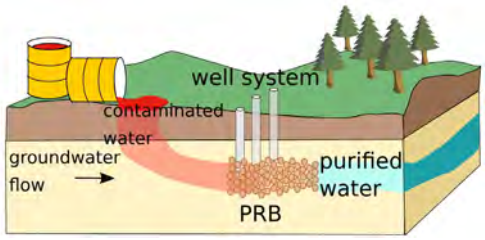



**Figure 1.** Scheme injection installation permeable reactive barrier (PRB) [1].

It is known that such properties are possessed by silsesquioxan-humic complexes [2]. These complexes are represented by polyelectrolyte complexes of humic substances and silsesquioxane oligomers.This work shows the possibility of their effective immobilization on coarsely dispersed substrates and extraction of such toxicants as azo dyes and copper ions.

## Materials and methods

**Synthesis of Silsesquixane-humic complexes (SSHC).** To synthesys SSHC 20 ml of a 5 g/l solution of humic substances (CHS-K, «Sakhalinsky» potassium humate) were prepared. Then the aliquot of APTES was added (50, 100, 200 mg for ChS-APTES-50, -100-200, respectively). This solution were directly used in further experiments. **Immobilization of SSHC complexes under dynamic conditions on a column with sand.** The glass column with a volume of 150 ml (length 30 cm, diameter 2.5 cm) was filled with a quartz sand suspension in water to 100 ml. The pH of SSHC solutions were adjusted to pH 8 with 1M HCl and left for 120 minutes (CHS + APTES-50), 40 minutes (CHS + APTES-100), and the CHS + APTES-200 solution was used immediately. The resulting mixtures were introduced into the column at a rate of 10 ml per minute and 10 ml of distilled water was passed at the same rate to displace the reaction zone closer to the center of the column. The flow rate was then reduced to 5 ml/hr and rinsed with distilled water for 12 hours. At the end of this time, the flow rate of the distilled water was increased to 1 ml / min to wash the reversibly bound complexes. Washing was carried out before the solution bleached at the column outlet. A more detailed synthesis procedure is described in the publications [2, 3].

**Sorption of azo dye red 81 on immobilized SSHC.** The solution of azo dye red 81 (Aldrich) in distilled water with a concentration of 25 mg/l digest at a rate of 80 ml/h (16 cm/h) was fed from top to bottom. During the test, water samples were taken from the column and the azo dye content was determined spectrophotometrically. When the concentration of the outgoing solution was equalized with the concentration of the incoming solution, distilled water was started to flow through the column at the same rate, until the washout from the column was discolored. The mathematical processing of the kinetic curves of sorption was carried out according to the Thomas equation:

$$\ln\left(\frac{C_0}{C_t}-1\right)=\frac{K_{th}\cdot q_0\cdot m}{Q}-\frac{K_{th}\cdot C_0\cdot V}{Q}$$

where $C_0$ is the concentration of the azo dye in the initial solution (mg/l), $C_t$ is the concentration at the column outlet at time t (mg/l), $K_{th}$ is the Thomas constant (ml * $min^{-1}$ * $mg^{-1}$), $q_0$ is the maximum sorption capacity (mg/g), m - mass of the sorbent, Q - flow rate passing through the column (ml/min), V - volume of solution passed through the column.

## Results and Discussion

APTES in the aqueous environment due to the

alkaline environment instantly hydrolyses with the formation of silsesquioxangumine oligomers, which interact with humic substances with the formation of polyelectrolyte complexes according to the scheme shown in Fig. 2.

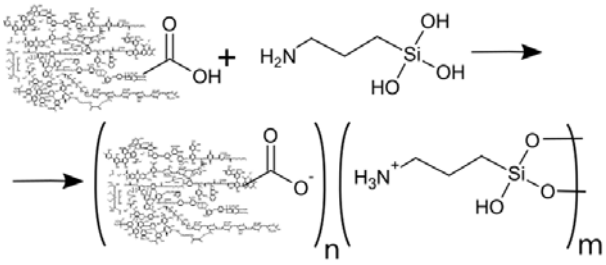


**Figure 2.** Scheme of interaction of HS with APTES in an aqueous medium.

The resulting complexes in the aqueous medium gradually condense with the formation of more curly and crosslinked structures, this process is schematically shown in Fig. 3. The fraction of active silanol groups decreases, the particles coalesce and a sol-gel transition occurs.

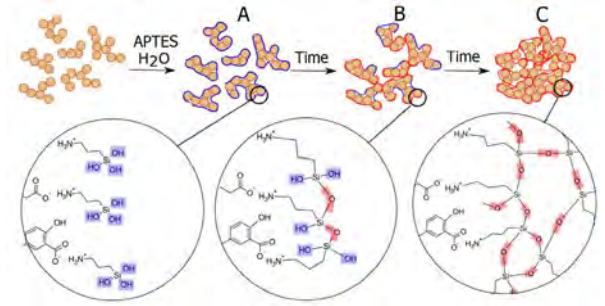



**Figure 3.** The conceptual model of formation and condensation of SSGK.

For effective immobilization of silsesquioxane-humic complexes, it is important that they are a sol, then they are able to efficiently form the gel already inside the column with sand and thereby establish a permeable reaction barrier. Testing of the obtained barriers was carried out with the use of direct red azo dye 81, a dangerous toxicant used in textile industry and copper ions. Figure 4 shows the kinetic curves obtained, and the calculated characteristics, according to the Thomas model, are given in Table 1.

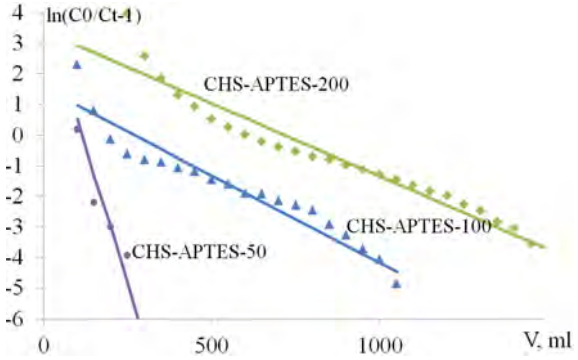



**Figure 4.** Kinetics of decay of absorbance at 515 nm for DPPH radicals [(DPPH) 0=30±0.1 μM] reacting with $SiO_2$[90]-HALP NPs.

As can be seen from the figure above and the table, the obtained barriers with immobilized silsesquioxane-humic derivatives are able to efficiently sorb the direct red azo dye 81, while the most effective are preparations with an excess of organosilane in the composition. When testing the columns on copper ions, similar results were obtained.

**Table 1.** Kinetic parameters of the Thomas equation for dynamic sorption of azo dye straight red 81 on immobilized SSGKs of different composition

| Sample | $K_{th}$, l/(min·kg) | $q_0$(Th), g/kg |
|---|---|---|
| Blank | 0.16±0.2 | 14±2 |
| CHS+APTES-50 | 3.8±0.5 | 12±2 |
| CHS+APTES-100 | 0.57±0.08 | 30±3 |
| CHS+APTES-200 | 0.24±0.04 | 133±15 |

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# Dissolution of silver nanoparticles in the environment: interactions with natural organic matter in aquatic systems

C. H. WATANABE [(a,b)]*, R.F.DOMINGOS [(b)], M.BENEDETTI [(b)], A.H. ROSA [(a)]

(a) Department of Environmental Engineering, Sao Paulo State University (UNESP), Sorocaba, Brazil
(b) Aquatic Biogeochemistry Team, Institut de Physique du Globe de Paris (IPGP), Paris, France.
* E-mail: claudia.watanabe@unesp.br

**Keywords:** silver nanoparticle, natural organic matter, aquatic systems

**Abstract.** Despite the growing interest and use of these NPs, the gathered knowledge on their environmental consequences is still scarce, since the large majority of the studies do not consider the effect of the presence of natural coatings around the particles. The present work aims to evaluate the dissolution rate of AgNPs in the presence of Natural organic matter (NOM).

## Introduction

Own to their size (in the range of 1-100 nm), nanoparticles (NPs) exhibit unique chemical, physical and eletronic properties differing from those of bulk materials, mainly owing to their higher surface-to-volume ratio [1]. The exponential appearance of a wide range of NPs, as led to their introduction in the environment, where they can interact with biotic and abiotic molecules yielding a transformed NP. The *in situ* transformation have relevant impacts on their toxicological effects, having new or additional risks still not studied. Due to the exposed facts the aim of the present work is to evaluate the dissolution rate of AgNPs in the presence of Natural organic matter (NOM).

## Materials and methods

### Natural organic matter production

Two different NOM were obtained for this study: humic substances (HSs) from waters and extracellular polymeric substances (EPS). The HSs were extracted from Sorocabinha river (São Paulo, Brazil), using recommended literature [2], whereas the EPS were produced from microalgae *R. subcapitata* following stablished protocols [3].

### Dissolution experiments

**Dissolution experiments.** Both NOM were used to evaluate the kinetic transformations of Ag NPs dispersed in two different medium (algae and daphnia) in absence and presence of both NOM. Ag NPs coated with citrate (diameter of 10 nm) named AgCit was exposed to all conditions described using two environmental relevant concentration in terms of Ag (1 and 100 µg $L^{-1}$) [5-6].

The dissolution rate were obtained using ultrafiltration system (3 kDa pore Amicon, Millipore) and centrifugation technique. For that 20 mL of test solution were prepared in polypropilene 50 mL falcon tubes for each condition by the time ($t_{exp}$ = 0, 72, 96, 408 and 720 h for Algae Medium [3] and 0, 72 h, 96, 192, 408 and 720 h for Daphnia Medium [4]). In terms of organic carbon, HSs (1 and 10 mg $L^{-1}$) and EPS (1 and 5 mg $L^{-1}$) concentration were also tested. All test solution were filtered using 3 kDa molecular weight membranes different exposition times. This specific case, the filtered portion correspond to the free portion Ag ($Ag^{+}$) plus all the small Ag complexes which have a correspondent size lower than the membrane pore, or 1-2 nm.

For the ultrafiltration process, 4 mL aliquots were taken from each sample and centrifugated during 35 minutes and 4,000 rpm. The portion was throwed away and 4 mL of test solution aliquot from the same flask tube was taken, centrifuged and again thrown away. This process was carried out 4 times (in total) and after the 4th cycle filtered portion was taken for analyis in High Resolution-Inductive Coupled Plasma Mass Spectrometer (HR-ICP-MS) (Element, ThermoScientific). After the data acquirenment from HR ICP-MS, UFREASI software [7].

## Results and Discussion

**Presence of HSs**. Fig. 1 show the dissolution results obtained when AgNPs were exposed in HSs presence.

Generally, at pH 6 and 7 and in absence of HS the dissolution of AgCit increases with time independently of the NPs concentration and on the medium dispersed, mainly until 408 h (17 days) from which decreases until 1 month of exposure. At pH 8, this increase of dissolution with time is much less prominent for both NPs concentrations and medium. In presence of 10 ppm of HS, and independently of the NP concentration or the medium, there is almost no Ag quantifiable in the filtrate.

In general, for all conditions and both medium, the dissolution of the AgCit NPs decreases with the increase of pH. The highest dissolution is found at pH 6, whereas at pH 8 the lowest dissolution of the NPs is obtained. For the AgCit in absence and presence of 1 ppm of HS and for both medium the highest dissolution was obtained for the highest concentration of the NPs, i.e., 100 ppb (with exception of the first time points for the Daphnia medium in absence of HSs). When in presence of the highest HS concentration there is no effect of the NPs concentration since there is no significant Ag concentration in the filtrate.

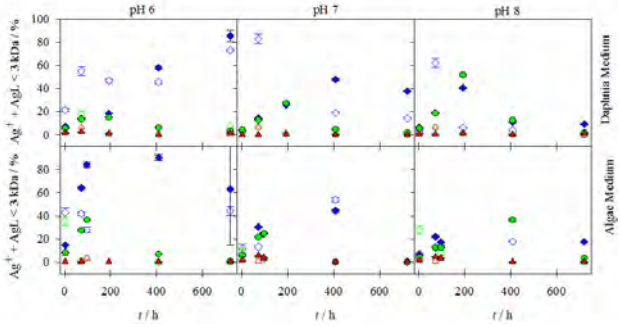



**Figure 1**. Percentage of free silver ($Ag^+$) plus silver complexes (AgL) with lower sizes than the cut-off of the membrane pores (< 3 kDa) as a function of exposure time for AgCit when dispersed in Dapnhia and Algae medium at pH 6, 7 and 8 in absence and presence of HS (◇) AgCit = 1 ppb, (○) AgCit = 1 ppb + HS = 1 ppm, (△) AgCit = 1 ppb + HS = 10 ppm, (◆) AgCit = 100 ppb, (●) AgCit = 100 ppb + HS = 1 ppm, (▲) AgCit = 100 ppb + HS = 10 ppm.

The presence of the HSs changes the dissolution rate and profile of the NPs independently of the medium and pH. The presence of even the lowest HSs concentration (1 ppm) reduces drastically the dissolution of the NPs, whereas increasing the HSs concentration 10× results in not significant concentration of Ag in the centricons filtrate. It is not straight forward to affirm which medium provokes more dissolution of the NPs. Whereas at pH 6 the algae medium seems more efficient on the particles dissolution, at pH 8 more Ag is found in the filtrates when the NPs are dispersed in the Daphnia medium.

**Presence of EPS**. Fig. 2 show the dissolution results obtained in HSs presence.

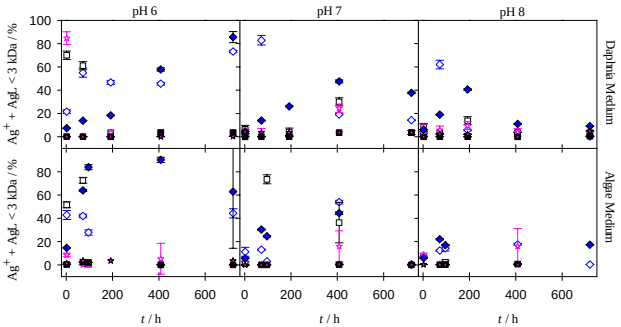



**Figure 2**. Percentage of free silver ($Ag^+$) plus silver complexes (AgL) with lower sizes than the cut-off of the membrane pores (< 3 kDa) as a function of exposure time for AgCit when dispersed in Dapnhia and Algae medium at pH 6, 7 and 8 in absence and presence (◇) AgCit = 1 ppb, (□) AgCit = 1 ppb + EPS = 1 ppm, (★) AgCit = 1 ppb + EPS = 5 ppm, (◆) AgCit = 100 ppb, (■) AgCit = 100 ppb + EPS = 1 ppm, (★) AgCit = 100 ppb + EPS= 5 ppm.

No significant Ag concentration values for both EPS concentrations was observed, except pH 6. It's possible to see the decrease of dissolution with the increase of pH for Algae Medium and, contrarily to AgCit, lower dissolution in Daphnia Medium than in Algae Medium. A large NPs aggregation is expected in the presence of divalent cations and pH values and in the presence of high concentrations of NP and/or low concentrations of NOM [8]. The solubility of the citrate-coated NPs as expected was higher in more acidic pH values. According to the results obtained from the present work it was possible to observe that the matrix (Algae or Daphnia medium) affect in the Ag-NP transformations. The percentage of dissolution is different in a Ag concentration of 100 ppb. Instead we have different dissolution rate at depending on the matrix, concentration and type of NOM the fate of NPs can influence in terms of Ag bioavailability.

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# Seasonal changes of iron in rivers and relation to structural features of humic acids in their sediments of the northwest of Hokkaido, Japan

M. YAMAMOTO [(a)] *, H. IWAI [(b)], A. YAMAGUCHI [(c)], D. LIU [(d)]

(a) Ocean Alliance, The University of Tokyo, Chiba 277-8564, Japan
(b) Department of Marine System Engineering, Osaka Prefecture University, Osaka 599-8531, Japan
(c) Saga Prefectural Ariake Fisheries Research and Development Center, Saga 849-0313, Japan
(d) Department of Creative Engineering, National Institute of Technology, Ariake College, Fukuoka 836-8585, Japan
* E-mail: mitsuo@aori.u-tokyo.ac.jp

**Keywords:** iron, humic acids, $\delta^{13}C$, $\delta^{15}N$, river, coastal environment

**Abstract.** Seasonal changes of iron concentrations in seven rivers of the northwest of Hokkaido, Japan have been monitored together with nitrogen and phosphate concentrations. Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in these seven rivers and structural features of humic acids (HAs) derived from representative river sediments were also investigated. The results suggested that the river environments including iron concentrations, $\delta^{15}N$ values, and structural features of HAs were influenced primarily by land use in the river basins.

## Introduction

Barren ground in coastal areas, which is a phenomenon of seaweed bed depletion, is a serious problem in Japan and around the world. A shortage of dissolved iron due to the decrease of iron originating from terrestrial areas is one of the reasons for barren ground in these coastal areas. An iron fertilization method has been developed for restoring seaweed beds and its effectiveness has been confirmed through field tests in Hokkaido, Japan [1]. However, the relationship between iron dynamics and seaweed bed distribution in these coastal areas has not been well understood . As iron supplied from rivers is related to organic matter, such as humic substances [2], it is important to evaluate the characteristics of iron concentrations and terrestrial organic matter in coastal areas.

The objective of this study is to evaluate seasonal changes of iron concentrations in rivers and their relation to structural features of humic acids derived from the river sediments in Mashike, the northwest of Hokkaido, Japan. Concentrations of iron, nitrogen, and phosphate in the rivers was monitored, and stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), and structural features of humic acids (HAs) derived from their sediments were analyzed.

## Materials and methods

**Sampling sites and methods.** From July 2016 to July 2017, fresh water sampling for analysis of total iron (T-Fe), dissolved iron (D-Fe, $< 0.45$ μm), inorganic nitrogen, and phosphate concentrations was conducted at the mouths of the sevem following rivers in Mashike: the Nobusha River (NS), the Shumonbetsu River (SM), the Hashibetsu River (HB), the Shokanbetsu River (SK), the Ponotarumanai River (PO), the Ninaibetsu River (NB), and the Oobetsukari River (OB) as shown in Figure 1. Nitric acid was added to T-Fe and D-Fe water samples immediately. Particulate organic matter (POM) was collected by filtrating water samples with GF/F filters in December 2016 and May 2017. Surface sediments were also collected from representative rivers: NS, SM, SK, and PO in December 2016.

**Analytical methods.** The concentrations of T-Fe and D-Fe were analyzed with the spectrophotometric method using 1, 10-phenanthroline [3]. Inorganic nitrogen and phosphate were measured with a nutrient analyzer (AACS-II, Bran+Luebbe). Analysis of $\delta^{13}C$ and $\delta^{15}N$ was conducted with an isotope ratio mass spectrometer (DELTA V Advantage, Thermo Fisher Scientific). Structural features of HAs, which were treated according to IHSS protocols, were evaluated by UV-vis absorptivities, FT-IR spectra and the analysis of acidic functional group content, in accordance with the previous studies [4].

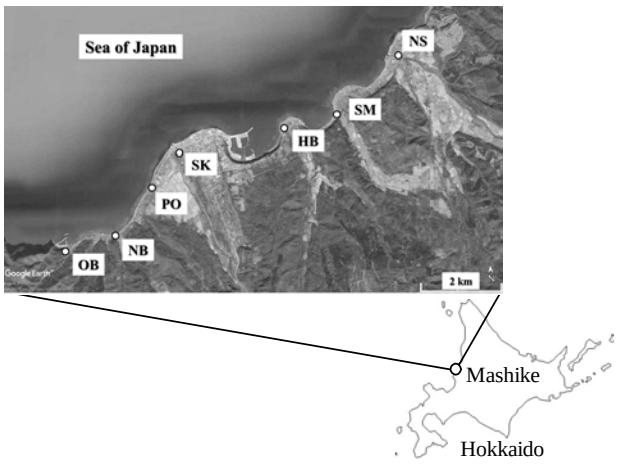



**Figure 1.** Sampling sites in Mashike, Hokkaido.

## Results and Discussion

### Seasonal changes in iron concentrations

Figure 2 shows the seasonal changes of T-Fe concentration in the seven rivers. T-Fe concentrations at SM and PO were found to be higher than those at the other sites during the period of investigation. D-Fe concentrations at PO also were higher, whereas the same

seasonal changes did not appear in those of inorganic nitrogen and phosphate concentrations.

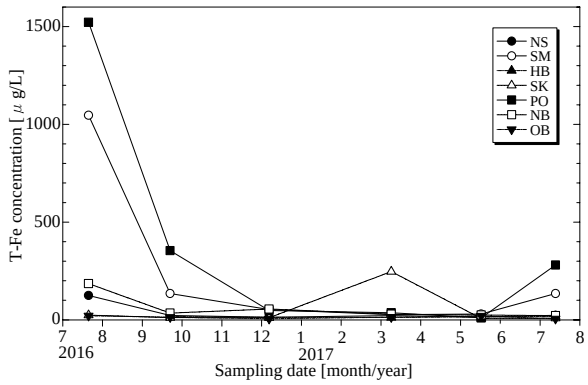



**Figure 2.** Seasonal changes of T-Fe concentrations in the rivers.

The $\delta^{13}C$ values of POM in the rivers are listed in Table 1. Similar values (-28 - -26 ‰) were obtained at all the sites except for OB. The $\delta^{13}C$ at OB could not be analyzed because of a malfunction of the isotope ratio mass spectrometer. The values obtained are consistent with typical values of terrestrial organic matter [5]. Meanwhile, the $\delta^{15}N$ values are different among the sites. The values at PO and SM were relatively high compared to those at the sites. Although the Ponotarumanai River (PO) is a branch of the Shokanbetsu River (SK), the characteristics of $\delta^{15}N$ were different.

In general, $\delta^{15}N$ values become higher in eutrophic conditions and are also increased by the effects of living wastewaters and biological activitiy [6]. In regards to land use, there are paddy areas in the basins of the SM and PO (Figure 1). There is a possibility that the manner of land use affected the river environments in this area.

**Table 1.** $\delta^{13}C$ values of POM

| Sampling site | $\delta^{13}C$ (‰) |
|---|---|
| NS | -26.4 |
| SM | -26.1 |
| HB | -28.0 |
| SK | -27.6 |
| PO | -26.9 |
| NB | -26.0 |
| OB | — |

**Structural features of HAs**

Yields, acidic functional group contents, and UV-vis absorptivities of HAs are summarized in Table 2. The yield values of SM and PO were significantly higher than those of NS and SK. The $E_{600}$ values of HAs were significantly low (0.135 - 0.554) compared to the case of mature HAs, indicating that the degree of their humification was also extremely low. The $E_{280}$ value of PO was significantly higher than those of other HAs. Meanwhile, the content of acidic functional groups in PO, including total, carboxyl and phenolic-OH groups, is the highest among HAs of the rivers. The acidic group content in SM was relatively higher than those in NS and SK.

**Table 2.** Yields, acidic functional group contents and UV-vis absorptivities of HAs

| | Yields[α] | Acidic functional group content (mmol $g^{-1}$) | | | UV-vis absorptibity[γ] | |
|---|---|---|---|---|---|---|
| | | Total | -COOH | Phenolic-OH[β] | $E_{600}$ | $E_{280}$ |
| NS | 3.53 | 3.91 | 0.889 | 3.02 | 0.126 | 6.04 |
| SM | 13.9 | 4.21 | 1.35 | 2.86 | 0.355 | 9.89 |
| SK | 7.34 | 3.76 | 1.05 | 2.71 | 0.135 | 4.73 |
| PO | 14.0 | 6.18 | 1.81 | 4.37 | 0.554 | 13.8 |

α The values were evaluated by dividing the HA (g, dw) obtained in a one-time extraction by initial taken sediment (kg, dw).
β The values were calculated by subtracting the carboxylic acid content from the total acidity.
γ These parameters were measured by 100 mg $L^{-1}$ HA in 0.1 M phosphate buffer (pH 8.00).

The trends of the acidic functional group contents and the UV-vis indexes were consistent with that of the yield values, suggesting that the river environments of SM and PO have conditions favorable to humification in comparison with those of NS and SK.

Concentrations of iron were found to correspond to $\delta^{15}N$ values and structural features of HAs. A higher level of total Fe concentration in SM and PO could be attributed to a large amount of acidic functional groups of HAs in SM and PO. These results suggest that the river environments were influenced mainly by the manner of land use in these river basins.

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# Assessing the impact of wildfires on the solubility of soil organic matter and formation of disinfection byproducts

Y. YU [(a)], A. RETUTA [(a)], F. ROSARIO-ORTIZ [(a)]*

(a) Department of Civil, Environmental and Architectural Engineering,
University of Colorado Boulder, Colorado, USA
* E-mail: fernando.rosario@colorado.edu

**Keywords:** dissolved organic carbon, disinfection byproducts, wildfires

**Abstract.** The frequency and intensity of wildfires have significantly increased over the past decade. Wildfire represents an acute perturbation to watersheds, and the major impacts include changes in ecosystems, sediment export, and overall quality of receiving waters. In this work, the effects of wildfires on soil organic matter were investigated. Specifically, soil samples were collected and heated to specific temperature endpoints. Subsequently, the solubility of soil organic matter and its potential to form drinking water disinfection byproducts were evaluated. These results provide preliminary insights into the impact of fire on the thermal transformation of soil organic matter and ultimately on water quality and treatment.

## Introduction

Wildfires are dramatic land disturbances that can severely change the ecological integrity of watersheds. Since the 1980's, changes in climate and land use have led to a near fourfold increase of wildfire frequency, an increase of burn area by 6.5 times, and a much longer wildfire season [1]. The frequency and severity of wildfires have significantly increased in recent decades and are expected to continue to increase.

For water managers who rely on high quality water from forested watersheds, wildfires present concerns regarding deteriorated quality and treatability of impacted water, especially when alternative water supplies are not available. One of the most commonly acknowledged consequences of wildfire is post-fire erosion and enhanced sediment export from fire-affected watersheds [2]. This is partially due to a decrease in soil stability after vegetation loss and the formation of a repellant layer that bypasses water from infiltration to overland runoff [3]. Post-fire erosion, coupled with thermal transformation of organic matter in surface soils, has been observed to cause elevated concentrations of dissolved organic matter (DOM) and nutrients in the receiving aquatic systems [4]. An increased amount of pyrogenic organic matter can be leached into the aquatic environment that may be a source for drinking water. Because DOM reacts with disinfectants producing disinfection byproducts (DBPs) that are currently under regulation, its concentration and character are of great interest to drinking water utilities. Changes in DOM character may have implications in precursor reactivity towards the formation of both regulated and non-regulated DBPs (e.g., N-DBPs) [5].

The main objectives of this study were to first assess the changes in desorption kinetics and solubility of soil organic carbon (SOC) and nitrogen (SON) due to thermal alteration and second, investigate the compositional transformations of soil organic carbon and nitrogen. The final key objective was to examine the DBP formation potentials of pyrogenic SOC and SON to understand the changes in their reactivity with chlorine and their potential role as DBP precursors.

## Materials and methods

**Soil sampling and processing.** For this study, six surface soil samples were collected in Colorado Front Range following litter removal to a depth of 5 cm beneath ground in September of 2016. All soil samples were dried at 100 ˚C for two hours immediately after collection to evaporate moisture and to suppress microbial activity that may compromise sample integrity during storage. Dried soils were then passed through a 2 mm (No. 10) and a 0.841mm (No. 20) sieve in series to remove rocks and plant debris before storage. Homogenized soils were then heated for two hours at temperatures ranging from 150˚C to 550˚C using a muffle furnace and were subsequently analyzed for total soil C and N contents.

**Soil leaching and leachate characterization.** After prescribed thermal treatment, each soil sample was leached in ultra-pure Milli-Q water at 5 g/L and was filtered through 0.45 μm membrane filter before leachate characterization. Leachates were then characterized in terms of dissolved organic carbon, total dissolved nitrogen, ammonia nitrogen, nitrate and nitrite nitrogen concentrations. DBP formation potential tests were performed at pH 7 with an initial free chlorine dose of 20 mg/L as $Cl_2$ and a suite of regulated and non-regulated DBPs were quantified after 72 hours of incubation following EPA methods 551.1 and 552.2.

## Results and Discussion

### Elemental analsyis and DOC release

Figure 1 shows the effect of increasing heating temperature on the carbon content in soil and also on the water-extractable fraction of the remaining SOC for two of the samples collected. Heating decreases the overall carbon content in the soil mainly through mineralization, specifically above 250 ˚C. In contrast, the solublity of SOC increased with increasing heating temperature and reached its maximum at 350 ˚C. Compared to the unheated control, the fraction of SOC that was extractable by water from soils heated at 350 ˚C was approximately one order of magnitude higher. Similar results were obtained for the enhanced solubilty of the SON (data not shown).

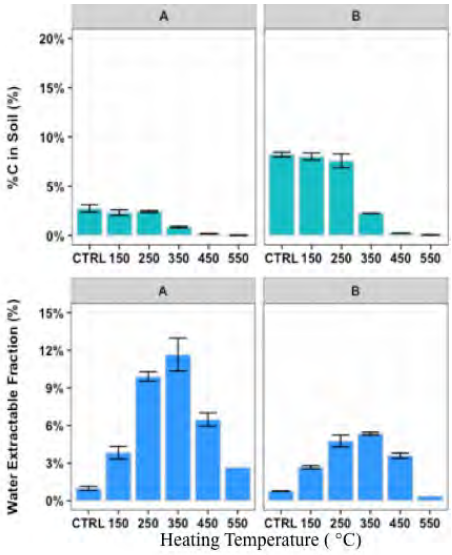



**Figure 1.** Effect of increasing heating temperature on the on the carbon content in soils A and B (top panel) and on the water-extractable fraction of the corresponding SOC (bottom panel). Experiments were conducted on leachates prepared by leaching 5 g of soil per liter of water.

### DBP formation potential

Figure 2 shows the DBP formation of from chlorinated leachates. The top panel shows chloroform formation potential as a function of heating temperature. In general, chloroform formation decreased with increasing heating temperature with the exception at 250 ˚C. The bottom panel shows the dichloroacetic acid (DCAA) formation potential. Contradictory to chloroform, DCAA formation increased with increasing heating temparture. These results indicate that the chemical reactivity of the leached orgnaic matter changed as the soils were thermally altered. Similar profiles were collected for N-DBPs (data not shown). Taken together, these results provide some preliminary insights into the thermal transformations of SOM and the impact of heating on its reactivity.

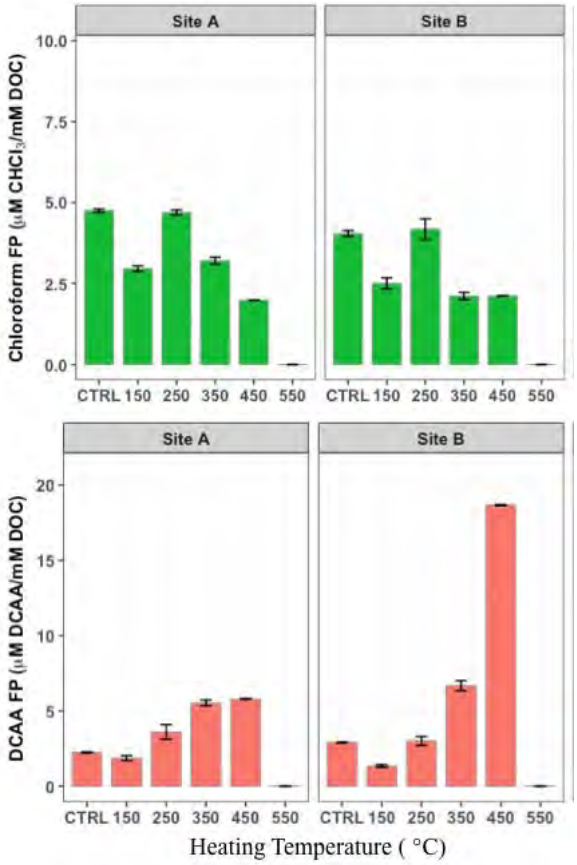



**Figure 2.** Specific yields of chloroform (top panel) and DCAA (bottom panel) from chlorinated leachates as a function of heating temperautre. Specific yields are calculated as concentration of formed DBP per milligrams of DOC.

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# Assessment of humification indices by monitoring humic products changes using UV-VIS, 2D-FTIR

Y. ZHANG [(a)], D. YUE [(a) (b)*], W. SONG [(c)]

(a) Key Laboratory for Solid Waste Management and Environment Safety (MOE), School of Environment, Tsinghua University, Beijing 100084, P.R. China
(b) Collaborative Innovation Center for Regional Environmental Quality, Beijing 100084, P.R. China
(c) Beijing Production and Marketing Service Station for Superiors Agricultural Products, Beijing 100029, P.R. China
* E-mail: yuedb@tsinghua.edu.cn

**Keywords:** humification degree, FLAs, HLAs, UV-Vis, 2D-FTIR

**Abstract.** Assessment of humification is pivotal in evaluating the nature of humic substances. The study aimed to evaluate the relationship of humic parameters by characterizing humification process using the widespread used UV-Vis parameters. Meanwhile, Two-dimensional infrared spectroscopy (2D-FTIR) was used to monitor the role of functional groups in forming humic acids. Results showed that $E_{254}$ has a relatively good correlation with humic-like acids (HLAs) amount, whereas $A_{465}/A_{665}$ ratio may have a positive linear relationship with the fulvic-like acids (FLAs) amount. Additionally, 2D FTIR showed the C-O stretching and C-O-H bending of ethers and phenols affected the formation of HLAs.

## Introduction

Biowaste recycled in compost is defined as an economic and environmental-friendly way for recycling nutrients. The composting process involves the degradation and transformation of biomass macro-molecules into stable and refractory dark-coloured humic substances (HSs). Therefore, compost is a product from humification process, and the humification degree of biowaste is closely associated with the maturation of the compost. During the progress of composting, chemical and structural performances of organic humic precursors are essentially important for the quality of composting products [1]. The humic precursors such as polyphenols, proteins and polysaccharides are hydrolyzed and further formed humic substances under the action of microbes or inorganic minerals. Especially the polyphenols are cable of forming the major part of HSs.

The assessment of humification degree is affected by many factors, such as the variation of pH, temperature, humic precursors concentration and inorganic ionic strength. Inorganic ammonium is often coexisted with the dissolved organic matter in aquatic systems with high concentration levels, whereas the effects on humification remain obscure. Furthermore, the extraction and quantify process of humic substances are complicated, which require the molecular structure of humic substances kept from damaging. Since there are many parameters reported in the research with some properties of humic substances, comparatively, Ultraviolet-Visible absorbance (UV-Vis absorption) spectra have been widely used for the characters of nondestructive evaluation as well as highly sensibility [2]. Nevertheless, the relationship between the procedure for the quantitative characterization of humic substances recommended by IHSS (International Humic Substances Society) and UV-Vis absorption spectra for characterising humification progress received little attention.

The objective of this study was to estimate the humification process associated with the products by using UV-Vis and 2D FTIR. A linear regression was conducted and the relationship was investigated by using the data obtained from sampling. Several indices of UV-Vis parameters has been widely used for assessing the formation of humic substances, including absorbance at 210 nm ($A_{210}$), 254 nm ($A_{254}$), 280 nm ($A_{280}$), 400 nm ($A_{400}$), 465 nm ($A_{465}$), 472 nm ($A_{472}$), 600 nm ($A_{600}$) and 664 nm ($A_{664}$), meanwhile divided corresponding DOC (dissolve organic carbon), $E_{254}$, $E_{400}$ and $E_{600}$. Additionally, the absorbance ratios are often referenced in literature, $A_{210}/A_{280}$, $A_{465}/A_{665}$, $A_{280}/A_{664}$ and $A_{280}/A_{472}$. The study was performed in two abiotic humification systems, one system containing glucose, glycine, and various catechol, and another system containing equivalent amounts of glycine and catechol with different concentrations of inorganic ammonium. All the systems were with a fixed amount of $MnO_2$ as an oxidation catalyst.

## Materials and methods

**Humification systems.** Based on the previous studies, the catechol is played a critical role in improving the formation of humic substances, and inorganic ammonia-N with high concentration negatively influence the yield of humic acids. Therefore, the study set two groups to investigate the changes of humification products: in the first system, powdered glycine and glucose were added at a concentration of 0.06 M and incubated with increasing catechol concentrations (0 M, 0.005 M, 0.01 M, 0.02 M, 0.04 M, 0.06 M, 0.08 M, 0.12 M). The second systems containing catechol and glycine concentrations of 0.1 M and 0.05 M, and powdered ammonium chloride was added into flasks to obtain concentrations of inorganic ammonia-N at 0, 400, 800, 1600, 2500and 4000 mg/L. Meanwhile, 2 g of $MnO_2$ was added to each system. All mixtures were in 200 mL of

phosphate buffer (pH 8.03), 0.02% (w/v) thimerosal, and operated at 35± 1°C in a rotatory shaker at 170 rpm for 290 h. In addition, control systems were buffer solution only and a mixture of buffer solution and $MnO_2$. All the experiments were carried out in triplicate.

**Characterization of humic substances.** Samplings of 1 mL were extracted and centrifuged (10,000 rpm, 5 min) at different sampling times for determining the concentrations of DOC and UV-Vis absorbance. Furthermore, Extraction and fractionation of fulvic-like acids (FLAs) and humic-like acids (HLAs) were conducted by using the method recommended by the International Humic Substances Society (IHSS). The relevant description has been published in detail in a previous report.

Additionally, the DOC was tested with a TOC-L CSN type analyzer (Shimadzu, Japan) to determine the amount of HSs. Absorbance values at a wavelength range of 200-800 nm were used to monitor the formation of darkening polymers using a UV-1800 type UV-Vis spectrophotometer (Shimadzu, Japan). The elemental composition (C, H, N, and O) of HLAs was determined with an Elemental Analyzer EA3000 (Eurovector, Italy). FTIR spectra were collected using an FT/IR 600-type spectrometer (Japan Spectroscopic Co. Ltd.) with KBr pellets, and 2D shige software was used to analyze the variations of functional groups after humification. Aromaticity was investigated using solid-state $^{13}C$ NMR analysis with cross-polarization and magic angle spinning (CPMAS).

## Results and Discussion

### UV-Vis parameters

Table 1 displays the different linear regression coefficients ($R^2$) between UV-Vis parameters and the concentration of humic substances associated with the number of double bond equivalents (DBE) and aromaticity under different reaction conditions. Obviously, $E_{254}$ has a relatively good correlation with HLAs, and showed a clear linear relationship with DBE, whereas no apparent effects between $E_{254}$ and FLAs. However, the $A_{465}/A_{665}$ has been widely accepted as an indicator of the molecular weight and degree of polymerization of humic substances. However, the results found in this study indicate that the $A_{465}/A_{665}$ ratio may have a positive relationship with the amount FLAs, rather than that of HLAs, and the result echoed previous findings. Additionally, what is interesting that the values which often represent the humification degree or the number of humic substances, whereas showed poor linear correlation. The reason may attribute to the appearance of the dark-coloured substances, and further re-polymerization has not occurred.

**Table 1.** Linear regression coefficients ($R^2$) for UV-Vis parameters associated with the DOC amount of FLAs and HLAs formed in systems containing different levels of catechol and inorganic ammonium.

| | $E_{254}$ | $E_{400}$ | $E_{600}$ | $A_{210}/A_{280}$ | $A_{280}/A_{472}$ | $A_{280}/A_{664}$ | $A_{465}/A_{665}$ |
|---|---|---|---|---|---|---|---|
| FLAs (mg DOC/L) | 0.0009 | 0.3962 | 0.0362 | 0.1312 | 0.1048 | 4.00E-07 | 0.5259 |
| HLAs (mg DOC/L) | 0.6681 | 0.4102 | 0.3881 | 0.0677 | 0.2065 | 0.1028 | 0.0336 |
| DBE | 0.8041 | 0.6031 | 0.3991 | 0.0215 | 0.1858 | 0.0035 | 0.0168 |
| Aromaticity | 0.2374 | 0.3461 | 0.0615 | 0.2104 | 0.2207 | 0.0556 | 0.2429 |

### 2-D FTIR of end humic acids

As shown in Fig.1., the synchronous maps display two major auto peaks at 1230 and 1740 cm-1 which were attributed to C-O stretching and C-O-H bending of ethers and phenols.

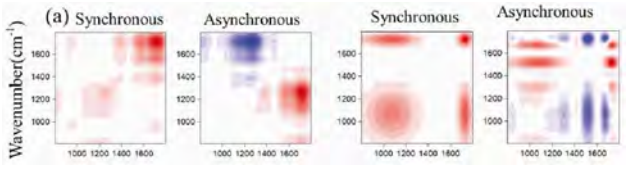



Figure 1. Synchronous and asynchronous 2D-FTIR maps of end humic acids generated from the unidimensional FTIR spectra of 1800-800 $cm^{-1}$ region: (a) Humic acids extracted from catechol system; (b) Humic acids extracted from the inorganic ammonia-N system. Red represents a positive correlation, whereas blue represents a negative correlation, and higher colour intensity indicates stronger correlation.

The results indicated that the variation of humification degree mainly attributed to the impacts of C-O stretching from ethers and phenols, which further influence the structure of HLAs. Additionally, the asynchronous maps demonstrated that the sequence of functional group in forming HLAs follows the sequence: carbonyl C=O C > phenol C-O > aromatic C=C.

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# TOPIC 4.
# “SOIL PHYSICAL CHEMISTRY - SOIL FERTILITY”

# Subsoil incorporation of crop residues enhances humic acid accumulation and its carbohydrates enrichment

H. ABDELRAHMAN [(a,b)*], D. OLK [(b)], D. DINNES [(b)]

(a) Soil Science Dept., Faculty of Agriculture, Cairo University, Giza 12613, Egypt
(b) USDA-ARS, National Laboratory for Agriculture and the Environment, Ames, IA, USA
* E-mail: hamada@cu.edu.eg

**Keywords:** crop residues, no-till, conventional tillage, SOM, humic substances

**Abstract. Can subsoil incorporation of crop residues promote soil C sequestration?** Soil organic matter (SOM) fractions were separated from four depths in three fields that were managed under either conventional incorporation of crop residues to 15 cm, subsoil incorporation to 35 cm, and no-tillage. The light fraction (LF), 53 um-particulate organic matter (POM), mobile humic acid (MHA) fraction, and Ca-bound humid acid (CaHA) fraction were separated and characterized for their amounts and carbohydrate content. Results showed that SOM fractional masses were greater with subsoil incorporation than with conventional tillage or no-tillage. Moreover, results showed an enrichment of carbohydrates in soil and SOM fractions. The carbohydrates ratio of (galactose + mannose)/(arabinose + xylose) indicated a major contribution of plant derived carbohydrates to soil, the MHA and the CaHA. Subsoil incorporation of crop residues seems to enhance the accumulation of SOM fractions and their carbohydrate enrichment.

## Introduction

Soil organic C (SOC) is a major pool of global C; it contains more than three times as much C as either the atmosphere or terrestrial vegetation. However, due to human activities, the annual net release of C from soils is increasing [1]. Thus, initiatives aiming to maintain and improve SOC stocks and the soil's capacity to store C [2] are promising measures to mitigate climate changes.

Subsoil incorporation of crop residues represents a potential mean to increase SOC, as the decomposition process in subsoil is slower than in topsoil, due to limited supply of oxygen. Moreover, stabilization of SOC in subsoil is considered more effective as subsoil generally contains higher clay contents that are mainly C deficient and is less exposed to environmental conditions [3]. Soil C loss can occur in subsoil due to the priming effect of the inputs of fresh residues [4], however, no evidence on the priming effect was found in a long term study [5].

The objective of this work was to evaluate the effect of different crop management practices on the amount and composition (carbohydrates) of SOM fractions.

## Materials and methods

### Site and sampling

Soil samples were collected from three nearby farmers' fields in Delaware County, Iowa, USA. Crop residues were managed differently in each field, namely: i) long-term conventional tillage (CT) practice where crop residues are tilled into the 0-15 cm soil depth; ii) no incorporation or no-tillage (NT) for the previous 7 yrs, and iii) subsoil (incorporation (SI) of crop residues down to the 35-cm depth for about 40 yrs. Fields were cultivated with maize (*Zea mays* L.) – soybean [*Glycine max* (L.) Merr.] rotations under rainfed conditions.

Soil samples were collected post-harvest from four depths (0–5, 5–20, 20–40, 40–60 cm) at two locations in each field. Collected samples were air dried, 2-mm sieved and then stored for subsequent analyses.

### SOM fractionation and characterization

Soil samples were subjected to a joint physical-chemical fractionation approach [7] to extract in sequence the light fraction (LF) by densitometric separation, 53-µm particulate organic matter (POM) by size separation and 0.25 M NaOH extraction for the mobile humic acid (MHA) and Ca-bound humic acid (CaHA) fractions before and after soil decalcification via 0.1 M HCl washes, respectively. Carbohydrate contents of the separated fractions were measured by anion chromatography–pulsed amperometric detection contents [8].

## Results and Discussion

### SOM response to crop residues management

The masses of the LF, POM, MHA and CaHa were higher in the topsoil, then decreased generally with depth. However, the LF and the POM masses were greater in the 40–60 cm layer than in the 20–40 cm layer.

The LF, POM, MHA and the CaHA masses were greater with SI than with CT or NT at each depth (Fig. 1).

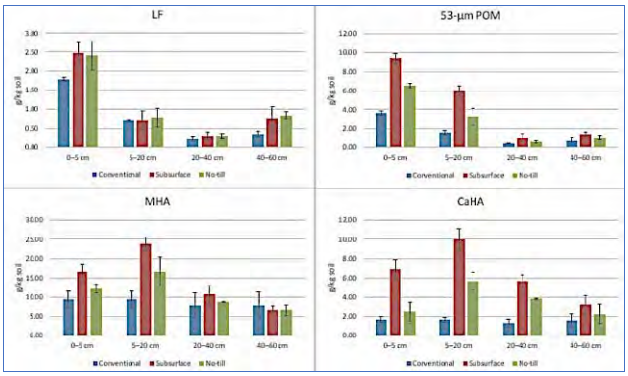



**Figure 1.** Amounts (g fraction $kg^{-1}$ soil) of the LF, POM, MHA, and CaHA at four soil depths for three crop residue management practices.

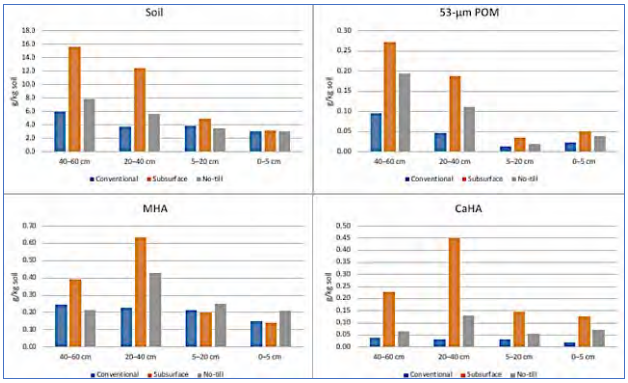



**Figure 2.** Carbohydrate accumulation (g carbohydrates $kg^{-1}$ soil) in total soil, the POM, MHA, and CaHA fractions at four soil depths for three residue management practices.

In the SI field, the total soil, POM, MHA and CaHA fractions (Fig. 2) had greater carbohydrate contents per kg soil than in the other residue management practices. Carbohydrate accumulation was due not merely to the increase in fractions mass but also to higher carbohydrate enrichment in both humic fractions. For example, carbohydrate concentration of the CaHA was 110–202% greater than for the CT system for the 20–60 cm depths (Fig. 3). In contrast, the POM fraction, being composed of plant-like materials, showed comparable carbohydrates content per unit mass among residue treatments for each depth of 20-60 cm (Fig. 3).

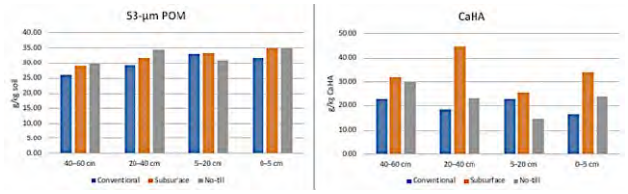



**Figure 3.** Carbohydrates enrichment (g carbohydrates $kg^{-1}$ fraction) in the CaHA in four soil depths from three different residues management systems.

Carbohydrate ratios are used as a useful indicator of SOM changes as a function of biotic and abiotic process in soil. The ratio of (Galactose + Mannose)/(Arabinose + Xylose) has been used to define the origin of a material [9]. When close to 1, the ratio indicates similar contributions of plant and microbial source materials to total soil carbohydrates. However, the carbohydrate ratio was 0.68 in the 20–40 cm depth reflecting the impact of SI of crop residues and indicating a majority presence of plant-derived carbohydrates (Table 1).

The carbohydrates ratio for the MHA and CaHA was $\leq$ 0.5, similarly indicating a larger contribution of plant-derived than microbial-derived carbohydrates.

**Table 1**. (Galactose + Mannose)/(Arabinose + Xylose) ratio of soil, MHA and the CaHA at different soil depths and different crop residue management

| | Depth, cm | CT | SI | NT |
|---|---|---|---|---|
| Soil | 0–5 | 0.88 | 0.89 | 0.76 |
| | 5–20 | 0.85 | 0.91 | 0.70 |
| | 20–40 | 0.86 | 0.68 | 0.75 |
| | 40–60 | 1.01 | 0.75 | 0.88 |
| MHA | 0–5 | 0.34 | 0.28 | 0.23 |
| | 5–20 | 0.31 | 0.28 | 0.28 |
| | 20–40 | 0.42 | 0.25 | 0.32 |
| | 40–60 | 0.35 | 0.20 | 0.24 |
| CaHA | 0–5 | 0.42 | 0.41 | 0.50 |
| | 5–20 | 0.41 | 0.43 | 0.37 |
| | 20–40 | 0.45 | 0.34 | 0.33 |
| | 40–60 | 0.42 | 0.43 | 0.43 |

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# Calcium improve humic acid adsorption by soil minerals

M. BARRETO [(a)*], M. RAMLOGAN[(b)], A. ROUFF [(b)], E. ELZINGA[(b)], L.R.F. ALLEONI [(a)]

(a) Department of Soil Science, University of São Paulo, Piracicaba-SP, Brazil
(b) Department of Earth and Environmental Science, Rutgers University, Newark-NJ, USA
* E-mail: barretomsc@usp.br

**Keywords:** carbon storage, tropical soil, ternary complex, organic matter stability

**Abstract.** Increasing organic matter contents in highly weathered soil is an important strategy to improve soil fertility and sequester carbon. Calcium (Ca) is frequently associated with bonding mechanism (e.g., cation bridging) between soil minerals and organic molecules. Ca concentration increased, we observed an increase of humic acid adsorption, mainly at higher pH. Improving Ca availability by lime and/or gypsum should be a tool for improving carbon storage in weathered soils.

## Introduction

The soil is a huge sink and source of $CO_2$ and sequesters more carbon (C) than vegetation and the atmosphere [1]. The mechanisms that drive the SOM stability are generally attributed to the biochemical complexity of SOM molecules, physical protection in soil aggregates and chemical stabilization by interaction SOM-minerals [1, 2, 3].

The shift on land use, mainly from native vegetation to agriculture, decrease the amount of SOM. Some practices such as soil tillage, pH correction, fertilization, and irrigation improve the accessibility and velocity of SOM decomposition by microorganisms [3]. The main strategy to improve SOM contents in agricultural systems adopted in highly weathered soils is the no-tillage, which improves soil aggregates stability and decreases SOM exposition to degradation [4].

Humic acid (HA) sorption on soil and iron minerals increases in the presence of Ca [5]. These results suggest that Ca should be an important player to SOC sequestration. In this study, we assessed HA adsorption by a mimic clay fraction of a weathered soil under different levels of Ca and pH.

## Materials and methods

**HA solution.** Standard Elliott Soil Humic Acid (1S102H) was obtained from the International Humic Substances Society and used without further purification.

**"Mimic" clay fraction.** The proportion of the more representative minerals was chosen based on the "Representative Pedon and Data" about Oxisol from Soil Taxonomy Classification. The mix, named here as "clay", had 70 % of kaolinite, 20 % of aged $\gamma$-$Al_2O_3$ and 10 % of goethite and it had particle size < 2µm.

**HA adsorption to clay.** The isotherm adsorption was carried using 1 g $L^{-1}$ of clay in 10 mM NaCl and HA concentration range 5-120 mg.$L^{-1}$. The variations were Ca levels (0, 0.1, 0.2, 0.4 mM.$L^{-1}$) and pH values (6.5 and 4.5, using 0.1M NaOH or HCl for pH adjustment). Solutions were equilibrated for 24 h. HA equilibrium solution was analised using UV-Vis spectroscopy. We used the absorbance at 280 nm ($E_2$) to quantification by standard curve and absorbance at 465 nm ($E_4$) and 665 nm ($E_6$) to compose the $E_2/E_4$ and $E_4/E_6$ index. The adsorption was described by Langmuir equation.

## Results and Discussion

**HA adsorption.** At the lower pH, we found the maximum adsorption estimated by Langmuir equation coefficient (Table 1). At low pHs, the positive surface charge of minerals in the clay fraction is greater than at higher pHs.

Ca had a synergetic effect on HA adsorption, been higher on higher Ca levels (Fig. 1).

This increases should be a result of the formation of Ca-bridging in the ternary complex (HA-Ca-clay). At higher pH, this mechanism was more evident likely by a decrease of positive charged surface sites in clay, especially on goethite and aged-$\gamma$-$Al_2O_3$ [5].

**Table 1.** Langmuir parameters used to model sorption of HA on clay

| pH | Ca (mM) | $q_{max}$ (mg HA $g^{-1}$ clay) | K | $R^2$ |
|---|---|---|---|---|
| 6.5 | 0 | 11.4 | 0.17 | 0.84 |
| | 0.1 | 18.1 | 0.13 | 0.88 |
| | 0.2 | 24.7 | 0.10 | 0.94 |
| | 0.4 | 27.7 | 0.10 | 0.96 |
| 4.5 | 0 | 19.5 | 0.12 | 0.94 |
| | 0.1 | 24.0 | 0.10 | 0.88 |
| | 0.2 | 25.2 | 0.11 | 0.90 |
| | 0.4 | 30.5 | 0.12 | 0.91 |

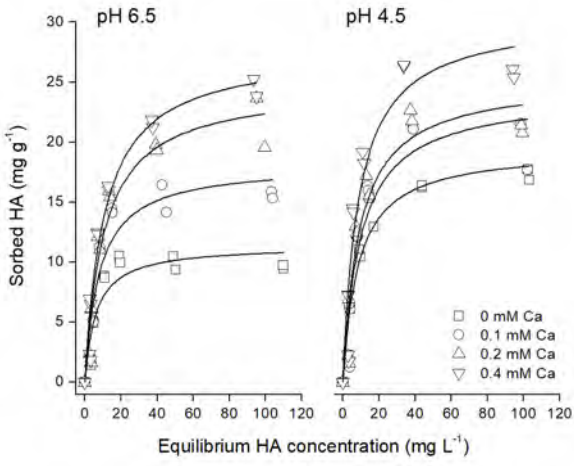



**Figure 1.** Sorption of HA by clay in function of pH and Ca levels. The solid lines represented Langmuir fitting.

### $E_2/E_4$ and $E_4/E_6$

The ratio $E_2/E_4$ and $E_4/E_6$ had a negative correlation with the degree of humification and aromatic condensation of humic acids [6]. The ratio started at a low value and increased until stabilization near to the initial value of humic acid (Fig. 2), for both pH values.

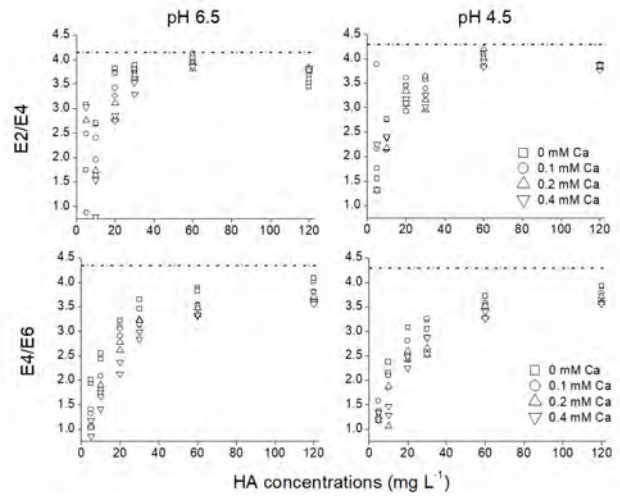



**Figure 2.** $E_2/E_4$ (upper) and $E_4/E_6$ (bottom) ratio of HA solution after equilibrium with clay at pH 6.5 (left) and 4.5 (right). The upper line in each graphic represents the value of initial 60 mg $L^{-1}$ of HA.

Even in high HA concentration, the equilibrium solution maintains the ratios lower than the initial values of $E_2/E_4$ and $E_4/E_6$. There is a preference for adsorption of less humificated compounds with slightly aromatic character, which could be carboxylic acids or polysaccharides. Thus, it is possible to infer a biochemical preference for these compounds in HA adsorption process.

Higher Ca levels lead to lower ratios values. Likely, the increment in HA adsorption by interactions with Ca is also related to less humificated compounds with low degree of condensation of aromatic nuclides.

In future studies, we will try to identify which compounds are preferentially adsorbed without and with Ca assistance and to asses the C thermal stability in the HA-mineral associations.

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# Eco-friendly iron-humic nano-fertilizers tested with $^{57}$Fe in calcareous soil

M. T. CIESCHI [(a)*], A. YU. POLYAKOV [(c)], D. S. VOLKOV [(b)], V. A. LEBEDEV [(b)], D. A. PANKRATOV [(b)], I. V. PERMINOVA [(b)], J. J. LUCENA [(a)]

(a) Autonomous University of Madrid. Department of Agricultural Chemistry and Food Science. Madrid. Spain
(b) Department of Chemistry. Lomonosov Moscow State University. Moscow. Russia
(c) Department of Materials Science. Lomonosov Moscow State University. Moscow. Russia
* E-mail: maria.cieschi@uam.es

**Keywords:** Nanofertilizers, iron nutrition, humic substances, leonardite, $^{57}$Fe, soybean.

**Abstract:** Three iron- humic hybrid nanomaterials were synthesized from leonardite humate and $^{57}$Fe isotope used in the form of $^{57}Fe(NO_3)_3$ and $^{57}Fe_2(SO_4)_3$. They were characterized using Mossbauer spectroscopy, XRD, EXAFS and transmission electronic microscopy and tested for iron availability in a calcareous soil pot experiment under controlled conditions. Three doses of each iron-humic material were applied and iron nutrition contributions were evaluated using $^{57}$FeEDDHA as a positive control and soybean as iron deficient plants. The test plants fertilized with iron-humic compounds exhibited continuous and long-term iron uptake and showed high shoot fresh weight. Moreover, the humic fertilizers remained available in the soil and were observed in fruits.

## Introduction

Iron fertilizers based on humic substances (HS) extracted from lignites, such as leonardite, are used in the Mediterranean area (as liquid concentrates) in drip irrigated fruit tree plantations [1]. This kind of iron fertilizers is more eco-friendly than synthetic iron chelates but they are less efficient in correcting calcium chlorosis. Field experiments have demonstrated that the synthetic chelate has a fast effect while the iron humate fertilizers provide increasing iron availability in the root-soil interface that allows a slow uptake of Fe by the plants [3]. Kulikova et al. [4] have demonstrated that only iron from very small and amorphous nanoparticles of ferric polymers incorporated into the humic matrix are readily taken up by plants. Therefore, iron humates should be optimized for developing efficient nano-fertilizers.

In this study, the stable isotope $^{57}$Fe was used as a tracer to study iron source uptake and its distribution in plants. Three $^{57}$Fe-humic nanomaterials were synthesized and the aim of this work was to test iron bioavailability of the iron-humic materials and distribution in the plant.

## Materials and methods

**Synthesis of $^{57}$Fe-humic hybrids materials**. Two products were obtained by interaction of potassium humate (70 g $L^{-1}$) with $^{57}Fe_2(SO_4)_3$ 0.2M (S at pH 10 and M at pH 9) and one by complexation with $^{57}Fe(NO_3)_3$ 0.2M (F at pH 10). pH was adjusted with 1M KOH and then the samples were lyophilized for three days. $^{57}$FeEDDHA was prepared by chelation of EDDHA with $^{57}Fe(NO_3)_3$ prior to pot experiments.

**Pot experiment**. For a long-term soil pot experiment, each $^{57}$Fe-humic nano-fertilizer (F, S, and M) was applied to iron deficient soybean plants (*Glycine max* AG1835) in three doses (150, 75 and 35 µmol $pot^{-1}$). The $^{57}$FeEDDHA dose was 50µmol $pot^{-1}$. Pots (600g) were filled with a mixture of calcareous soil and sand 70:30 w:w. Two samplings were done (15 and 48 days after fertilization).

**Analytical procedures**. Biometrics (shoot, root and fresh weight, shoot and root length and SPAD index) were determined and $^{57}$Fe content in soil and plant was measured by ICP-MS after the acidic mineralization of the samples.

## Results and discussion

**Samples characterization.** All three iron humates synthesized in this study were characterized by the presence of nanoparticles of ferrihydrite of rather small sizes. (Fig. 1). This was also confirmed by EXAFS. At the same time, they had crystalline character (the presence of 2nd coordination sphere in EXAFS spectra).

**Biometrics.** Plants fertilized with humic fertilizers presented higher shoot and root fresh weight than those with FeEDDHA. No significant differences were observed for fruit fresh weight.

**Iron content in plants and soil**. The $^{57}$Fe content was measured in plants and soil.

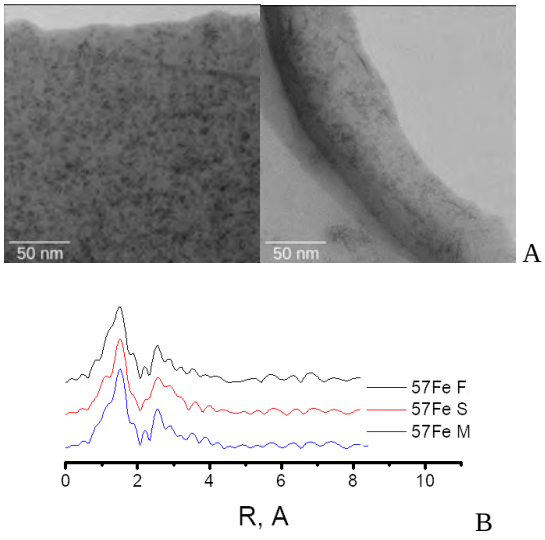



**Figure 1.** Zero-loss TEM images (A) and EXAFS of the samples synthesized in this study.

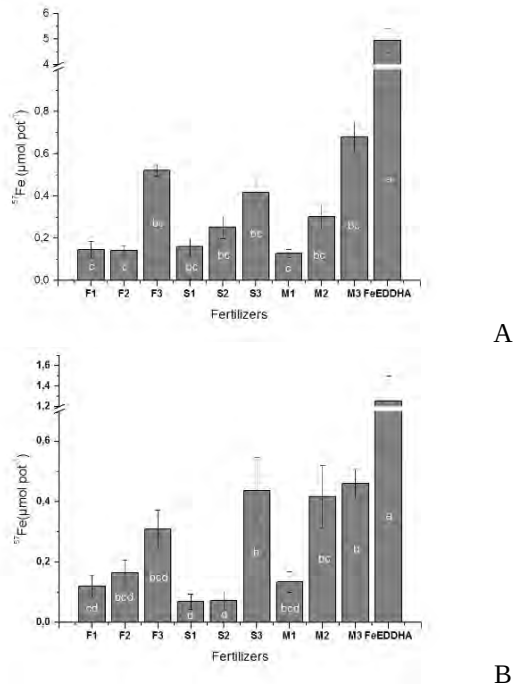



**Figure 2.** $^{57}$Fe content after 48 days of fertilization: in the shoots (A); in the fruit (B).

The $^{57}$Fe content in shoots and fruits from the $^{57}$Fe-humic nano-fertilizers is shown in Fig. 2. The products S and M, prepared with $^{57}Fe_2(SO_4)_3$, delivered more iron to shoot and fruit than the product F along with an increase in the dosage.

As shown in Fig. 3, almost 10% of $^{57}$Fe among samplings was detected in leaf for plants treated with S2 and M2. At the same time, the plants fertilized with FeEDDHA consumed iron immediately and did not show a further increase. Most of $^{57}$Fe provided by the humic products remained available in soil (Fig. 4).

Iron-humic fertilizers are apparently less efficient than iron chelates in soil because of kinetics effects although they promote a long-term effect in iron nutrition as opposed to the short-term effect of the iron chelates.

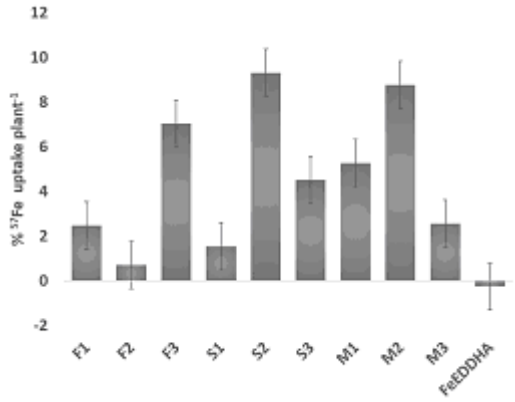



**Figure 3.** Percentage of $^{57}$Fe uptake in leaf per plant among samplings.

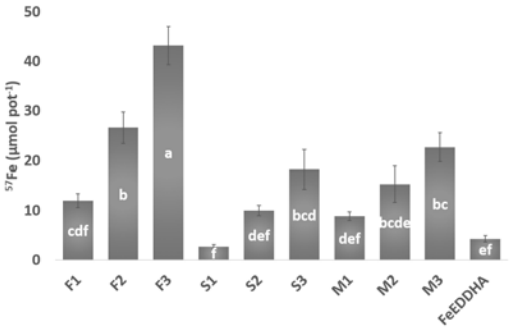



**Figure 4.** The $^{57}$Fe content in the available soil fraction after 48 days of fertilization.

Our study demonstrates the availability of iron from the tested $^{57}$Fe-humic materials to soybean plants grown in calcareous soils. This shows goods prospects for a use of iron-humic nanomaterials as nano-fertilizers.

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# Labile carbon of manure-based biochar accelerates soil denitrification process and increases $N_2O$ emission

Z. DAI [a,b], Y. LI[a,b], J. XU [a,b]*

(a) Institute of Soil and Water Resources and Environmental Science, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China
(b) Zhejiang Provincial Key Laboratory of Agricultural Resources and Environment, Zhejiang University, Hangzhou 310058, China
* E-mail: jmxu@zju.edu.cn

**Keywords:** manure biochar, nitrous oxide, pyrolysis temperature, denitrification, *nirK*, *nosZ*

**Abstract.** The responses of $N_2O$ emissions and microbial N cycling community to the application of manure-based biochar remain unknown. We produced a variety of manure biochars with contrasting organic matter availability. Biochars with relatively high labile C content increased the abundance of denitrification genes, i.e. *nirK* and *nosZ*, and consequently increased $N_2O$ emission, compared to biochars with high aromaticity. Thus, the labile carbon fraction of manure biochar can accelerate soil denitrification process and increase $N_2O$ emissions, which may offset the carbon sequestration in soil ecosystems.

## Introduction

Dinitrogen monoxide ($N_2O$) is a greenhouse gas and considerably produced via denitrification during the reduction of $NO_3^-$ to $N_2O$, and to $N_2$ under anaerobic conditions. Biochar is a carbon-rich solid material obtained from pyrolysis of biomass in an oxygen-limited environment [1]. A number of studies have reported that lignocellulose-based biochars (e.g. derived from woody and crop residues) mitigate $N_2O$ emissions in soil ecosystems [2]. Conversion of manure to biochar has been supported to be an potentially effective and economical approach to reduce a large volume of manure generated, long-term soil fertility improvement and contaminated soil remediation [3]. However, the responses of $N_2O$ emissions and microbial N cycling community to the application of manure-based biochar remain unknown.

In general, the changes in soil $N_2O$ emission and N cycling microbial community mediated by biochars are mainly determined by 1) bioavailable C and N availability of biochar [1]; 2) electron exchange capacities [4]; and 3) changes of soil properties, e.g. pH, aeration etc. [5]. To find the underlying mechanisms, we produced a variety of manure biochars with contrasting organic matter availability. The objectives were to investigate the effects of manure biochars that contain different labile C on 1) soil $N_2O$ emission, and 2) the structure and function of denitrifier genes. We hypothessed that the $N_2O$ emission and denitrification genes increased with decreasing biochar aromaticity and decreased when the labile C was removed.

## Materials and methods

**Biochar preparation.** Two manure biochars were produced by pyrolysing at 300°C (B300) and 700°C (B700). The dissolved organic carbon (DOC300) and acetone extractable C (AeC300) from B300, both of which were called labile carbon (LC), were then added to B700, resulting in B700+DOC300 and B700+AeC300, respectively.

**Soil incubation.** Four biochars (B300, B700, B700+DOC300 and B700+AeC300) and N source (e.g. sodium nitrate) were added to a paddy soil for incubation at 25 °C and in dark. The application rate was 3% (w/w). Soil moisture was adjusted to 130% of field capacity. Soil samples were incubated for 82 days. The $N_2O$ fluxes and the abundance of *nirS*, *nosZ* genes were determined.

## Results and Discussion

### Biochar properties

The molar H/C among four biochars were in the order: B300 > B700+AeC300 and B700+DOC300 > B700 (Table 1), indicating the addition of labile carbon (LC) to biochar decreased biochar aromaticity. Similar contents of mineral N between four biochars were observed, suggesting the mineral N did not differentially affect soil N cycling processes mediated by these biochars.

**Table 1.** Molar H/C and mineral N contents of four biochars

| Biochar type | Molar H/C | Mineral N (mg $kg^{-1}$) |
|---|---|---|
| B300 | 1.33 | 229 |
| B700 | 0.32 | 168 |
| B700+AeC300 | 0.55 | 174 |
| B700+DOC300 | 0.59 | 206 |

### Soil $N_2O$ emissions

The $N_2O$ fluxes among biochar treatments were in the order: B300 > B700+AeC300 > B700 > CK (Figure 1), indicated that biochar application significantly increased soil $N_2O$ emissions, and the addition of LC to biochar also increased soil $N_2O$ emissions. This result was inconsistent with previous studies showing lignocellulose-based biochars can mitigate $N_2O$ emission. The relatively higher LC content of manure-based biochar probably accelerated soil denitrification process compared to lignocellulose-based biochar, as LC is the energy source for denitrifier metabolism.

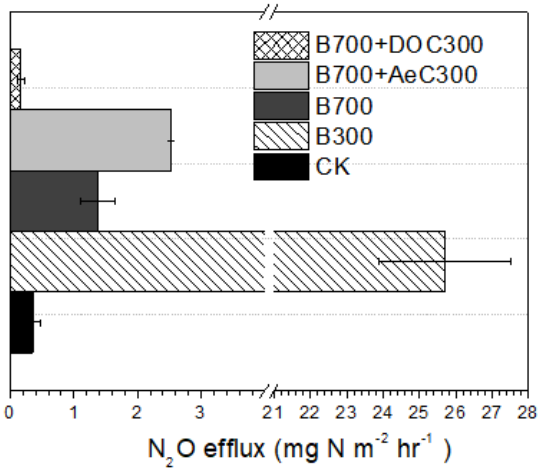



**Figure 1.** The peak of $N_2O$ fluxes from the control (CK) and biochar amended soils under the moisture condition of 130% field capacity and with sodium nitrate additions.

### Denitrifier functional genes

The abundance peak of *nirK* gene responsible for the conversion of $NO_3^-$ to $N_2O$ in B300 was highest, followed by B700+AeC300 and B700+DOC300, and the B700 and CK lowest (Figure 2). The abundance peak of *nosZ* gene responsible for the conversion of $N_2O$ to $N_2$ in B300 was highest, followed by B700+AeC300 and B700+DOC300, and the B700 and CK lowest (Figure 3).

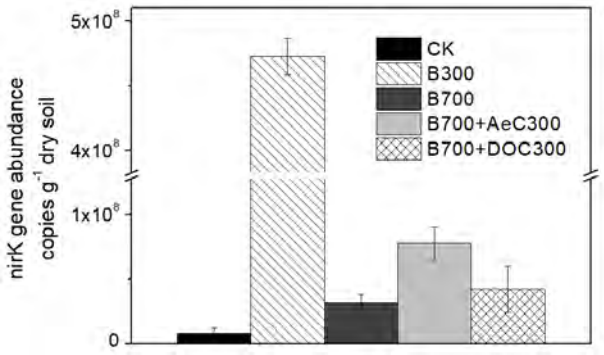



**Figure 2.** The peak of *nirK* gene abundance from the control (CK) and biochar amended soils under the moisture condition of 130% water holding capacity and with sodium nitrate additions.

The reduction of $NO_3$- by denitrifiers requires a readily available C supply to complete the whole denitrification [6]. Thus, the LC of manure biochars could be directly utilized by *nirK* containing microorganisms coupled with the reduction of $NO_3^-$ to $N_2O$. In addition, the *nosZ*-encoded bacteria could be affected by LC sufficient anaerobic condition, and be accelerating the completion of denitrification [4]. It was consistent with the results that the addition of more labile C (DOC300) to B700 increased *nosZ* gene abundance (Figure 3) and decreased $N_2O$ emission (Figure 1). In conclusion, conversion manure to biochar is an efficient way for manure disposal and soil fertility improvement while pre-treatment is necessary to reduce the LC from manure biochar before application.

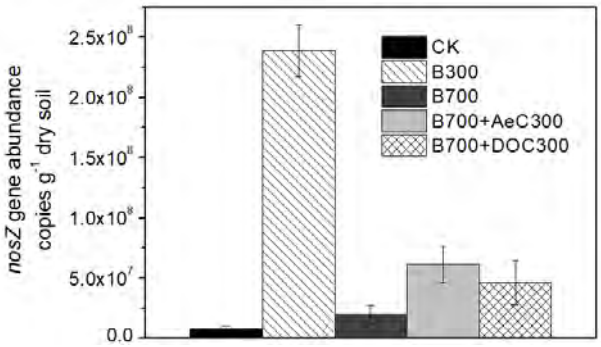



**Figure 3.** The peak of *nosZ* gene abundance from the control (CK) and biochar amended soils under the moisture condition of 130% field capacity and with sodium nitrate additions.

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# Molecular changes of SOM due to cropping system as revealed by humeomics

M. DROSOS [(a)*], A. PICCOLO [(a)]

(a) Centro Interdipartimentale di Ricerca sulla Risonanza Magnetica Nucleare per l'Ambiente, l'Agroalimentare ed i Nuovi Materiali (CERMANU), Università di Napoli "Federico II", via Università 100, 80055 Portici, Italy.
* E-mail: drosos.marios@gmail.com

**Keywords:** Soil, SOM, Humeomics, Fractionation, Maize, Wheat

**Abstract.** Humeomics is a sequential chemical fractionation that uses in the order organic solvent extraction, transesterification with boron trifluoride in methanol, methanolic alkaline hydrolysis, cleavage of ether and glycosidic bonds with HI, and final alkaline extraction. The Humeomic procedure was applied directly on a Maize and a Wheat cropped soil and revealed a series of soil organic matter (SOM) fractions. Gravimetric analysis revealed a significantly larger ratio of organosoluble *vs* hydrosoluble components in the maize cropped soil. Thus, humeomics fractionation can differentiate humic molecules even after only one year of cultivation.

## Introduction

Recent advances in humus chemistry indicated that the traditional macro polymeric view of humic matter is to be substituted with that of non-covalently linked supramolecular association [1] of relatively small heterogeneous molecules of biological and plant origin, which are stabilized in soil by metal bridges, hydrogen bonds and hydrophobic interactions [1-2]. Consequently, a detailed chemical fractionation, called Humeomics, coupled with advanced physical-chemical identification of separated molecules, was recently developed to characterize the molecular composition of humified matter [3-4]. This technique progressively isolates from the humic matrix and identifies either unbound apolar molecules or both hydrophilic and hydrophobic compounds released from hydrolysis of esters and ethers, thereby providing both structural and conformational information on humic superstructures. Humeomics was successfully applied on soils to either elucidate the molecular composition of an otherwise unextractable humin from a volcanic forest soil [5] or to identify, in a tilled agricultural soil, 2.35 times more OC than by the common alkaline extraction [2].

Herein we applied the Humeomics fractionation to elucidate molecular alteration of humic material due to the change of cropping system after one year of either maize or wheat cultivation.

## Materials and methods

### Soil Sample

An undisturbed sandy loam soil was sampled near Torino (Italy) and classified as Typic Ustifluvent after one year of maize and wheat cropping under traditional tillage.

### SOM extraction (eSOM)

Soil was subjected to extraction with $NaOH/Na_4P_2O_7$ solution. The extract was filtered and the pH was adjusted to 7 using HCl and freeze-dried. The material was dialyzed against distilled water using Amicon C membrane (1*k*Da) to remove residual salt and freeze-dried to collect eSOM. Prior to fractionation, the soil was then treated with 0.1M HCl overnight, centrifuged and air-dried.

### Humeomics Fractionation

The sequential fractionation provided an organosoluble unbound fraction (ORG1), a weakly ester-bound organosoluble fraction (ORG2), and a hydrosoluble fraction (AQU2), a strongly ester-bound organosoluble fraction (ORG3), a strongly ether-bound hydrosoluble fraction (AQU4), and a residual OM fraction from the soil (RESOM) extracted with alkaline solution as for eSOM. The protocol of the soil characteristics and Humeomics extraction is described in detail in [2].

### Elemental Analysis

Elemental Composition (C, H, N) of powdered sample was determined with a Fisons Instruments EA 1108 Elemental Analyzer, using Eager 200 Ver. 3.09 calculation software.

### GC-MS Spectrometry

1 mg of ORG1, ORG2 and ORG3 was derivatized before GC-MS analysis using acetyl chloride/methanol as methylating agent, followed by silylation using N,N-bis [trimethylsilyl] trifluoracetamide / 1% trimethylchlorosilane, spiked with 20 µg of nonadecanoic methyl ester as internal standard.

### ESI-Orbitrap-MS Spectrometry

2 mg of eSOM, AQU2, AQU4 and RESOM were weighed and spiked with 20 µg each of the two internal standards 16-d3-hexadecanoic acid and ring $^{13}C$ labelled hydroxybenzoic acid. Mass spectra were obtained with an LTQ Orbitrap (Thermo Electron, Waltham, MA) equipped with a HESI-II source, using negative mode, 140- 2000 m/z mass scan range, and 1.0 s scan time.

## Results and Discussion

The original soil had almost 1% OC. The eSOM under Maize crop yielded 150 mg of OC with minimum losses of material during extraction (Table 1). However, under Wheat crop the Humin material was much lesser and, even though we obtained more mg OC in eSOM, we lost a large amount of hydrophilic OC during the extraction (375 mg). The comparison of Van Krevelen

plots of these two eSOM materials shows a larger hydration degree on the humic ethers and esters for Wheat crop (Figure 1).

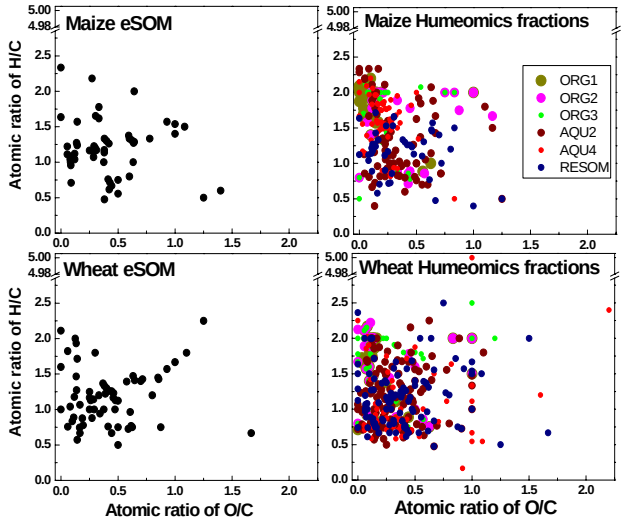



**Figure 1.** Van Krevelen plots of eSOM and Humeomic Fractions of Maize and Wheat cropped soil.

On the other hand Humeomics provided total fractions accounting for almost 550 mg OC for the Maize cropped soil (~3.7 times more than eSOM) with no material losses, and about 370 mg OC for the Wheat cropped soil (~2.0 times more than eSOM) with a loss of almost 194 mg OC, yet less than that found for eSOM (Table 1). It is known that a larger degree of hydrophobicity can result in a more sequestered humic matter. Organosoluble materials are much more hydrophobic by definition. Since Humeomics can deliver both organosoluble and hydrosoluble material, it provides a measure of the degree of organic matter chemical protection, such as the ratio of OC in the organosoluble/hydrosoluble material. This ratio was 0.75 in the case of Maize crop and it dropped to 0.49 for the Wheat crop.

Van Krevelen plots of Humeomics fractions showed a much greater definition of the Humeome complexity, while they revealed a larger oxidation and heterogeneity under Wheat crop (Figure 1).

In more details, GC-MS analysis revealed that fatty acids were the main compounds contributing to the hydrophobic character in all ORG fractions. Furthermore, the ORG1 unbound fraction in Maize crop comprised also of alkanes, which were oxidized to alcohols under Wheat. ORG2 was comprised also of sugars for both crops, although for Wheat they were 50% less than for Maize.

**Table 1.** Carbon yields (mg OC/100 g Soil).

| SAMPLE | Maize | Wheat |
|---|---|---|
| SOIL | 1080 | 1050 |
| eSOM | 150.4 | 192.2 |
| HUMIN | 842.8 | 482.7 |
| LOSS | 86.8 | 375.1 |
| ORG1 | 7.6 | 8.8 |
| ORG2 | 209.4 | 103.3 |
| ORG3 | 17.3 | 10.4 |
| AQU2 | 11.4 | 6.4 |
| AQU4 | 246.2 | 196.8 |
| RESOM | 56.7 | 45.9 |
| RES | 530.6 | 484.7 |
| LOSS | 0.8 | 193.7 |

Finally ORG3 was found to also consist of hydroxy and phenolic acids in the case of Maize, which were reduced to fatty acids and phenols under Wheat.

On the other hand, ESI-Orbitrap-MS revealed that the main compound of AQU and RESOM fractions were heterocyclic nitrogen groups, that were mainly complexed with iron hydroxides. AQU4 was the only exception since it also consisted of amines mostly complexed with iron hydroxides. The difference found in OC of AQU4 between Maize and Wheat (Table 1) was due to the amides which were absent under Wheat but stabilized under Maize, although without being complexed with iron hydroxides.

These findings prove that the complex supramolecular arrangement of the soil Humeome is extremely dynamic and is related to the effects of different agricultural or ecosystem management.

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# Physico-chemical characterization of commercial lignohumate

V. ENEV [(a)], J. KROUSKÁ [(a)], M. PEKAŘ [(a,b)*]

(a) Materials Research Centre, Faculty of Chemistry, Brno University of Technology, 612 00 Brno, Czech Republic
(b) Institute of Physical and Applied Chemistry, Faculty of Chemistry,
Brno University of Technology, 612 00 Brno, Czech Republic
* E-mail: pekar@fch.vut.cz

**Keywords:** lignohumate, physico-chemical properties

**Abstract.** Physico-chemical properties of two commercial products Lignohumate A and Lignohumate MAX were studied by different methods - simple solubility tests, acidobasic titration, surface tension measurements, microcalorimetric dissolution and titrations, elemental analysis, ICP-OES and FTIR spectroscopy.

## Introduction

Lignohumates are specific commercial products designed especially for agricultural applications. They are produced from technical lignosulfonates by the thermal hydrolytic-oxidative conversion aunde gigh pressure [1]. They can be viewed as semi-synthetic products which means that they are obtained by an artificial (synthetic) processing of natural raw material. Structure, producers and distributors report on the results of pot of field tests – i.e. on efficiency in the plant production. Little is known about their physico-chemical properties which could be also important for their practical appliactions. This contribution reports an overview of results obtained in multi-technique study of these properties.

## Materials and methods

Samples of two commercial products, Lignohumate A (LHA) and Lignohumate MAX (LHM), were kindly provided by Amagro company (Czech Republic). Lignohumate A is a solid product in powder form which can be viewed as a basic lignohumate material in potassium form. Lignohumate MAX is essentialy an aqueous solution of the powder product enriched in nutritional microelements and is delivered as a brown-black liquid.

Solubility of the solid product was estimated by simple mixing of increasing weighted amount with constant volume of water and checking for solid residue after 24 h and filtration.

Acidobasic properties of Lignohumate A were investigated preparing solution at the concentration of 1 % w/w and titration with 0.01 M HCl and recording pH using a pH-meter SevenMulti™ by Mettler Toledo®.

Surface activity of Lignohumate A was studied by measuring the surface tension of its solutions in water using a ring method. Its potential aggregation in aqueous solution was adressed by isothermal titration calorimetry (TAM III, TA Instruments). Lignohumate solution (1 or 10 $g \cdot dm^{-3}$) was titrated into water (1 $cm^3$) in 5 µl steps up to the total addition of 250 µl.

Heat of Lignohumate A dissolution at 25°C was measured in the SolCal module of TAM III instrument.

Dry and organic matter contents, ash content in the liquid product were determined by standard gravimetric procedures, humic acids contents similarly after their precipitation by HCl.

Elemental composition of the lignohumates was determined using a CHNS/O analyzer Euro Vector EA 3000. Powder samples (0.5–1.5 mg) were packed in tin capsules. The oxygen content was calculated by difference.

Trace elements were determined by ICP-OES (Horiba Jobin Yvone, type Ultima 2). For ICP-OES measurments, samples were prepared from freeze-dried lignohumates extracted by Mehlich III solution.

FTIR spectra were obtained by means of DRIFT technique using a Nicolet *i*S50 spectrometer. Samples of lignohumates were prepared a mixture of 4–5 mg of powder sample and 100 mg of dried spectrometry grade KBr.

## Results and Discussion

### Solution properties

The solubility of the solid product in deionized water at laboratory temperature was estimated to be at least 10 g in 100 ml of water; the sample containing 20 g per 100 ml of water contained undissolved solid residues.

The pH-value of Lignohumate A aqueous solutions was very slightly dependent on the concentration in the tested range 1-8 g/100 $cm^3$ and changed from 8.43 to 8.46 with increasing concentration. The acidobasic titration showed featureless decreasing trend with no clear equivalence point. This indicates that semi-synthetic lignohumates are also a (rather complex) mixture of moieties with acidobasic properties.

Surface tension data showed certain surface activity of dissolved Lignihumate A – the surface tension is reduced in investigated solutions (1-8% w/v) in average to about 55 mN/m without a clear dependence on the concentration. No typical break of amphiphilic surfactants was found, however we cannot exclude its existence in more diluted solutions.

Isothermal titration calorimetry (ITC) was used to study the aggregation and dilution behavior in water at 25°C. There was not any significant difference between the two used concentrations and an example of the obtained data is shown in Fig. 1. Except the first three or four peaks only heat of dilution after each injection into water (exothermic character) was observed. The first

peaks might indicate also a contribution from some final stage of aggregation; taking together with the results of the surface tension measurements future work should address potential aggregation behavior at concentrations below 1 %.

Heat of Lignohumate A dissolution was determined to be slightly endothermic (14.8 ± 1.5 $J \cdot g^{-1}$, data not shown).

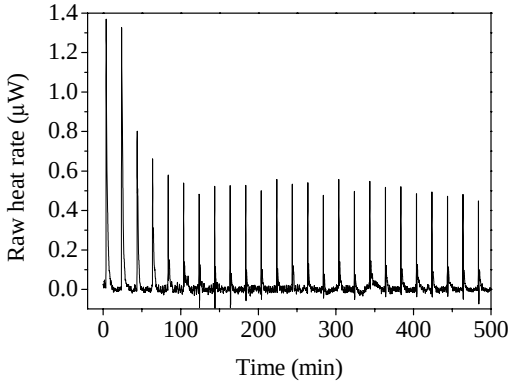



**Figure 1.** ITC data for Lignohumate A at the concentration of 10 $g \cdot dm^{-3}$.

### Elemental composition

Dry matter content in Lignohumate MAX was found to be 19 weight %, and it contained 61 weight % of organic matter. The organic matter contained 31 weight % of humic acid fraction and 69 weight % of the fulvic fraction (besides fulvic acids this fraction may contain also low molecular weight organic acids and fragments of easily hydrolysable substances).

The elemental compositions are presented in Table 1 in atomic percent. Sulphur is either below the detection limit or represents a minor component of lignohumate. The H/C atomic ratio is considered as an indicator of aromaticity. The aromaticity of Lignohumate A was the higher of the Lignohumate MAX. O/C ratio ranges from 0.26 to 0.40 in lignohumates. The higher value for the O/C ratio for LHM can be attributed to the high amount of oxygen-containing groups.

**Table 1.** Results of elemental analysis (atomic %), and calculated ratios

| sample | C | H | N | S | O | H/C | O/C |
|---|---|---|---|---|---|---|---|
| LHA | 40.6 | 44.6 | 3.7 | 0.8 | 10.5 | 1.10 | 0.26 |
| LHM | 38.4 | 45.6 | 0.8 | 0.0 | 15.3 | 1.19 | 0.40 |

### ICP-EOS

The highest content determined by ICP-OES analysis was found for potassium ($138 \cdot 10^3$ $mg \cdot kg^{-1}$). The content of biogenic elements of the Lignohumate MAX was following: sodium ($49 \cdot 10^3$ $mg \cdot kg^{-1}$), calcium (198 $mg \cdot kg^{-1}$) and magnesium (26 $mg \cdot kg^{-1}$). The highest content was determined for zinc (153 $mg \cdot kg^{-1}$) and copper (146 $mg \cdot kg^{-1}$) for our set of trace elements. Other trace elements decrease in the following order: Co > Mn > Si > Sn > Mo > Fe. The lowest content determined from Lignohumate MAX was found for arsenic (0.47 $mg \cdot kg^{-1}$). From environmental point of view, the arsenic concentration in Lignohumate MAX does not represent a toxic risk. Heavy metals (Cd and Pb) were below the detection limit of ICP-EOS analysis.

### FTIR spectroscopy

The FTIR spectra of lignohumates are presented in Figure 2. The FTIR spectra of lignohumates were similar to that of lignohumates from other commercial products. The main differences among the spectra of lignohumates appear in the region 1540–1300 $cm^{-1}$. The region is characterized by a most intensive band at 1412 $cm^{-1}$ corresponding to the C–O vibration in carboxylate anion. The band centred at about 1358 / $cm^{-1}$ indicates the presence of amides II. In comparison with LHA the lignohumate MAX did not contain the vibration at 1358 / $cm^{-1}$.

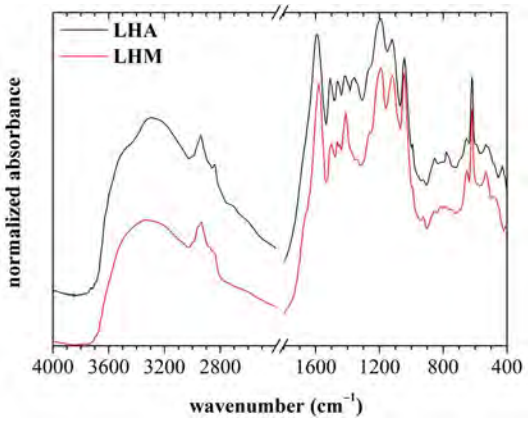



**Figure 2.** FTIR spectra of commercial lignohumates.

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# Content and composition of SOM after long year's field experiment at organic vs mineral fertilization

M. HRISTOVA[(a)*], I. MITOVA[(b)], N. DINEV [(c)]

(a) Department of Genesis, geography and classification of soils; N. Poushkarov ISSAPP, 1080 Sofia, Bulgaria
(b) Department of Agrochemistry; N. Poushkarov ISSAPP, 1080 Sofia, Bulgaria
(c) Department Soil Chemistry; N. Poushkarov ISSAPP, 1080 Sofia, Bulgaria
*E-mail: marihristova@mail.bg

**Keywords:** SOM; organic vs mineral fertilization, a field experiment

**Abstract.** Soil organic matter (SOM) is one of the most important soil characteristics. The intensive and continuous cultivation of arable land lead to a reduction of organic matter in soil. The aim of the paper is to evaluate the quantity and quality of SOM after long time field experiment with crops rotation in three variants of fertilization: the organic and mineral alone, and combination of both of them. The conclusion is that recommended practice for soil health is using of mix organic and mineral fertilization instead of alone variants.

## Introduction

Soil organic matter (SOM) is one of the most important soil characteristics. Over the last ten years, SOM has been gradually reduced in EU countries. The intensive and continuous exploitation of arable land can have led to a reduction of organic matter in the soil is reflected in the EU Soil Strategy as one of the most important issues [1].

By investigating the results of 32 years field experimental work show that the most important factor for stabilizing the SOM is rotation crops with legumes [2]. Other scientists have pointed out that organic fertilizers are the most effective management practice for improving soil physical characteristics compared to mineral fertilizers in the soil root zone [3].

Fertilizer inputs are usually used primarily to increase crop yields. In the long-term practice the application of only N fertilization is not appropriate. The group of researchers recommend the application of other nutrients. They recommended as best way to do is a combination of mineral and organic fertilizers and in amounts which are adequate for the plants as well as for healthy soil life and fertility [4].

The aim of the investiagtion was to evaluate the quantity and quality of SOM after long years field experiment with crops rotation in different variants of fertilization: organic, mineral and the both of them.

## Materials and methods

**Soil sample set.** The experimental field (EF) was conducted in the village of Tsalapitsa, Plovdiv district. The experiment was done many years with crops rotation. The village is situated in the Central region of South Bulgaria. It has an altitude of 186 m. The climate in the area is warm with mild winters, early and warm spring and hot summer. The predominant soils are alluvial meadows, clay-sandy.

The soil samples set were presented from three variants of fertilisation: 1$^{st}$ alone organic, 2$^{nd}$ alone mineral fertilization and 3$^{rd}$ organic: mineral in ratio 50:50. There was control of alone soil without fertilization. The manure has follow contents of nutrients: 1.9% nitrogen (N), 3.91% phosphorus (P), 0.79% potassium (K). The methodology of organic and mineral fertilization is based on an equal amount of nitrogen.

**Determination of total organic carbon.** The content of the organic soil was determined by a modified Turin's method. The method contains the oxidation with $K_2Cr_2O_7$ and $H_2SO_4$ at 125º C for 45 min. The determination of the content of nonoxidative organic carbon was measured by titration with $(NH_4)_2SO_4FeSO_46H_2O$, catalyst $Ag_2SO_4$ and indicator phenyl anthranilic acid [5, 6].

**The fractional composition of SOM.** It's chemical extraction procedure containing the following fractions: The first fraction determines the total content of humic and fulvic acids soluble in the mixed alkali solution of 0.1 M $Na_4P_2O_7$ and 0.1 N NaOH. The second fraction determines the quantity of humic and fulvic acids "free" and bounding with mobile forms of $R_2O_3$, soluble in the solution of 0.1 N NaOH.

**The absorbance of HAs.** Spectrophotometric analysis at different wavelengths estimates the extinction due to the absorption of light from the humic substances in the solutions. Wavelengths 465 and 665 nm recorded the extinction of the HAs adsorption spectra ($cm^{-1}$). The extinction depends on the dissolved organic carbon concentration. An index of the optical density of HAs, their degree of aromaticity and condensation of humic substances is the ratio $E_4/E_6$.

## Results and discussion

The study of the content and composition of soil organic matter in different fertilization variants of field experience shows that accumulation SOM is the most sensitive in the 2nd variant or 100% organic fertilization (OF). The accumulation of SOM covers the whole studied depth up to 40 cm, but its quantitative values are on the lower limit or medium class of SOM [7, 8, 9].

Figure 1 shows that variants the insoluble fraction of SOM-humin is predominant in all fertilization. This fraction increases with organic fertilization. In the variant of mineral fertilization tend shows the reduction of humin fraction. The variant of mineral and organic fertilization also have the reduction of humin compear to the control.

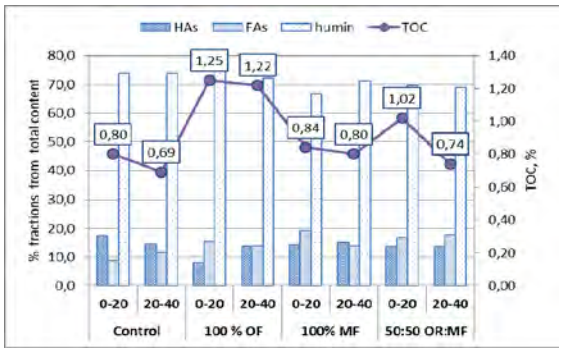



**Figure 1**. TOC, Humic acids, Fulvic acid and humin.

The predominant fraction of organic acids represented by the ratio of humic acids and fulvic acids are shown in Figure 2. The control is humic type on the depth of 0-20 cm to the fulvic - humic type up to 40 cm. The composition of SOM changes into a humic-fulvic type in all variants of fertilization [7, 10]. It was found an increase of fulvic acids in all fertilization variants for the 0-20 cm.

Figure 2 shows the second trend of an optical density of humic acids.

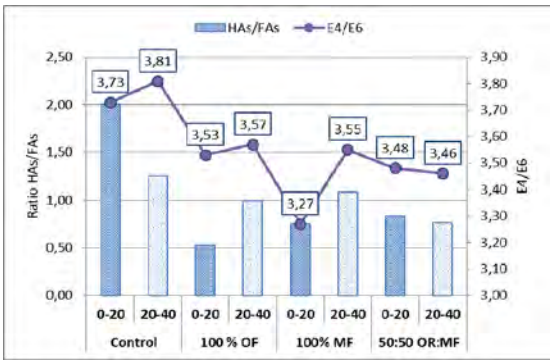



**Figure 2.** Ratio HAs/FAs and ratio $E_4/E_6$.

The tendency of SOM consumption is very clear. Irrespective of the type of fertilization, fulvic acids increase proportionately with the humic acids decreases. Therefore, the residual fraction humic acids show a higher optical density compared to the control without fertilization.

It has been established that the combined best introduction of manure and mineral fertilizers has the best effect on the quantity of production and quality parameters. The unilateral introduction of mineral fertilizers leads to the accumulation of nitrate at the end of vegetation, which after the fall is reduced [11, 12, 13].

The advantages of organic fertilization are mostly related to maximum exports of nitrogen, phosphorus and potassium. As a drawback, the nutrient content in the soil and mainly in nitrogen, phosphorus: potassium in manure variants is reported [11, 12, 13].

The input of organic, mineral fertilizers or a mixture of them leads to changes in the content and composition of SOM in soil. The accumulation of SOM gives priority to organic fertilization in comparison to mineral fertilization. The composition of SOM is changed from humic type to humic-fulvic type whether fertilization is an organic, mineral or a combination of both. The consumption of SOM is associated with a reduction of humin and less quantity humic acids with higher density and aromatic. Our conclusion is that the better practice for soil health is using of mix organic and mineral fertilization instead of alone variants.

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# Application of biological activators and organic fertilizers in a pot experiment with marigold (*Calendula officinalis L.)*

D. IVANOVA [(a)*], Z. PETKOVA [(b)]

(a) Institute of Soil Science, Agrotechnology and Plant Protection "Nikola Pushkarov"
7, "Shose Bankya" Str., 1080, Sofia, Bulgaria
(b) Agricultural academy, 30 Suhodolska Str., 1373 Sofia, Bulgaria
* E-mail: dimkaivanova19@gmail.com

**Keywords:** Calendula officinalis L., organic fertilizers, biological activators-Aminosol and Amminostim bio

**Abstract.** A pot experiment was carried out in 2016-2017 of the medicinal plant *Calendula officinalis L.* on different soil types - Leached Vertisols from the region of Bozhurishte and Fluvisols from the region of Tsalapitsa. This study investigated the effects of soil improvers in comparison to the traditionally used manure. The combination of soil and foliar fertilization is a practice that ensures optimal plant nutrition and high quality of the biologically active substances of marigold. So it is shown that the examined new organic fertilizers (Humipromoter and Biopromoter) in combination with 2 types of foliar bio stimulators liquid fertilizers containing amino acids (Aminosol and Amminostim bio) give a positive effect to plant growth. The ANOVA analysis for yield of biomass (g/pot) indicates that the influence of soil factors and different fertilization treatments is statistically significant at a variation level P <0.1%. Data variation due to soil variation is 44,9%, and for different types of fertilizers - 21,8%. The yields obtained from the soil type Leached Vertisols are nearly twice as high as those of the Fluvisols, as the differences are proved at P <0.5%.

## Introduction

The study was carried out with the medicinal plant *Calendula officinalis L.* of the Asteraceae family, which is approved as herbal medicine and homeopathic ingredient in the European Union [1]. Marigold is approved in the United States for use in cosmetics, food supplements, homeopathic medicines, and is generally recognized as safe for use in foods (GRAS § 182.10) [1]. In Bulgaria, it is used in herbal supplements and ointments. In recent studies on the medicinal properties of marigold antiviral [3], anti-tumor [5] and anti-inflammatory effects, have been reported. The use of organic fertilizers in the cultivation of medicinal plants is an alternative to improving phytochemical quality.

Compost and organic fertilizers are applied to improve soil fertility, pest prevention and pest control in many agricultural systems [4]. Biostimulators are defined as a substance that contains living microorganisms and is known to promote growth of the root system and better seed germination.

The aim is to evaluate the efficiency of the newest improvers (Humipromoter and Biopromoter) of soil in comparison with the traditionally used manure and establish their effect on plant production. It is important also to clarify the influence of the combination of these 3 organic fertilizers with 2 foliar biostimulators on growth of plants during the vegetation.

## Materials and methods

The medicinal plant *Calendula officinalis L.* was tasted on two soil types - Leached Vertisols from the region of Bozhurishte and Fluvisols from the region of Tsalapitsa. The organic fertilizers Humipromoter and Biopromoter consist of poultry and farmyard manure, compost of plant origin containing natural enzymes (alpha-amylase proinsymes, beta-amylases, pentosanases, lipases, glucoamylases, celluloses, lactases, proteases, phosphorylase, hemicelluloses, pectinases, etc.), microflora selected from controlled fermentation, biostimulators of natural origin, organic nitrogen (N) 3%. There are treatments with a combination of the mentioned 3 different organic fertilizers with 2 foliar bio stimulators - Aminosol and Amminostim bio. The amino acids are biological substances that stimulate metabolism and metabolic processes by increasing yield [2]. The soil improvers were compared with the 2 treatments - 1) with manure (0.65 % org. N) and 2) with NPK (ratio NPK = 1:2:1). The experiment comprised 11 treatments of fertilization in 3 replicates: T1) Control; T2) NPK; T3) Humipromoter; 4) Humipromoter + Amminostim bio; T5) Humipromoter+Aminosol; T6) Biopromoter; T7) Biopromoter+Amminostim bio; T8) Biopromoter + Aminosol; T9) Manure; T10) Manure + Amminostim bio; T11) Manure + Aminosol.

The rate of fertiliser application are calculated according to N.100 $mg.kg^{-1}$ soil in order to create the same conditions in different treatments. The rate of Aminosol (9% org.N) is 0.01 ml/pot and of Amminostim bio (6% org.N) is 0.015 ml/pot, spraying 3 times during the vegetation in 2 weeks. The calculations for foliar fertilizers also is made according to the quantity of N contained therein.

The ANOVA analysis for obtained data of plant biomass was made.

**Soil agrochemical analyses.** In soil samples taken before seeding and after harvesting of marigold, the following agrochemical analyzes were carried out: determination of pH, humus, total N, mineral N, exchange $P_2O_5$ and $K_2O$.

## Results and Discussion

The agrochemical properties of Leached Vertilsol are more favorable, innorganic N is 20 $mg.kg^{-1}$, 21 and 50 $mg.100\ g^{-1}$ mobile $P_2O_5$ and $K_2O$, total N of 0.165% and humus of 4.38%. These values for Fluvisol are lower -

innorganic N 8 mg.kg$^{-1}$, 9 and 22 mg.100 g$^{-1}$ mobile $P_2O_5$ and $K_2O$, total N of 0.102% and humus of 2.24%.

We observed that the quantity of innorganic N in the fertilized treatments is higher than in the control in the two soil types as shown in Fig.1 and 2.

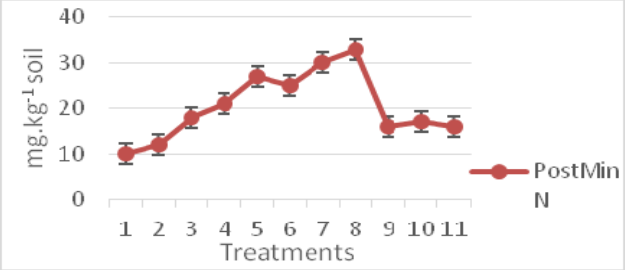



**Figure 1**. Quantity of inorganic N after harvesting of plants in **Leached Vertisols** (mg.kg$^{-1}$ soil).

The results of the analyzes show that Humimopter and Biopromoter provoke the availability higher quantity of mineral nitrogen compared to manure. Biopromoter is better than Humipromoter to the formation of innorganic N. Obviously the complex of specific microorganizms which is added with the organic fertilisers increases mineralization processes in the soil. The curve in Fig. 2 forms the peaks in the treatments 7 and 8, comprising Biopromoter plus fertilizers with amino acids.

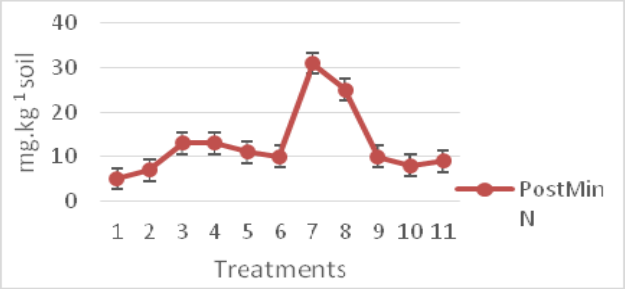



**Figure 2**. Quantity of inorganic N after harvesting of plants in **Fluvisols** (mg.kg$^{-1}$ soil).

On Fig. 3 the total nitrogen value at the end of the experiment differs for both soils – in Fluvisolsl is almost unchanged, but in Leached Vertisols vary more strongly. The treatment with Biopromoter have the highest quantity of total N.

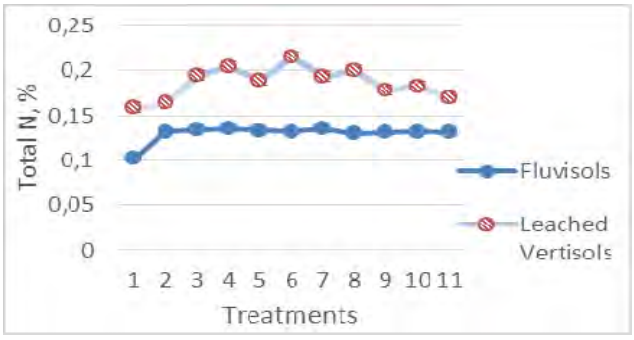



**Figure 3.** Quantity of total N at the end of the experiment in **Fluvisols** and **Leached Vertisols** (%).

Foliar bio stimulators with amino acids (Aminosol and Amminostim bio) give a positive effect to plant growth (fig.4). The ANOVA analysis of the yield of the plant biomass (g/pot) indicates that the influence of soil factors and different fertilization variants is statistically significant at a variation level P <0.1%. Data variation due to soil variation is 44.9%, and for different types of fertilizers - 21.8%. The yields obtained from the soil type Leached Vertisols are nearly twice as high as those of the Fluvisols, as the differences are proved at P <0.5%.

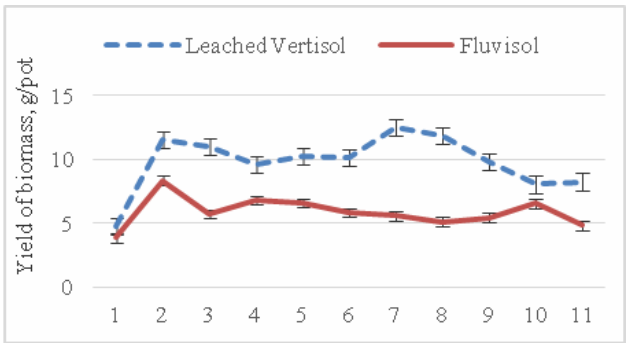



**Figure.4.** Yield of Marigold – air dried plants in g/pot under conditions of pot experiment (11 treatments).

The application of various organic and inorganic fertilizers in the cultivation of *Calendula officinalis L.* shows a pronounced effect on the yield. The results of the values of min. N and total N of both soil types after harvesting are indicative of their increase in tested variants with added new soil improvers Humipromoter and Biopromoter in combination with foliar fertilizer Amminostim bio and Aminosol. Therefore we could conclude that the above-mentioned fertilizers are highly efficient.

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# Fraction composition of humus compounds and optical properties of humic acids as indicator of quality of soil with biochar amendment

M. MIERZWA-HERSZTEK [(a)*], K. GONDEK [(a)] , A. BARAN [(a)]

(a) Department of Agricultural and Environmental Chemistry, University of Agriculture in Krakow, al. Mickiewicza 21, 31-120 Krakow, Poland
* E-mail: monika6_mierzwa@wp.pl

**Keywords:** biochar, humus compounds, soil, miscanthus straw

**Abstract.** The aim of this study was to evaluate the effect of the addition of miscanthus straw and miscanthus straw biochar (in rates 1% and 2%) to soil on quantitative and qualitative parameters of humus compounds. It was found that after applying 1% and 2% of biochar (MSB) to the soil, the content of Corg in the soil significantly increased compared to the same doses of non-pyrolysed straw (MS). Changes in the optical density of humic acids solutions indicated that humic acids in the soil of treatments amended with biochar in 1% and 2% doses, were characterised by lower A 2/6 and A 2/4 ratios, compared to control treatments.

## Introduction

Due to the indisputable significance of humus in many biochemical processes as well as its increasing deficit particularly in light soils, alternative sources of substrates for the reproduce of this constituent should be sought. Considering the physical, chemical and biological stability, the solid product of thermal transformation of biomass called biochar may be such a source [1, 2].

The use of different types of exogenous organic matter, such as biochar, has a great potential in stabilising humus content in soil [3, 4]. Frequent literature references to quality standards of humus and the soil itself do not describe individual factors such as quantitative and qualitative parameters of humus compounds. Identification of changes to biochar, taking account of the above aspects may play a key role in detoxification and restoration of soil fertility. The aim of this study was to evaluate the effect of the addition of miscanthus straw and biochar obtained from this biomass to soil on quantitative and qualitative parameters of humus.

## Materials and methods

B**iochar production.** The plant material (miscanthus straw) was pyrolysed at 300°C, for 15 minutes, under a limited supply of air [5]. The process parameters were configured so as to obtain the lowest possible losses of carbon [2].

**Experiment design and soil sample collection.** The pot experiment was carried out on brown soil with the granulometric composition of light loamy sand (85% sand, 9% silt, and 6% clay). The experiment comprised 6 treatments carried out in 3 replications: soil without mineral fertilisers and organic materials (C), soil with addition of mineral salts (M), soil with addition of MS and miscanthus straw in doses 1% and 2% (M+MS 1% and M+MS 2%) and soil with addition of MS and miscanthus straw biochar in doses 1% and 2% (M+MSB 1% and M+MSB 2%). The control treatments were C and MS (which allowed to demonstrate the real effect of organic materials). The test plant was perennial ryegrass.

## Fraction composition of humus compounds

Content of humus compounds: the carbon content of humic acids (CHA) and fulvic acids (CFA), the content of carbon in the extract (Cext) and non-hydrolysing carbon (CNH) was determined by Kononova and Bielczikowa method [6]. Light absorbance was then measured in the obtained solutions of humic acids at the wavelength of 280, 465, and 665 nm and ratios (A 2/6, A 2/4, A 4/6) was computed. Organic carbon in soil and extracts was determined by oxidative-titrating method.

## Results and Discussion

### Organic carbon content and fractional composition of humus compounds

Regardless of the type of organic material and its dose, the content of Corg in soil increased significantly compared to C and M treatments. Also the content of Cext and CKH significantly increased in the soil of treatments with 2% additions of MS and WSB compared to the content determined in the soil of C and M treatments. Researchdemonstrated diversified dynamics of the transformations of organic substance supplied to the soil conditioned by fractional composition of humic substances and properties of humic acids. In the author's own investigations the content of fulvic acids carbon was bigger than the content of humic acid carbon, irrespective of the applied fertilization and dose of MS or MSB.

### The value of CHA:CFA ratio and optical parameters of humic acids extracts

It has been generally accepted that humus with a higher value of Cha:Cfa ratio is characteristic for more fertile soils [6]. The values of CHA:CFA ratio, regardless of the type of organic material and dose were relatively low (0.22-0.37). It was found that the larger addition of both WS and WSB resulted in lower values of A 2/6 and A 4/6.

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# Long-term low-input nutrition regime of haplic chernozems and its influence on the productivity of *T. AESTIVUM L.* cultivars

M. NANKOVA [(a)]*, V. SAVOV [(b)], K. CHAKALOV [(c)], E. FILCHEVA [(d)]

(a) Dobroudja Agricultural Institute, 9520, General Toshevo, Bulgaria
(b) Biological Faculty of Sofia University "St. Kliment Ohridski", 8. "Dragan Tsankov" Bld.
(c) Balkan Plant Science Ltd., 25A, Tsar Osvoboditel Bld, Sofia, Sofia, Bulgaria
(d) Institute of Soil Science, Agrotechnology and Plant Protection "N. Poushkarov"
* E-mail: nankova_margo@abv.bg

**Keywords:** wheat cultivars, mineral and organic fertilizers, low-input nutrition, sowing material treatment (SMT)

**Abstract.** The annual soil fertilization with organic and mineral fertilizer contributed to higher productivity of wheat under unfavorable parameters of agronomy factors (previous crop and no nitrogen nutrition prior top, or at the beginning of permanent spring wheat vegetation). The sowing material treated (SMT) with a humic preparation Plantagra Average (2014-2017) and all trail variants contributed to receive extra 446.4 kg/ha grain yield. For cultivars Nahodka, Pchelina, Rada and Tina these values are between 673.8 and 537.6 kg/ha. Nearly 50% from the tested common wheat cultivars are suitable for growing with the above "low-input" practices.

## Introduction

Intensification and industrialization of agriculture in recent decades has led to great increases in food production but also to numerous undesirable environmental impacts such as biodiversity loss, soil degradation, and soil, water, air, and food pollution [1, 2, 3]. Integrated Pest Management (IPM) principles are combined in innovative Low-Input Cropping Systems (LI-CS) to preserve a realistic and systemic management strategy and to study carry over effects of management strategies. This holistic approach takes into account the complexity and functioning of agro-ecosystems. LI-CS approach allows for multiple combinations of IPM techniques [3, 4, 5, 6]. This high-input system includes herbicides, witch represent high percent of the total quantity of pesticides, mineral N fertilization that represents 30% of the total GHG emissions in maize production and other agricultural practices [7].

A fundamental goal of alternative agriculture including organic and low-input farming systems is to reduce non-renewable resourse use and environmental degradation while maitaining productivity and profitability [8].

The aim of this investigation was to follow the response of the new common winter wheat cultivars developed at Dobrudzha Agricultural Institute, under conditions of continuous monoculture cultivation and low-input technology using different ways for application of nutrients.

## Materials and methods

The investigation was carried out in a stationary field trial with three replications in the experimental field of Dobrudzha Agricultural Institute – General Toshevo on Chernozem. The field has been used for growing of continuous mono crop since the year 2000. The trail involved a total of 11 new common winter wheat cultivars. The cultivars were grown against 4 backgrounds of mineral fertilization: 1. Untreated control; 2. Superfos (350 kg/ha); 3. Superfos + Excel$^{Orga}$ (175+175 kg/da) and 4. Excel$^{Orga}$ (350 kg/da). Superfos is a granulated combined fertilizer containing 12% N; 24% $P_2O_5$; 0.5% Mg; 14% Ca; 25% S. Excel$^{Orga}$ (Triple-Action Fish-Guano Organic Fertilizer) is an organic fertilizer produced by Angibaud & Specialites, France. The sowing material was treated with the tested humine preparation Plantagra, produced by ROMB Ltd – Sofia (SMT).

## Results and Discussion

The results obtained from the variance analysis showed that the effect of the all factors on the yield was significant to a maximum degree during the investigated years in two variants - without and with SMT with Plantagra.

The values of the meteorological elements in the years of investigation significantly differed between themselves. The meteorological condition of 2016 through growth season of wheat ranked the best following year 2017. In these two years we received the highest yields (Figure 1).

The SMT lead to yield increasing average for the tested period with 15.03% that is 481.9 kg/ha.

The meteorological condition of the years are the main determining yields factor. Its average strength of effect is 66.15%.

The established agronomic effect of the application of the fertilizers was significantly influenced by the way of introduction in all cultivars during the years of investigation. The annual main soil fertilization with Suprefos contributed to higher productivity of wheat - average for trail with 24.56% for period 2014-2017 (Figure 2).

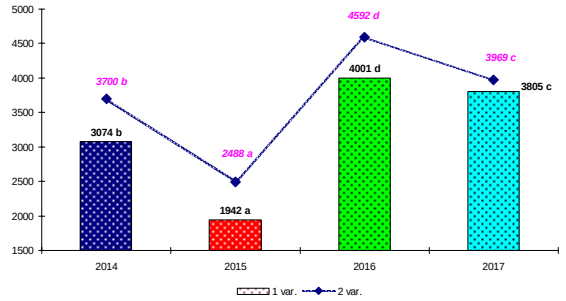



**Figure 1.** Mean productivity by years in variants without and with SMT-Plantagra, kg/ha.

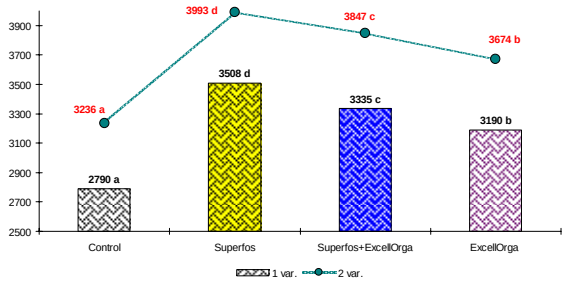



**Figure 2.** Mean productivity of wheat cultivars according to kind of fertilizer without and with SMT-Plantagra, kg/ha.

Under conditions of permanent drought during the grain filling stage, the combined application of main soil fertilization with the organic Excel$^{Orga}$ and SMT with Plantagra contributed to 15.17 % increase of the mean wheat productivity, or 484.0 kg/ha Averaged for all variants of the trial the strength of fertilization as a factor is 7.93%.

It was found that under unfavorable parameters of some main agronomy factors, such as previous crop and no nitrogen nutrition prior top, or at the beginning of permanent spring wheat vegetation, the choice of a suitable variety was important for the formation of productivity. Significant redistribution in the strength of effect of the tested factors on the productivity of the wheat cultivars was found. The strength of effect of the Genotype as a factor is 8.51% average (Table 1).

**Table 1.** Mean productivity of wheat cultivars, kg/ha

| Genotypes | Without SMT-Plantagra | With SMT-Plantagra |
|---|---|---|
| Bojana | 2976 b | 3279 a |
| Dragana | 3436 d | 3949 de |
| Enola | 3469 d | 3956 de |
| Kalina | 3676 e | 4057 e |
| Kiara | 3085 c | 3670 c |
| Kristy | 3442 d | 3939 de |
| Nahodka | 3382 d | 3924 d |
| Pchelina | 3137 c | 3516 b |
| Rada | 2822 a | 3248 a |
| Stoyana | 3056 bc | 3488 b |
| Tina | 2778 a | 3535 b |

Maximum average yield value was obtain from cv. Kalina - 3676 kg/ha without SMT and 4057 kg/ha with SMT. Average for the period and all trail variants SMT contributed to receive extra 446.4 kg/ha grain yield. For cultivars Nahodka, Pchelina, Rada and Tina these values are between 673.8 and 537.6 kg/ha. Nearly 50% from the tested common wheat cultivars demonstrated high productivity in combination with very good physical properties of the grain. These varieties are suitable for growing with the above “low-input” practices for plant nutrition.

Varietal response was highly expressed at all levels. The surplus from SMT with Plantagra to the control for each cultivar ranged from 303.0 kg/ha (cultivar Bojana) to 757.0 kg/ha (cultivar Tina).

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# Detritus role in soil organic matter formation

I. PACHEV [(a)*]

(a) Institute of Viticulture and Enology, Pleven, Bulgaria
* E-mail: Iv_Pachev@abv.bg

**Keywords:** humus, detritus, organic matter, soil, true humic substances

**Abstract.** The humus losses under soil cultivation have attained particularly great size during the last years which reflects on agricultural production. Therefore in both theoretical and applied soil science great attention is always paid to the problems of organic part of soil and its main specific component, humus. The practical application of this problem has increased still more during the last years because of the great humus loss from the cultivated lands where the complex of measures for its conservation has not been applied. Therefore naturally the interest for study of the organic matter nature and properties, the mechanism of its formation and transformation under agricultural use of lands becomes stronger.

An attempt is made to show the place and systematize the role and content of the detritus in the soil.

## Introduction

Soil humus is a dynamically open system that is constantly being renovated and always in a thermodynamically equilibrium with environmental conditions. The plant and animal residues transformation leads to accumulation of enormous amounts of bound organic carbon. The soil inclusion in the crop rotation changes the processes of humus formation and the soil organic matter quantity and quality. As a result of the virgin soil upturning, the organic carbon content of the fallow lands drops down sharply, and the humus systems gradually stabilize and reach a new equilibrium state in line with the changed environmental conditions. The soil organic matter loss is at the expense of the accelerated mineralization of its more labile components (detritus) upon the organic materials intake in the soils.

The organic part of the soil includes a wide variety of organic and organic-mineral compounds. Three groups could be distinguished in the organic matter composition:

1. Not decomposed plant residues and organic matter of animal origin.
2. Intermediate decomposition products.
3. True humic substances of a specific nature.

The first and the third groups differ greatly in their properties and significance in the soil formation processes and plants nutrition. The second (known as "Detritus") takes an intermediary position and it is difficult to be separated from the third one. These are the smallest particles of the organic residues that cannot be separated from the total mass of the soil. "Detritus" is called the dead organic matter that comes from the organism death or as a result of their vital activity.

The detritus serves as a food for various detrophages that are saprophytes and consume dead organic matters and decompose (or remineralize) the organic material.

Unfortunately, the studies on "detritus" are very scarce and there are almost no publications on this issue in the literature. Its composition and role in the soil organic matter, accumulation conditions, and further processing by soil microorganisms is even less known [1].

The true humic substances are primarily associated with the mineral part of the soil and are characterized by a certain resistance to microbiological decomposition.

By the analytical methods used, the soil substances are identified together with the humus and are included in the term "humus".

The plant residues, the intermediate products of their decomposition, and the newly formed humus substances have a high rate of transformation. For their determination, the "Reduction Test" method proposed determining the quantities of the most easily oxidized organic matter (detritus) for some basic soils in Bulgaria could be used. The analytical data showed that the highest rates were obtained for the surface horizons. That was the result of the readily oxidizable carbon, a constituent of the newly formed, low molecular organic compounds, product from the plant residues humification.

The period of their almost complete renewal was measured in months and not so often in years. It was that matter causing the significant biological activity of the soil as reported by Pachev [2], and participated in the soil structure formation and plant nutrition. The transformation rate of the fresh organic residues and the detritus were close. United them into one group, the group of the labile organic matter in soil (LOM) [3].

During the research it was found that the Alluvial-meadow soil had lower content of plant residues compared to the Chernozems and the mountain soils. That phenomenon could hardly be explained by the assumption that smaller amounts of plant residues are entering these soils than the rest ones.

The carbonate Chernozem formation was related to the steppe vegetation, distinguished for its powerful and deeply penetrating root system. It underwent decomposition and humification under the conditions of moderate humidity and moderately high temperature.

In the region of the leached Chernozems the climatic conditions were favourable both for the development of the steppe and wood vegetation. Due to the better wetting conditions, the steppe vegetation developed powerful root system, leaving plant residues at

a greater depth, resulting in a more potent humus horizon compared to the one of the carbonate Chernozems.

The favourable conditions (temperature and relatively high precipitation) enhanced the decomposition and humification processes of the plant residues Firg. 1.



Figure 1. Picture of Leached Chernozem, depth 0 - 20 cm, corn, detritus in process of decomposition. Magnification x 100.

The hydrothermal regime in the pseudo-podzolic soil showed that a sufficiently intensive microbiological activity was possible from the end of April almost to the second half of October. The decomposition and humification of plant residues occurred throughout this period in the presence of surface over-wetting of the soil that prevented the condensation processes.

The decomposition and humification process in the Brown forest soil was delayed by the unfavourable conditions. The hydrothermal regime was almost identical with the pseudo-podzolic soil. Considering the altitude and the relatively low temperatures in the spring and autumn, the period of microbiological activity was short but rather intensive because of the good moisture of this soil profile and the fine natural drainage.

The mountain-forest dark-colored soil had a similar hydrothermal regime as the Brown forest soil but with a rather shortened favourable period lasting only about 2-2.5 months of the year and very depended on the altitude.

The studies revealed that, compared to wheat and corn, alfalfa determined the formation of significantly higher amounts (0.35-1.10%) of humus in the surface 0-40 cm by increasing the total amount of carbon in the soil and improving the humic acids quality. In all soil types alfalfa significantly influenced the increase in the relative amount of humic acids (6-61%), indicating that the humus-formation processes were strongly affected by the available amounts of nitrogen-containing organic compounds in the soil. In alfalfa there was a clear tendency of increasing the plant residues by 50-80% in all types of soil at both depths (0-20 and 20-40 cm). Alfalfa raised the content of detritus by 40-60% compared to wheat and corn as it reached and was almost equal with the rates of the fallow [4, 5].

Finally, it could be concluded that the detritus content in the main soil types of Central North Bulgaria under different types of land use has been studied compared to the fallow land as a natural ecosystem. The impact of different land-use systems and crops on the nature of the organic matter has been established. A model has been proposed to optimize the amount of labile groups of soil organic matter in order to maintain a high level of soil fertility. The influence of the main climatic and meteorological peculiarities on the dynamics of the plant residues and detritus transformation in the nature and the character of the humus systems has been traced.

Unfortunately, the agronomic role and importance of detritus in the soils had not been well studied as a component of the soil organic matter, where the genetic nature of the humus formation process should be reflected.

It is necessary to be studied the subtypes of Chernozem, Dark gray, Dark brown and other soils being cultivated for agricultural production, where humus reduction is at the expense of the detritus mineralization.

The detailed study of the detritus and its involvement in the process of the soil structural formation could give us an answer how to influence the unstructured soils to improve their water and air and nutritional regime. In order to avoid low humus and soil degradation, it is important the role and function of the labile forms of organic matter that ensure the nitrogen deficiency of plants to be investigated.

The effective soil fertility regulation is done by providing sufficient quantities of labile forms of organic matter. Thus the mineralization of the true humic substances would be prevented, and the level of their absolute quantities would be increased.

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# Test of organic foliar fertilizer made from composted plant residues with manure

Z. PETKOVA [(a)*], D. IVANOVA [(b)]

(a) Agricultural Academy, 30 Suhodolska Str., 1373 Sofia, Bulgaria
(b) N. Poushkarov Institute of Soil Science, Agrotechnology and Plant Protection, 7, Shossee Bankya Str., 1080 Sofia, Bulgaria
* E-mail: petkova17@yahoo.com

**Keywords:** rucola, wheat, foliar fertilizer "Extra Force", plant residues, manure

**Abstract.** "Extra Force" made from composted manure - 63% and plant residues -37% has been tested in a pot experiments with rucola (*Eruka sativa, L.)* and wheat (*Triticum Aestivum, L.*). The fertilizer is obtained by extraction of humic substances from a bio-compost under appropriate conditions (pH, temperature, homogenization) allowing for their better extraction. It is found that the tested fertilizer is a valuable product that has a beneficial effect on rocket salad and wheat growth. A major component is humate that supply physiologically active substances which are involved in plant growth. It is established the increase in the yield from rucola as a result of applied foliar feeding with organic fertilizer which is about 7.23% in the treatments with low norm (1000 ml.ha$^{-1}$) and increases with an increase in the fertilization norm (3000 ml.ha$^{-1}$) to 19.41%. A similar trend is also established for the second crop - wheat. Biomass of wheat increases sequentially with an increase the norms of fertilization.

## Introduction

Together with traditional organic fertilizers, new organic fertilizers in liquid form have become more and more popular [1]. They are mainly used for foliar feeding because, in addition to easily digestible nutrients, they also contain biologically active components. The importance of foliar fertilization is that the nutrition of plants is optimized. The direct absorption from the leaves of the plants for their metabolic and energy needs make possible do not burden the soil. With proper and timely use, foliar fertilizer reduces the effects of adverse factors: abiotic - drought, cold, over-humidity, etc. and biotic - an attack by diseases and enemies. It could help to overcome also the stress caused by applied chemical preparations for plant protection.

The purpose of the study is to test the effectiveness of organic foliar fertilizer. It is obtained by aqueous extraction of humic substances from a bio-compost under appropriate conditions (pH, temperature, homogenization) allowing for a more complete extraction.

## Materials and methods

The vegetation tests with rucola (*Eruka sativa, L.)* and with wheat were set and sew on 22.09.2016. The starting soil for both cultures is Epicalcic Mollic Vertisol (WRB 2006) [2] from the experimental field in Bozhurishte. The treatments are: three-leaf foliar feeding with 3 rising rates of foliar organic fertilizer compared to a control treatment (without fertilizing) and a treatment with the addition of ammonium nitrate, triple superphosphate and potassium sulphate in N: P: K ratio = 1: 0.8: 0.8.

There is also a treatment with the introduction of the average rate of fertilizer direct into the soil for optimum homogenization of the fertilizer with the soil. The pots have a capacity of 1,800 grams soil.

The plants were grown for 30 days for the rucola and 150 days for the wheat, after which the yield of fresh and dry biomass was recorded. The dry content of the leaves is determined by weight. Foliar fertilizer is applied at a concentration of 2 ml/l.

Feeding was performed with 50, 75 and 100 ml solution per a pot, and these amounts were calculated according to 1000, 2000 and 3000 ml.ha$^{-1}$ rates. Spraying solution was done each 7 days during the vegetation and started 2 weeks after sowing rucola and each 10 days during the vegetation of wheat.

## Results and Discussion

The obtained data of the amount of mineral nitrogen (ammonium and nitrate) and the available forms of phosphorus and potassium at the beginning and the end of the rocket salad test show, that the Epicalcic Mollic Vertisolis was slightly affected by the applied foliar fertilization (treatments 3, 4, 5 and 7). The amount of mineral nitrogen varies from 45.8 to 62 mg/kg with a predominant amount of ammonium form of nitrogen at the start of the experiment and from 33 to 58 mg.kg at the end of the rocket vegetation.

This is logical and expected, because the total amount of nitrogen in the manure is not high - 0.2%. The amount of mineral nitrogen is highest (334 mg/kg) in treatment 2, where mineral fertilization with nitrogen, phosphorus and potassium is applied.

The proportion of ammonia nitrogen (268) in the total amount of mineral is higher about 4 times, and the amount of nitrate is 66 mg/kg. This tendency is maintained at the end of the rocket, respectively - 253 and 63 mg/kg. (Table 2). The $pH_{H2O}$ and $pH_{KCL}$ values indicate that there is no change in the different treatments, which supports the observed low response from the applied foliar feeding.

The Epicalcic Mollic Vertisolis is well stocked with available amounts of phosphorus and potassium. Poor variations are found in accessible forms of phosphorus - from 37.15 mg.100 g$^{-1}$ in the variant with low-norm of fertilizer up to 37.68 mg.100 g$^{-1}$ in those with the highest norm of fertilization.

The lowest is the quantity in control variant 36.33 and 36.03 mg.100 $g^{-1}$. Therefore, leaf feed with the test foliar fertilizer does not significantly affect the soil phosphorus content. Similar is the content of the available forms of potassium in the soil both at the beginning and at the end of the rocket vegetation. A similar trend is also found in wheat variants.

The increase in rucola yield as a result of applied foliar feeding with organic fertilizer is about 7.23% in treatments with low rate and increases with an increase in the fertilizer rate to 19.41% for treatments with the highest rate. The foliar feeding of the rocket with the average organic fertilizer rate gives almost the same results as those obtained for the treatments in which the liquid organic fertilizer was introduced into the soil (Table 1). The Rucola has a short vegetation period. In connection with the tendency of growing new and non-traditional crops and expanding and enriching the species composition of agricultural crops, it might be advisable for the Bulgarian producer to draw attention to such crops - fast-growing, easy to grow, containing very valuable nutrients and diversifying Human nutrition. Similar results have been found in other studies [3-6].

**Table 1.** Increase of yield of rucola in a pot experiment, 2016.

| Treatments | | Increase | |
|---|---|---|---|
| | g/pot | g/pot | % |
| 1 Control | 10.147 | | |
| 2. Soil + NPK - (N: P: K = 1: 0.8: 0.8) | 10.534 | 0.387 | 3.81 |
| 3. Soil + foliar spraying with liquid organic fertilizer - 1 norm | 10.881 | 0.734 | 7.23 |
| 4. Soil + foliar sprayed organic fertilizer - 2 norms | 11.222 | 1.075 | 10.59 |
| 5. Soil + foliar sprayed organic fertilizer - 3 norms | 12.116 | 1.969 | 19.41 |
| 6. Soil + sprayed with water | 10.413 | 0.266 | 2.62 |
| 7. Soil + soil intake - organic fertilizer - 2 norms | 11.305 | 1.158 | 11.41 |

**Table 2.** Increase of Wheat biomass in vegetation experiment. 2016-2017.

| Treatments | | Increase | |
|---|---|---|---|
| | g/pot | g/pot | % |
| 1 Control | 25.38 | | |
| 2. Soil + NPK - (N: P: K = 1: 0.8: 0.8) | 26.27 | 0.89 | 3.51 |
| 3. Soil + foliar spraying with liquid organic fertilizer - 1 norm | 28.78 | 3.4 | 13.40 |
| 4. Soil + foliar sprayed organic fertilizer - 2 norms | 31.71 | 6.33 | 24.94 |
| 5. Soil + foliar sprayed organic fertilizer - 3 norms | 34.01 | 8.63 | 34.00 |
| 6. Soil + sprayed with water | 27.67 | 2.29 | 9.02 |
| 7. Soil + soil intake - organic fertilizer - 2 norms | 32.4 | 7.02 | 27.66 |

## Conclusions

The organic fertilizer used as a compost extract (with source materials - 63% manure and 37% plant residues) is a valuable product that has a beneficial effect on plant growth and productivity.

The formulation allows direct application as foliar fertilizer. A major component is humates that contain physiologically active substances involved in plant growth. Supplying plants with them. especially during active vegetative growth. during flowering and fruiting. provides higher yields and high quality production.

Humates could improve the condition of the soil by increasing the humus content of the soil. They are also suitable for introduction into the soil through irrigation. drip irrigation and leaf-feeding plants. No phytotoxicity was observed at the study standards.

The increase in yield of rocket salad as a result of applied foliar feeding with organic fertilizer is about 7.23% in the low-rate variants (1000 $ml.ha^{-1}$) and increases with an increase of the fertilizer norm (3000 $ml.ha^{-1}$) to 19.41% for treatments with the highest rate. A similar trend is also found for biomass of wheat - about 13% for low-rate treatments. and increases sequentially with an increase in the fertilizer rate to 34% for treatments with the highest rate.

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# Sequestration of organic carbon (OC) in three agricultural soils by *in situ* photo-polymerization of humic molecules under biomimetic catalysis

A. PICCOLO [(a)*], R. SPACCINI [(a)]

(a) Centro Interdipartimentale di Ricerca sulla Risonanza Magnetica Nucleare per l'Ambiente, l'Agroalimentare ed i Nuovi Materiali (CERMANU), Università di Napoli Federico II, via Università 100, 80055 Portici, Italy
* E-mail: alessandro.piccolo@unina.it

**Keywords:** soil humus, carbon sequestration, SOM polymerization, photo-catalysis, iron-porphyrin

**Abstract.** Facing an exploding population growth with consequent increase of agricultural intensification new chemical technologies are being sought to limit organic matter losses. Here we report an effective carbon sequestration in different cropped soils of Italy is obtained by an *insitu* photo-oxidative coupling among soil humic molecules, when catalyzed under solar irradiation by a water soluble iron-porphryn catalyst. A 3-years long field study showed that the catalyst-assisted photo-polymerizatio of humic matter enabled a yearly sequestration of SOC ranging from 2.2 to 3.9 t $ha^{-1}$ $y^{-1}$, despite the soil distrubance due to conventional tillage.

## Introduction

The new awareness of the chemical and conformational arrangement of humic molecules in the soil matrix as supramolecular structures [1, 2] conveys the possibility of controlling the dynamics of humus by chemical technologies that would enhance SOC persistence. We showed that a free-radical driven coupling reaction among small humic aromatic molecules can be induced by a synthetic biomimetic metal-porphyrin catalyst [3]. The formation of covalent C-C and C-O-C bonds among humic molecules was found to occur also under solar irradiation and in the presence of both homogeneous and heterogeneous metal-porphyrin catalysts [4-7]. We thus conceived that an oxidative polymerization of SOM can be conducted directly *in situ* in soil by the activity of a biomimetic photo-catalyst under field solar irradiation. The catalytic formation of multiple covalent bonds among humic molecules and the consequent increase in chemical energy in SOM would result in a reduction of its bio-accessibility to microbial mineralization and in a concomitant OC accumulation in soil.

Here we applied the *in situ* photo-catalysed polymerization technology of carbon sequestration directly on different agricultural soils during a three years field experiment and followed the changes in SOC content.

## Materials and methods

**Catalyst.** A water-soluble iron-porphryn (FeP) synthesized in the lab as meso-tetra (2,6-dichloro-3-sulphonatophenyl)porphyrinate of iron (III)chloride [Fe-(TDCPPS)Cl] [3, 5].

**Soils and field sites.** Three different sites along an Italian North-South climate gradient were selected for field experiments at experimental stations of the Universities of Torino, Piacenza and Napoli. The field testswere conducted for three consecutive years on four replicates of 1x1 m plots in larger (7x7 m) plots cropped with wheat. Treatments were: 1. Control soils under conventional tillage and 130 kg $ha^{-1}$ of urea fertilizer; 2. As control but added with a water solution of FeP (10 kg $ha^{-1}$). After the cropping season, 1 kg of surface soil (30 cm) was sampled from both treatments, air dried and the OC content determined by elemental analyzer.

**Aggregate-size distribution.** Water-stable aggregates (WSA) were isolated from bulk soils by applying a wet sieving technique [5].

## Results and Discussion

The field-scale effectiveness in OC fixation in bulk soils and WSA by a water-soluble FeP is shown in Fig. 1 for three different sites under conventional tillage and wheat production for three consecutive years. The FeP tretment stored more OC in bulk soil in the 1st and 2nd year, while no OC increase was noted in the 3rd year. Conversely, the separate WSA showed OC fixation in the 2nd and also 3rd year. For the Piacenza soil, a significant SOC increase by FeP treatment was shown only for the 2nd year, while the SOC increase in the sum of WSA with photo-catalysis was significant for all three years. In the case of the Napoli soil, the significant enhancement of SOC by the catalytic photo-polymerization tretment occurred for both the 2nd and 3rd year for either the bulk soils or the sum of WSA. The differences in fixed OC among soils are likely to depend on the specific soil properties and local evapotranspiration rates and rainfall events. In fact, adsorption/desorption of the FeP catalyst adsorbedon the surface of soil aggregates determines its photocatalytic activity in the soil solution at the soil-atmosphere interphase, where maximum is the photo-chemical induction of natural light [7].

Despite the variability in OC fixation due to the heterogeneity of soil matrices, and site conditions, and inherent randomness of representative samplings, the carbon sequestered in bulk soil by the photo-activity of the FeP catalyst for three consecutive years reached an average of 3.54, 3.90, and 2.24 Mg $ha^{-1}$ $y^{-1}$ for the Torino, Piacenza, and Napoli experimental sites, respectively (Table 1). Similar or even larger OC fixation was reached also for the sum of WSA (Table 2). These significant yearly amounts of fixed SOC were achieved under conventional tillage (CT) management, whereas the current highly experimented no- (NT) or reduced tillage (RT) practices are far from reaching such a large OC increase in cropped bulk soils even after several years.

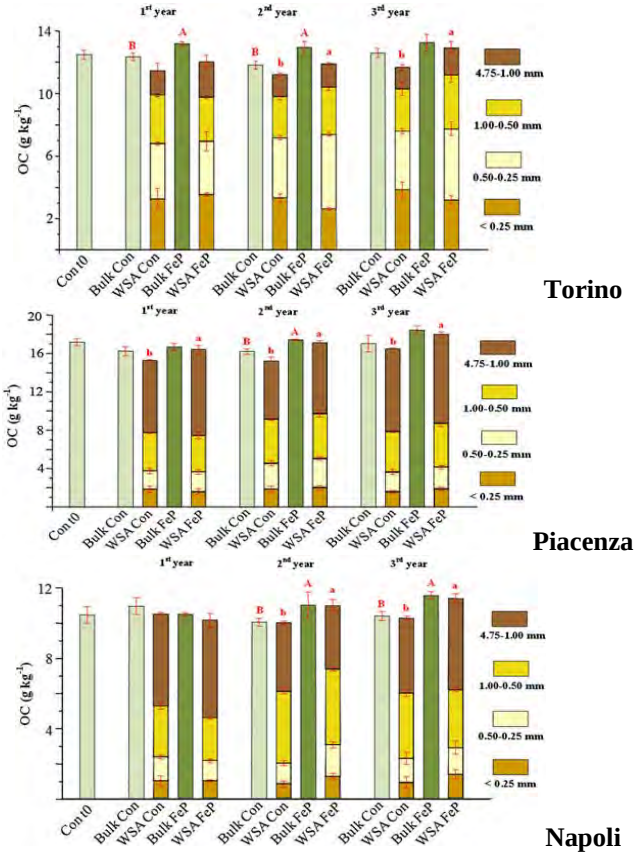



**Figure 1.** TOC content in bulk soils and sum of separated water-stable aggregates (WSA) for original (t0), control, and FeP-treated soils.

**Table 1.** Sequestered OC (mg $ha^{-1}$ $y^{-1}$) in bulk soils.

| Bulk soil | | | |
|---|---|---|---|
| 1st year | 2ndyear | 3rd year | Average |
| Torino | | | |
| 3.15* | 4.72* | 2.75* | 3.54 |
| Piacenza | | | |
| 1.56 | 4.68* | 5.46* | 3.90 |
| Napoli | | | |
| -2.10 | 4.20* | 4.62* | 2.24 |

**Table 2.** Sequestered OC (mg $ha^{-1}$ $y^{-1}$) in sum of WSA

| Sum of WSA | | | |
|---|---|---|---|
| 1st year | 2ndyear | 3rd year | Average |
| Torino | | | |
| 1.96 | 2.75* | 4.71* | 3.14 |
| Piacenza | | | |
| 4.29* | 7.41* | 5.85* | 5.85 |
| Napoli | | | |
| -1.26 | 4.20* | 4.62* | 2.52 |

*significant difference from control at $P < 0.05$ as for Tukey's test

This considerable SOM incorporation even under CT managemet was not due to a reduced exposure of SOC to microbial respiration, as in NT and RT management but rather to an enhanced chemical and biochemical stabilization of SOM achieved by the *in situ* catalyzed photo-oxidative coupling among humic molecules. The phenolic or other molecules susceptible to undergo the catalyzed coupling reaction in soil [5, 7] are likely to originate from the large mass of carbon moieties circulating in the soil solution during and after the crop growing season. These may be either soluble humic molecules in equilibrium with those adsorbed on soil particles, or lignin monomers released from heterotrophically respired crop residues, or exudates of plant roots, or their transformed metabolites by the intensified microbial metabolism.

All that soluble carbon in the soil solution may periodically become the substrate of the polymerization reaction at the soil-atmosphere interphase, under the photocatalysts, that is concomitantly desorbed in the soil solution from the surface of soil particles. This photo-oxidatively coupled carbon in catalyst-treated soils can thus be removed from solution and protected from microbial mineralization due to a thermodynamic shift, referred to as the "hydrophobic effect" [5], towards adsorption on aggregates surfaces. Conversely, in untreated control soils, most of the OC present in the soil solution is rapidly consumed by microbial metabolism and released as $CO_2$ to the atmosphere. In fact, these results indicate that a significant part of OM molecules is fixed in soil each year due to the FeP treatment, and is, thus, subtracted to the OC losses that occur in control soils.

Here we showed that the innovative in situ photopolymerization of soil humic molecules under FeP catalysis was highly effective in fixing OC from 2.24 to 3.90 mg $ha^{-1}$ $y^{-1}$ more than control soils.

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# Study of the influence of peat pre-processing on hydrodynamic characteristics of peat humates

S. SELYANINA [(a)*], A. ORLOV [(a)], I. ZUBOV [(a)], O. YARYGINA [(a)] , V. TATARINTSEVA [(a)]

(a) N. Laverov Federal Centre for Integrated Arctic Research, Severnoi Dviny Emb., 23, 163000, Arkhangelsk, Russia
* E-mail: gumin@fciarctic.ru

**Keywords:** peat humates, hydrodynamic diameter of particles, intermolecular interactions

**Abstract.** Particle size distribution in the aquatic solutions of humus biopolymers, extracted from high-moor peat, was studied involving the method of dynamic diffusion of light. The essential influence of the peat pre-processing on hydrodynamic characteristics of the aquatic solutions of peat humates was established. Condensing oxidative processes carry during sample drying and that should be taken into account while researching the structure and the physicochemical properties of peat and peat humates.

## Introduction

Organic substance accumulation in the crust as a result of drastic reductive-oxidative transformations of plants residues is essential prime of synthesis of solid combustible.

Humic acids (HA) - polymolecular polydisperse compounds are master components of peat. Herein humification conditions are decisive in formation of composition, structure and physicochemical characteristics of HA (particularly its ability of interaction by reductive-oxidative, ion-ion, adsorptive, donor-acceptor mechanisms and tendency of organization of supermolecular features) [1, 2].

According to the last researches [3,4], physicochemical properties of peat humates could largely depend on presence of minor low-molecular dirt, particularly bitumen components. To the contrary, researchers seem to be insensitive to the influence of peat pre-processing, such as drying and debituminizing, on peat properties.

One of the essential parameter which should be introduced is hydrodynamic diameter of particles. It is determined by not only composition, structure and functional nature of higher-molecular components but also the presence of low-molecular dirt. The high-moor peat and its components (especially humic substances) which participate actively in capillary-colloidal structure formation of peat could be the example of such systems.

Thereby the purpose of the work is to study the influence of peat pre-processing on hydrodynamic characteristics of extracted HA.

## Materials and methods

### Experimental

The objects of the research: the aquatic solutions of the peat humates that was stripped from the samples of high-moor peat. The latter were sorted by formation depth (0-10, 25-45, 100-110 и 143-150 cm), then debituminized with petroleum-ether and finally processed with alkali - 0,1 N NaOH. Sampling area – Arkhangelsk region, Mezen district, 65°52′8.2″ n.l., 44°14′44.3″ e.l.). Peat humidity was modified to air drying and averaged. The samples were sorted to various stages of water removal:

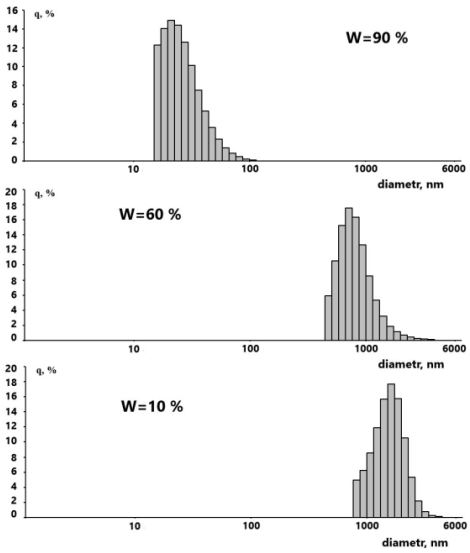
a. after mechanical pressing (85–86 %),
b. after 2 days (54–64 %),
c. after 10 days (17–24 %),
d. air-dry samples (10–12 %).

The aquatic solutions of peat humates were purified with dialysis using cellophane until pH is 7.5-8.0. Then solutions were cut back with di$H_2O$ to concentration 100 mg/l before measuring operation. Particle size distribution in obtained humates samples was estimated with the method of dynamic diffusion of light using laser particle analyzer Horiba LB550.

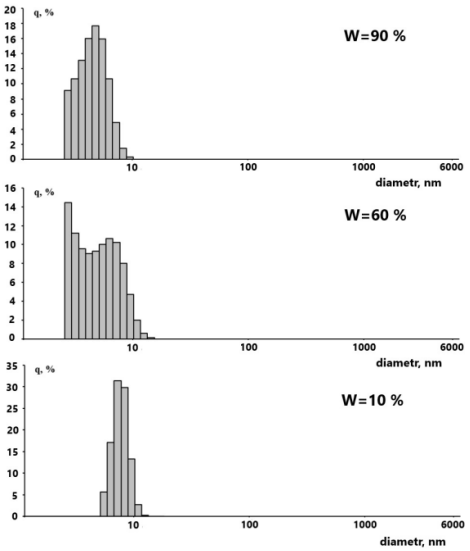
## Results and Discussion

Experimental data (Fig. 1) illustrate the influence of peat pre-processing on hydrodynamic characteristics of humic substances.

It is impressive to see that not only particle size but also its polydispersity change due to drying out. Older peat humates tend to decline polydispersity whereas humates of low-decomposed peat are opposite. Such effects could be explained primarily by proceeding processes of oxidative condensation. Peat bitumens, recovery degree of which rises while sample humidity becomes lower, could make some contribution to these processes. On the one hand, bitumens are able to prevent high-molecular components of peat from processes of oxidative condensation [3]. On the other hand, sodium salts of bitumens components dissolve into the solution as well as humates. The sodium salts of bitumens are surface-active substances which are able to form micellar aggregates increasing samples polydispersity and decreasing hydrodynamic particles size. Such effect was described in work [5] as exemplified by colloidal systems of the lignin and extractive substances of wood.




a)




b)

**Figure 1**. Hydrodynamic characteristics of peat humates, extracted from peat with various humidity:
a) h = 0-10 cm; R = 10-15 %;
b) h = 143-150 cm; R = 35-40 %.

It is notable that humates, extracted from low-decomposed peat, are distinct in high degree of polydispersity. Even under conditions of low concentrations (100 mg/l) it tends to form huge supermolecular foundation (d = 800 – 4000 nm). Humus that was extracted from older peat within the analyzing concentrations are represented with certain macromolecules (d = 5 – 20 nm). This could be explained by specificity of its functional composition and structure that is stipulated by processes of more drastic biotransformation.

## Conclusions

To conclude, the results of the research demonstrate that the pre-processing operations of peat, particularly drying and debitumizing, effect on hydrodynamic characteristics of peat humates. The processes of oxidative condensation are recorded during samples preparation. This fact should be taken into account while studying structure and physicochemical properties of peat and its humates.

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# The effect of organic sludge application on the elemental composition and spectroscopic parameters of soil humic acids

K. SKIC [(a)], P. BOGUTA [(a)], Z. SOKOŁOWSKA [(a)], K. SZEWCZUK-KARPISZ [(a)]

(a) Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna str. 4, 20-290 Lublin, Poland
* E-mail: p.boguta@ipan.lublin.pl

**Keywords**: humic acid, organic sludge, elemental and spectroscopic analyses

**Abstract.** In this work, the structural properties of humic-like fractions isolated from organic sludge of different origin, and the humic acids isolated from soil treated at various rates of these sludge were evaluated. Humic acids isolated from organic sludge were characterized by a higher content of N and H and smaller degree of oxidation in comparison to the humic acids isolated from the soil. Application of organic sludge to the soil influenced changes in the adsorption parameters of soil humic acids. The transformation of humic acids structure was observed due to addition of weakly humified material of low molecular weight and further polimerization.

## Introduction

Nowadays, the application of sewage sludge to soil has become a common practice due to its significant quantity generated from municipal and agro-industrial sectors, reduction of available disposal sites and its potential to increase soil fertility. It is found that application of sewage sludge into the soil may improve the level of soil organic matter stability, biological activity, and nutrients content [1, 2]. However, before applicationsewage sludge requires an adequate control of the chemical quality and of the effects that these materials may have on the status, quality, chemistry and functions of the soil and soil humic substances [3, 4]. Uncontrolled and cyclic use of sewage sludge can lead to unfavorable changes in the soil environment associated with accumulation of harmful components e.g. heavy metals, pathogenic microorganisms or phytotoxic organic compounds [5, 6].

The objective of the study was to determine the elemental and spectroscopic parameters of humic acids developed over the decomposition of organic sludge in soils.

The research was based on approaches involving field applications. These allow us to gain information in real cropping system, which undergo to environmental variability.

## Materials and methods

The experiment sites were situated in the vicinity of Krasnystaw (South-East Poland). At this location, soil was classified as a *Dystric Cambisol*, according to the WRB classification (2014). Two doses (4.5 and 9.0 Mg dm·ha$^{-1}$) of organic sludge with different characteristics and origin (dairy wastewater treatment plant (DS), fruit industry (FS) and agricultural biogas plant (PS) were applied at the beginning of the experiment. During two years, soil samples were taken 7-times from the depth of 0-25 cm and 25-40cm.

The humic acids were isolated according to Schnitzer [7] procedure with Gonet [8] modification.

The elemental analysis (C, H, N) of humic acids was performed using a Perkin-Elmer CHN 2400 device. The oxygen content was calculated from the difference 100% - (H%+C%+N%). Atomic ratios H/C, O/H, O/C, and C/N and the degree of internal oxidation (expressed as $\omega = [(2O + 3N) - H] / C$) were calculated from the obtained data.

A UV/Vis V-530 spectrophotometer (Jasco Inc.) was used to obtain spectra of 40 mg·dm$^{-3}$ humic acid solutions adjusted to pH 8.3 [9]. The values of the absorbance measured at 465 and 665 nm allowed determining the $E_4/E_6$ ratio.

## Results and Discussion

Elemental data showed that fractions of humic acids derived from sludge were characterized by lower values of O/H, O/C atomic ratios and lower degree of internal oxidation in comparison to soil humic acids. These results (with atomic ratio H/C > 1.5) suggest a smaller degree of humification and the presence of weakly oxidized structures (low in O-containing and acidic functional groups) [10]. In addition, the sludge humic acid molecules were rich in N - containing compounds suggesting incorporation of protein decomposition products into humic acid formation during sludge stabilization process.

The highest changes of $E_4/E_6$ parameter were observed for humic acids extracted from soil with addition of digestate and dairy sludge whereas humic acids from soil fertilized with fruit sludge were affected in minor extent.

As shown in Fig. 1 the application of dairy sludge into the soil influenced the structure of soil humic acids in different extent, depending on soil layer. This transformation was related with addition of the organic material of a low molecular weight and weak polymerization or compounds that do not represent the feature of humic substances. Long-term impact of dairy sludge application caused decrease in humification degree of soil humic acids in the layer of 0-25 cm. This may indicate the beginning of the resynthesis process of the decomposed organic substrate to humic compounds.

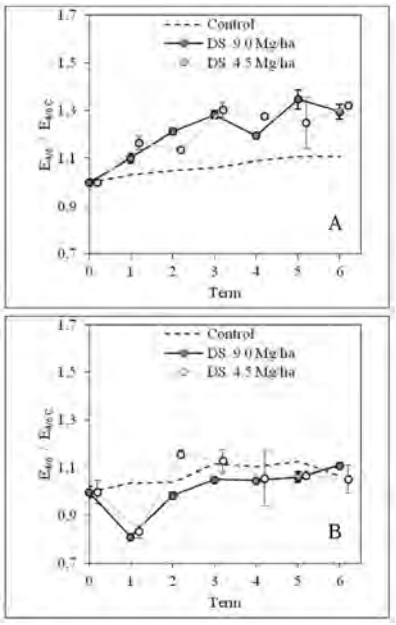



**Figure 1.** Relative changes in the $E_4/E_6$ ratio obtained for humic acids fraction extracted from soil fertilized with dairy sludge. A - soil layer of 0-25 cm, B - soil layer of 25-40 cm. Bars represent standard deviation.

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# Decomposition of green and rooibos tea in different soil types investigated by high-resolution NMR and FT-ICR-MS

Y. ZHU, A. SMITH, W. BEISHUIZEN, K. CHEN, D. FORSTER, Y. JI, L. KNOX, N. G. A. BELL[(a)*]

[(a)] School of Chemistry, University of Edinburgh, EH93FJ, Edinburgh, UK
* E-mail: Nicholle.Bell@ed.ac.uk

**Keywords:** NMR, FT-ICR MS, green tea, rooibos tea, NOM, tea bag index

**Abstract.** Understanding the composition of natural organic matter (NOM) is vital to our understanding of how soils form and respond to climate or land use changes. NOM is a complex mixture of organic compounds produced by the biotic and abiotic decomposition of plant and animal remains. The shear complexity of NOM hinders drawing structure-function relationships needed to explain these processes. In this work, the decomposition of green and rooibos tea, as a potential proxy for the processes taking place in soils, is analysed in different soils by high-resolution analytical techniques.

## Introduction

Understanding soil decomposition is vital to understand how soils form and respond to climate and land use changes. The main ingredient of soil to be considered here is the natural organic matter (NOM), the composition of which defines the structure-function relationships of soils. However, the complexity of NOM hinders the direct mapping of its compositional changes as a response to natural or anthropogenic factors. Tea, a less complex organic mixture, can be used as a proxy to characterise such changes and link them to different soil types.

The tea bag index is a simple method for assessing the decomposition and stability of different soils [1]. The method utilises the different decomposition rates of green tea and rooibos tea caused by different production process: green tea leaves are simply heated and dried, while rooibos tea leaves undergo an additional oxidation step. Thus chemically, in addition to metabolites from the plant itself, oxidation products are present in the rooibos tea.

This study aims to follow the molecular changes that occur in tea buried in different soil types utilising FT-ICR MS and NMR [2], thus going beyond the simple weight loss measurements.

## Materials and methods

**Sampling sites**. Altogether 5 sites were investigated. A raised peat bog sites located in the Red Moss in Balerno outside of the City of Edinburgh, Scotland. In the last 30 years, this damaged peat bog has undergone restoration. Two sites were examined, one damaged and one undergoing restoration.

A forest site located 100 m to the west of the Red Moss peat bog near Threipmuir reservoir, which is part of the Pentland hills regional park.

Two species-rich grassland sites (~20 m apart), located on the south side of the Pentland hills - both on the edge of arable plots used for scientific studies by Scotland's Rural College – were also investigated.

**Tea bag burial and extraction.** Eleven sets of rooibos (Lipton, EAN 87 22700 18843 8) and green tea bags (Lipton, EAN 87 22700 05552 5) were weighed and buried at the two-peat bog sites and the forest site, while 12 sets were buried at the grassland site in November 2018. One rooibos tea bag and one green tea bag were buried 15 cm apart, both at a depth of 8 cm. The GPS coordinates of each sample were recorded. The samples were collected after 96 days. Samples for NMR and MS analysis were prepared by hot water extraction. 150 mg of ground tea was heated to 80 °C in Milli-Q® water for 30 minutes using a microwave. The supernatants were filtered using glass fibre filters and freeze-dried for NMR and MS analysis.

**NMR spectroscopy.** For $^{1}$H NMR analysis (Bruker 600 MHz AVANCE III with a 5 mm TCI cryoprobe), 5 mg of each extract was dissolved in 600 µL of $D_2O$ (100%, Sigma Aldrich). A 1D NOESY based water suppression technique was used to collect the spectra accumulating 64 scans and using 9 s relaxation delay, 1 s acquisition time, 100 ms mixing time and pulsed field gradients (80 %) during the mixing time.

**FT-ICR MS.** 1 mg samples of tea extracts were prepared in 50:50 MeOH/$H_2O$ and diluted to a final concentration of 0.1 mg/ml. The analysis was performed on a 12 T SolariX FT-ICR MS (Bruker Daltonics) with an infinity cell. Negative mode ESI was used and the sample was sprayed at 200 µl/hour. Ion accumulation time and time of flight were set to 0.2 s and 0.6 ms, respectively. 200 transients at 4 MW were summed. Spectra of 3 unburied and 30 buried green tea samples were collected and calibrated using 12 known compounds. The signal to noise ratio > 5 and the assignment error threshold of ± 0.5 ppm was used. The peaks were assigned molecular formulae using python scripts written by W. Kew (https://wkew.co.uk/ftmsvisonline/) using the following elemental formula constraints 12C0-80 1H0-160 16O0-40 14N0-8 32S0-1.

## Results and Discussion

Only the results of green tea are discussed here.

### $^{1}$H NMR spectra of green tea

Exemplar $^{1}$H NMR spectra of green tea unburied and buried in different soils are shown in Figure 1.

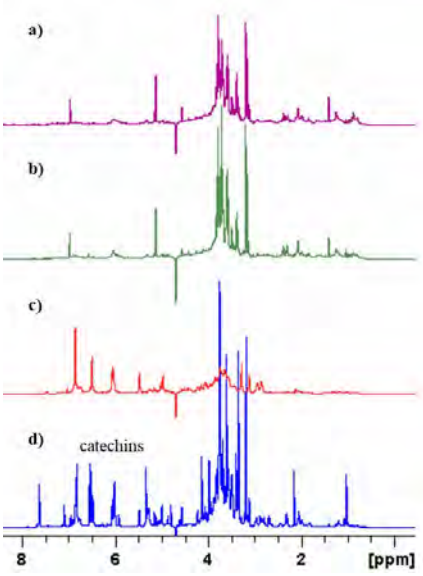



**Figure 1.** 600 MHz $^{1}$H spectra of tea buried in a) grassland, b) forest, c) peat bog and d) unburied green tea.

Green tea has been well characterized and many of the $^{1}$H NMR signals assigned [3]. These correspond to phenolics, terpenoids, alkaloids, fatty acids, amino acids, and carbohydrates. The intense peaks in the aromatic region are those of caffeine and catechins. In comparison, the spectra of the buried tea show clear differences. The similarity between the grassland and forest soil samples is evident, with signals of the catechins missing. To the contrary, these signals are present in the spectrum of the tea buried in the peat bog, indicating much slower decomposition rate in this soil type. Experiments characterising aggregation propensities of all samples are currently ongoing, together with the chemometric analysis of the $^{1}$H NMR spectra.

### FT-ICR-MS analysis of green tea

Using the molecular formulae assigned to each MS spectra, van Krevelen plots relating the C/H *vs* O/C ratios were created (Figure 2). The bubble size represents a formula's relative intensity within each site and is not comparable between the sites.

As demonstrated by Figure 2, the complexity increased and relative abundance of compounds changed upon burial. Principal component analysis of the MS data showed clear differences between the unburied samples and samples buried in the peat, while the grassland and forest sites grouped together in one area of the score plot.

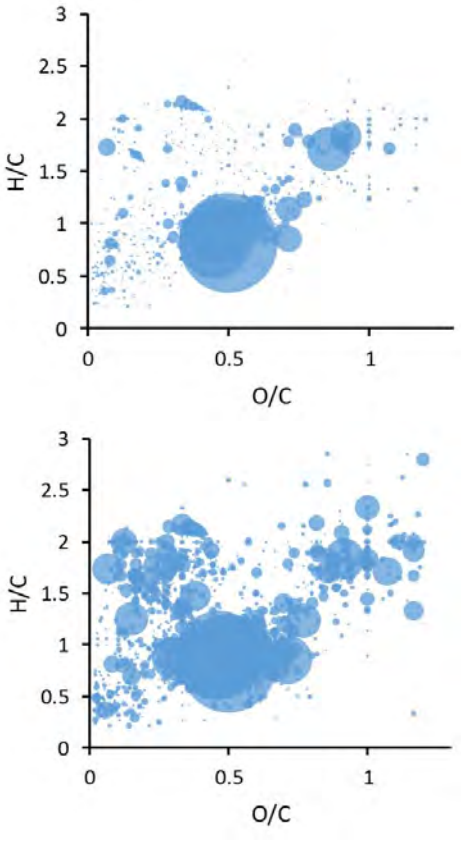



**Figure 2.** Van Krevelen plots of unburied (top) and forest soil buried green tea (bottom). For details see text.

The NMR and MS data thus both show similar trends in the decomposition of the green tea in different soils.

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# TOPIC 5. “HUMIC BASED TECHNOLOGIES - CROP PRODUCTION”

# Effects of percolate recirculation frequency on the organic matter composition after solid-state anaerobic digestion of straw and pig slurry

O. CAVALLO [(a)], J. M. DE LA ROSA [(b)*], J. A. GONZÁLEZ-PÉREZ [(b)], C. ZACCONE [(c)], D. PEZZOLLA [(d)], G. GIGLIOTTI [(d)], H. KNICKER [(b)], M. R. PROVENZANO [(a)]

(a) Department of Soil, Plant and Food Sciences, University of Bari, 70126 Bari, Italy
(b) Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), 41012 Seville, Spain
(c) Department of Sciences of Agriculture, Food and Environment, University of Foggia, 71121 Foggia, Italy
(d) Department of Civil and Environmental Engineering, University of Perugia, 06125 Perugia, Italy
* E-mail: jmrosa@irnase.csic.es

**Keywords:** pig slurry, solid-state anaerobic digestion, percolate, Py-GC/MS, TGA, O-alkyl C/alkyl C ratio

**Abstract.** Restoring humus in soil represents a key factor for improving soil quality. Digestates from anaerobic digestion (AD) can be successfully applied as soil amendments. Here, we investigated changes of the organic matter (OM) composition of digestates from solid-state anaerobic digestion (AD) of straw and pig slurry at different percolate recirculation frequency. Atomic ratios, thermostability indexes, O-alkyl C/alkyl C ratio and Py-GC/MS results consistently showed a positive effect of the highest number (4) of percolate recirculations per day on OM features, resulting in a more stable OM in digestate.

## Introduction

AD technologies allow converting organic residues into biogas and digestate, thus recycling materials and replacing energy from fossil fuels with renewable energy. Digestate has been proven to be a good soil amendment [1], useful to restore OM in soil. As such, digestate should consist of stabilized OM to avoid phytotoxicity, greenhouse gases and odorous emissions, and nutrient losses.

In the present study, we investigated changes of the OM composition of digestates obtained from solid-state anaerobic digestion (SS-AD) of straw and pig slurry at different percolate recirculation frequency. Percolate (*i.e.* the liquid fraction of digestate) recirculation was performed to minimize inhibition phenomena during SS-AD, as it could promote the solubilization and leaching of certain inhibitory organic compounds (*i.e.* volatile fatty acids, [2]). The initial mixture and digestate samples were analyzed by elemental analysis (CHNS-O), pyrolysis coupled to gas chromatography and mass spectrometry (Py-GC/MS), thermogravimetric analysis (TGA) and cross polarization magic angle spinning $^{13}C$ nuclear magnetic resonance (CPMAS $^{13}C$ NMR) spectroscopy.

## Materials and methods

**Experimental set-up.** The experiments were performed in 3 polyethylene reactors of 15 L capacity equipped with a percolate recycling system. The initial mixture was composed of pig slurry and straw (3:1 w/w); the inoculum, was added at the same amount of pig slurry to the initial mixture along with 2 L of demineralized water. Percolate leaching through the mixture was collected at the reactor bottom and recycled by the system. Three tests were carried out using 1, 2 and 4 recirculations per day, producing digestate samples *1SS-AD*, *2SS-AD* and *4SS-AD*, respectively. A control test (*i.e.* without percolate recirculation) was also performed, producing digestate *control.*

**Elemental analysis.** The elemental composition (CHNS-O) was determined in triplicate by flash combustion, using sulfanilic acid as standard; results were expressed as C/N, H/C and O/C ratios.

**Py-GC/MS.** The analysis was performed using a double-shot pyrolyzer attached to a gas chromatograph. Samples (1–2 mg) were introduced into a pre-heated furnace at 400°C for 1 min and the evolved gases directly injected into the GC/MS. The GC was equipped with a low-polarity fused silica capillary column and used helium as carrier gas at a flow rate of 1 mL $min^{-1}$. Mass spectra were acquired with a mass selective detector at 70 eV ionizing energy. The identification of individual compounds was achieved by single ion monitoring (SIM), low-resolution mass spectrometry and comparison with stored (NIST and Wiley libraries) and previously published data [3, 4]. The relative abundance of each pyrolysis product was expressed as a percentage (%) of the total chromatogram area, after peak integration.

**TGA.** About 20 mg sample were heated from 30 to 830°C at 30°C $min^{-1}$ at a flow rate of 30 mL $min^{-1}$ synthetic air. Weight loss (%) was calculated within the temperature ranges 250-350°C (WL1) and 350-550°C (WL2). The temperature at which 50% (TG-$T_{50}$) of the mass loss occurred was also calculated as well as the ratio WL2/WL1, as they are sensitive to OM changes due to biological processes [5, 6].

**CPMAS $^{13}C$ NMR spectroscopy.** The solid-state $^{13}C$ NMR spectra were obtained by collecting over 5000 scans $sample^{-1}$ with a Bruker Avance III HD 400 MHz and using zirconium rotors of 4 mm OD. The cross polarization magic angle spinning (CPMAS) technique was applied during magic-angle spinning of the rotor at 14 kHz. A ramped $^{1}H$-pulse was applied during a contact time of 1 ms. The $^{13}C$ chemical shifts were calibrated relative to tetramethylsilane (0 ppm) with glycine (COOH at 176.08 ppm). Spectra were quantified by integration of the following chemical shift regions: alkyl C (0-45 ppm), N-alkyl/methoxyl C (45-60 ppm), O-alkyl C (60-110

ppm), aromatic C (110-140 ppm), O-substituted aromatic C (140-160 ppm) and carboxyl/amide C and carbonyl C (160-220 ppm). The relative abundance of each typical C resonance region was expressed as relative percentage of the total C resonance area (assumed to be 100%) [7].

## Results and Discussion

**Elemental analysis.** When compared with the initial mixture, all digestate samples showed: (i) a decrease of the C/N ratio, particularly for 2SS-AD and 4SS-AD; and (ii) a decrease of the O/C ratio, with the lowest value found for 4SS-AD, which is in line with the lowest content of O-containing functional groups of this sample. Both ratios suggested that the most efficient OM degradation had been attained using 4 percolate recirculations per day.

**Py-GC/MS.** The identified pyrolysis products were grouped into 7 families, *i.e.* aromatics (Ar), lignin-derived compounds (Lig-), polysaccharide-derived compounds (Ps-), N-compounds (N), ketones, *n*-alkane/n-alk-1-ene doublets and alkanols (AKA), *n*-alkanoic acids (FA) and sterols and terpenoids (ST).

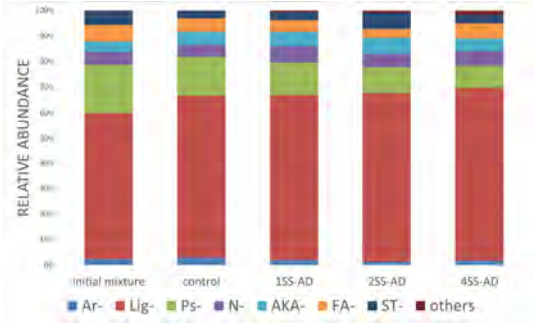



**Figure 1.** Changes in the relative abundance (%) of the chemical families identified by Py-GC/MS.

As compared to the initial mixture, all digestate samples showed a decrease in Ps-derived compounds and a relative increase in Lig-derived compounds. Increasing percolate recirculation frequency resulted in: (i) a major decrease in Ps-derived compounds (4SS-AD < 2SS-AD < 1SS-AD < control); (ii) a greater relative increase in Lig-derived compounds (the highest in 4SS-AD; Fig. 1). The lower content of more labile organic compounds in digestates (the lowest for 4SS-AD) reflect a higher OM stability (the highest for 4SS-AD).

The third most abundant chemical compound class (13-16%) comprises lipids-derived compounds, including AKA, ST and FA (Fig. 1). Extracting the ion chromatogram m/z 74, fatty acids methyl esters (FAMEs) distribution among samples was studied. A marked decrease in L-FAMEs ($C_{21}$-$C_{30}$) was observed in digestate samples with respect to the initial mixture. An opposite trend was found for S-FAMEs ($C_{13}$-$C_{20}$). These results indicate that AD promoted a chain shortening with L- to S-FAMEs conversion (*i.e.* by chain cracking during SS-AD).

**TGA.** The initial mixture showed *ca.* 51% of WL1. Because of carbohydrates consumption during AD, WL1 decreased with digestion, reaching its minimum value in 4SS-AD (35%). In the initial mixture, control and 1SS-AD, WL2 contributued with 33% to the total weight loss. WL2 increased slightly only in 2SS-AD and 4SS-AD. These results indicate that AD has caused a decrease of the more labile fraction of OM and a relative enrichment of the more stable one. This is in particular evident after increasing percolate recirculation frequency to 4 times per day, in agreement with Py-GC/MS results. The thermostability index WL2/WL1 enhanced from 0.6 in the initial mixture to a maximum of 1 in 4SS-AD. Also TG-$T_{50}$ increased in all digestate samples with increasing percolate recirculation frequency, reaching the highest value in 4SS-AD (424°C). Based on thermostability indexes, 4SS-AD shows the highest recalcitrance to thermal degradation compared to the other digestate samples.

**CPMAS $^{13}$C NMR spectroscopy.** All samples showed high contents of O-alkyl C, commonly ascribed to carbohydrates. Their relative abundance decreased from about 70% in the initial mixture to 55% in the control, and 59, 55 and 48% in 1SS-AD, 2SS-AD and 4SS-AD, respectively, which agrees result shown by Py-GC/MS. Digestate samples also showed a relative increase in the alkyl C contribution, with the highest value in 4SS-AD. As a result, O-alkyl C/alkyl C ratio decreased in the digestates. This ratio, used as an index of the extent of OM decomposition, shows the lowest value in 4SS-AD, confirming a greater degree of OM stability with increasing the percolate recirculation frequency, which is in line with Py-GC/MS and TGA results.

The overall results showed a positive effect of 4 percolate recirculations per day on the OM stability of digestate, in terms of relative increase of the recalcitrant Lig domain at the expenses of the more labile Ps content, suggesting a great potential of 4SS-AD to be successfully used as soil amendment.

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# Influence of bio humates on some physiological answer of poinsettia

K. CHAKALOV [(a)*], T. POPOVA[(a)], V. SAVOV [(a)], G. DELEVA [(a)], C.METODIEVA [(a)]

(a) Balkan Plant Science Ltd., 25A, Tsar Osvoboditel Bld, Sofia, Bulgaria
* E-mail:konstantinchakalov@yahoo.com

**Keywords:** bio humates, poinsettia

**Abstract.** Most of the studies show that microbial extracellular enzyme systems go in interaction with metal chelates and break the macromolecules in leonardite. The aim of this study is characterization of humic substances biosolubilized with Cu and Zn chelates, and their influence on poinsettia. Only the biosolubulization with *Pseudomonas putida* slightly increases the proportion of humic acids. The pure humates, and this biosolubilized with *Ps. putida*, increase the anthocyanins twice and biohumates 3 times. The products with more humic acids have stronger action than the ones with more fulvic acids.

## Introduction

In our previous work we discussed characterization of humic substances from different deposits biosolubilizated with our strain microorganisms and their influence on plants [1, 2]. The aim of this study is determination of characteristics of humic substances bioisolubilizated with Cu and Zn chelates, and their influence on some physiological parameters of treated with them poinsettia plants.

## Material and methods:

Scheme of the test with Leonardite – IHSS GASCOYNE BS104L

10 g Leonardite in 100 ml; diluted extract to 500 ml; Urea – 3 g; Inoculates – *Bacillus pasteurii, Pseudomonas putida*

Variants – BH:

1. Control + background (BG)-chemical extraction
2. BG+*B. pasteurii*
3. BG+*P.putida*
4. BG+B.p + P.p.
5. BG+B.p + P.p.+5 ml/100 ml 10%EDTA
6. BG+B.p.+ P.p.+5 ml/100 ml 5% Cu EDTA
7. BG+B.p.+ P.p.+5 ml/100 ml 5% Zn EDTA

## Results and Discussion

The experiment shows that the biosolubilization of leonardite only with *B. pasteurii* reduces the extractable humic substances compared to the chemical extraction (Control). The introduction of chelated Cu and Zn increases the yield of humic substances with about 10-11%, but the HA:FA ratio decreases by half. The humic acids are most likely less aromatic and less condensed. Only the biosolubulization with *Ps. putida* slightly increases the proportion of humic acids.

For the plant experiment with foliar spray (1 ml/L) are chosen variants 3 to 7 with controls HA, FA, and K humate.

Scheme of the test: 1.Control-without humates; 2. F-1 (From K-humate); 3. H-2 (From K-humate); 4. K humate; 5. BH3; 6. BH4; 7. BH5; 8. BH6; 9. BH7.

**Table 1.** Characteristic of bio humates, in %

| No | Total C | | HA | | FA | |
|---|---|---|---|---|---|---|
| | * | ** | * | ** | * | ** |
| 1. | 36.65 | 0.713<br>100 | 29.40 | 0.588<br>82.47 | 6.25 | 0.125<br>17.53 |
| 2. | 24.85 | 0.497<br>100 | 19.75 | 0.389<br>78.27 | 5.40 | 0.108<br>21.73 |
| 3. | 36.40 | 0.728<br>100 | 30.60 | 0.612<br>84.07 | 5.80 | 0.116<br>15.93 |
| 4. | 24.40 | 0.488<br>100 | 19.10 | 0.382<br>79.51 | 5.30 | 0.106<br>20.49 |
| 5. | 35.1 | 0.702<br>100 | 28.15 | 0.563<br>80.20 | 6.95 | 0.139<br>19.80 |
| 6. | 45.10 | 0.902<br>100 | 30.80 | 0.616<br>68.29 | 14.30 | 0.286<br>31.71 |
| 7. | 40.10 | 0.802<br>100 | 28.10 | 0.562<br>70.07 | 12.00 | 0.240<br>29.93 |

* from the sample; **in extract

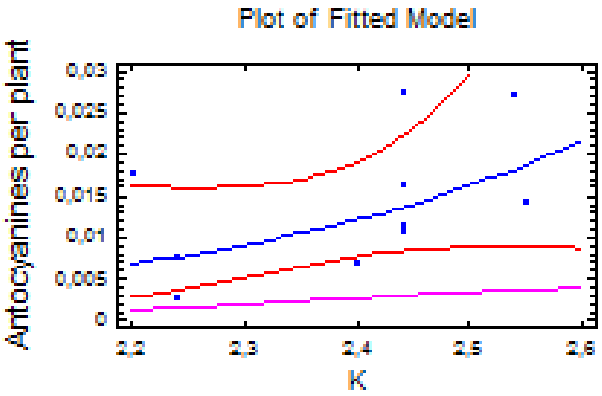



**Figure 1.** Regressions analyze.
Coefficient = 0.527; (P=0.0496);
Antocyanines per plant = exp(-11.3351 + 2.88631*K)

**Table 2.** Biometrical data of poinsettia plants

| Exp. | d | Fresh shoot biomass | Fresh root biomass, | Green leaves area | Red leaves |
|---|---|---|---|---|---|
| | mm | g | g | $cm^2$ | % |
| Control | 6.86 | 55.8 | 6.74 | 1049.9 | 54.58 |
| F 1 | 7.15 | 53.37 | 8.7 | 1007.1 | 54.45 |
| H 2 | 8.29 | 62.41 | 9.41 | 1014.9 | 56.87 |
| K humate | 7.48 | 61.53 | 9.48 | 1028.9 | 56.03 |
| BH 3 | 7.91 | 60.46 | 4.00 | 923.8 | 56.83 |
| BH 4 | 7.86 | 55.43 | 3.84 | 1006.9 | 55.24 |
| BH 5 | 7.33 | 57.32 | 6.58 | 1129.0 | 54.49 |
| BH 6 | 6.73 | 55.75 | 6.65 | 1016.4 | 54.47 |
| BH 7 | 7.65 | 56.47 | 8.82 | 1023.2 | N.D. |

N.D. – not determined

The data presented in Table 1 show that pure humic acids H-2 increase the stem diameter to 21%. The higher the share of FA, the weaker the effect of treatment for this parameter. An exception is variant BH5, with more humic acids and weak effect. Probably the HA in this variant have a low degree of condensation and aromatization.

The fresh biomass follows the same trend. Regarding the dry biomass, however, variant 5 has 28% more than the Control, while the H-2 variant – only 12% more.

In the case of root biomass it was found that the pure HA and FA from variants 2, 3, and 4 have a stronger impact and the increase of fresh root biomass is 29-41%. The biosolubilized humates with Zn chelate in the medium also increase the fresh root biomass – with 32%. The dry root biomass of almost all variants is higher than that of the Control, it's slightly lower only for the biosolubilized variants BH3 and BH6.

The total leaf surface, the surface of green and red leaves are comparable to these of the Control, since there is no significant difference. However it should be noted that the pure HA and BH7 have relatively more red leaves than the Control.

The influence of the treatment on the formation of anthocyanins in plants is significant. Only the pure humates – H-2, and the humates biosolubilized with *Ps. putida* (BH3), increase the anthocyanins – H-2 – twice, and BH3 – almost 3 times. All other treatments reduce the anthocyanins 2 to 3 times.

The mineral nutrition of plants with nitrogen is not very different from that of the Control. The biosolubilization with the two strains decreases the N assimilation, while the addition of Cu chelate increases it. The P assimilation follows the same trend; only BH4 and BH5 decrease the assimilation. The K, Ca, and Mg assimilation is almost the same as that of the Control. Only the K humate increases significantly Mg assimilation. Only K relatively influence on anthocyanins content.

## Conclusion

The method for leonardite biosolubilization with *B. pasteurii* and *Ps. putida* allows the preparation of products with different HA: FA ratio.

The products with more HA have stronger physiological action than the ones with more FA.

The products with more FA are suitable for vegetative growth stimulation and the ones with more HA – for generative processes stimulation and for increasing yield quality.

The *B. pasteurii* strain should prevail over the *Ps. putida* strain during the process of biosolubilization.

**Table 3.** Mineral nutrition and anthocyanin content of poinsettia plants, % to the dry biomass

| Exp. | N | P | K | Ca | Mg | Anthocyanines $.10^{-3}$ |
|---|---|---|---|---|---|---|
| Control | 2.896 | 0.609 | 2.42 | 1.384 | 0.378 | 161.27 |
| F 1 | 2.870 | 0.601 | 2.343 | 1.46 | 0.39 | 121.12 |
| H 2 | 2.811 | 0.566 | 2.241 | 1.425 | 0.392 | 385.37 |
| K humate | 2.556 | 0.579 | 2.345 | 1.428 | 0.433 | 74.48 |
| BH 3 | 2.936 | 0.59 | 2.173 | 1.385 | 0.355 | 466.32 |
| BH 4 | 2.859 | 0.56 | 2.246 | 1.33 | 0.377 | 89.37 |
| BH 5 | 2.64 | 0.482 | 2.116 | 1.312 | 0.327 | 154.15 |
| BH 6 | 3.117 | 0.536 | 2.242 | 1.387 | 0.375 | 57.64 |
| BH 7 | 2.926 | 0.554 | 2.246 | 1.35 | 0.651 | 97.15 |

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# Growth and nutritional quality of lettuce plants as affected by humic conditioners

D. P. DICK [(a)*], A. C. BENDER [(b)], D. HANKE [(c)], E. L. S. DE SÁ [(b)]

(a) Department of Physical Chemistry, Federal University of Rio Grande do Sul, UFRGS, Av. Bento Gonçalves, 9500, Porto Alegre – RS – Brazil.
(b) Department of Soil Science, UFRGS, Av. Bento Gonçalves, 7712, Porto Alegre – RS – Brazil.
(c) Department of Soil Science, Federal University of the Pampa, Street 21st April, 80, Dom Pedrito – RS - Brazil
* E-mail: debby.dick@gmail.com; deborah.dick@ufrgs.br

**Keywords:** fulvic acid, humic acid, nutrient absorption, loamy sandy soil

**Abstract.** This work evaluated the effect of humic conditioners (HC) on growth and nutrient uptake of lettuce plants at two soil pH levels in comparison to mineral fertilizer. At pH 4.9, fresh biomass did not differ between HC treatments and NPK, while contents of P, Ca, K and Fe were greater in treatments where HC applied in combination with NPK. At pH 5.5, this difference was less pronounced. Our results indicate that NPK doses can be reduced when HC is applied and that a synergic effect between HC and NPK occurs.

## Introduction

Humic substances (HS) have been reported in the literature as bioactivators of metabolic processes in plants. One of the main observed effects is the increase in the absorption of nutrients by the plants treated with HS. Because of this and other effects of HS on plants, there is a steady increase in the market for HS-based commercial products. In addition, with the growing demand for more sustainable food production, HS-based products have become an alternative for farmers seeking more sustainable and profitable production due to reduced use of mineral fertilizers. Therefore, the objective of this work was to test two commercial conditioners based on humic acids (HA) and fulvic acids (FA), with different doses of NPK on the growth and absorption of nutrients such as P, K, Ca and Fe by lettuce plants.

## Materials and methods

The pot experiment was conducted at the greenhouse of the UFRGS, Porto Alegre, Brazil. Samples of a Red Acrisol was used at its natural pH (4.9) and after liming (pH 5.5), using lettuce as a bioindicator. Besides the mineral fertilizer NPK, the conditioners Growmate Soil® (GS), rich in humic acids (1.44% w/w) and Growmate Plant® (GP) rich in fulvic acids (1.35% w/w) were used. The following treatments were performed in each pH condition: CNT (no addition of fertilizer); NPK, 100% of its recommended dose; GS, at recommended dose; GS+100% of NPK; GS+75% of NPK, GS+50% of NPK; GP on the recommended dose; GP +100% of NPK; GP+75% of NPK; GP+50% of NPK. NPK was added to the soil one day prior to seedling implantation, and soil conditioners were applied immediately prior to seedling implantation, according to the recommended dose of 4 L $ha^{-1}$. Biomass was monitored along the experiment and nutrients content of lettuce plants was determined at 14 and 28 days after transplant (DAT). For this, 1 g of lettuce dry mass was digested according to the plant tissue analysis method of Tedesco [1]. The concentrations of P, K, Ca and Fe on the extracts were determined by coupled plasma optical emission spectrometry (ICP-OES).

## Results and Discussion

Biomass of lettuce leaves showed no interaction between treatments and soil pH level. However, it presented a difference between the different treatments (Figure 1). Biomass from treatments CNT, GP and GS was lower than in the other treatments. The treatments with addition of GP and GS together with NPK showed biomass production equal to NPK treatment. This indicates that it is possible to reduce the dose of mineral fertilizer for the same lettuce production. The content of nutrients P, K and Ca showed a significant interaction between soil pH and different treatments (Figure 2). Fe content in plants differed between treatments but not for pH level. For CNT, GP0 and GS0 treatments, values of P (<0.50 $mg.kg^{-1}$), K (<11.00 $mg.kg^{-1}$), Ca (<7.00 $mg.kg^{-1}$) and Fe (<1.00 $mg.kg^{-1}$) were very low and differed from those obtained in other treatments. These results demonstrate that without mineral fertilizers, humic conditioners have no effect on nutrient uptake by plants. However, by combining GP and GS conditioners and mineral fertilizer, nutrient uptake by lettuce plants is significantly greater than NPK treatment. For instances, at pH 4.9, P content in lettuce leaves was greater in all treatments with addition of GP or GS. In the soil with pH 5.5, P content in leaves (Figure 2a) was higher only in GP 75 and GP 100 treatments in relation to NPK treatment. Treatment GS 100 showed the greatest accumulation of K in leaves at pH 4.9 (Figure 2b), differing from NPK treatment. K content of treatments GP 100, GS 50 and GS 75 did not differ from that of GS 100 and NPK. In soil with pH 5.5, K content in leaves was greater in treatments GP 75, GP 100 and GS 100 than in NPK. The other treatments presented intermediate values to the mentioned treatments. Ca content in leaves was greater in the soil with pH 4.9 for treatment GS 100, while in the soil with pH 5.5 the absorption of Ca was the same for all the treatments (Figure 2c). In soil with pH 5.5, it is assumed that the highest absorption of Ca by all treatments is due to the application of limestone and not to the effect of humic or NPK conditioners. However, in soil with pH 4.9, the importance of the addition of HA and in association with the 100% NPK (GS 100) dose is visible,

because in this treatment plants absorbed more Ca than other treatments. Fe content in leaves showed no interaction between soil pH and the different treatments. In relation to the treatments, the combination of the humic conditioners and 100% of the NPK doses (GP 100 and GS 100) promoted a greater Fe absorption in the leaves in comparison to NPK. The other treatments did not differ from each other (Figure 2d).

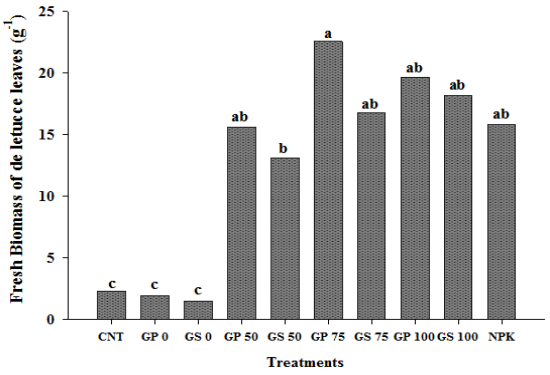
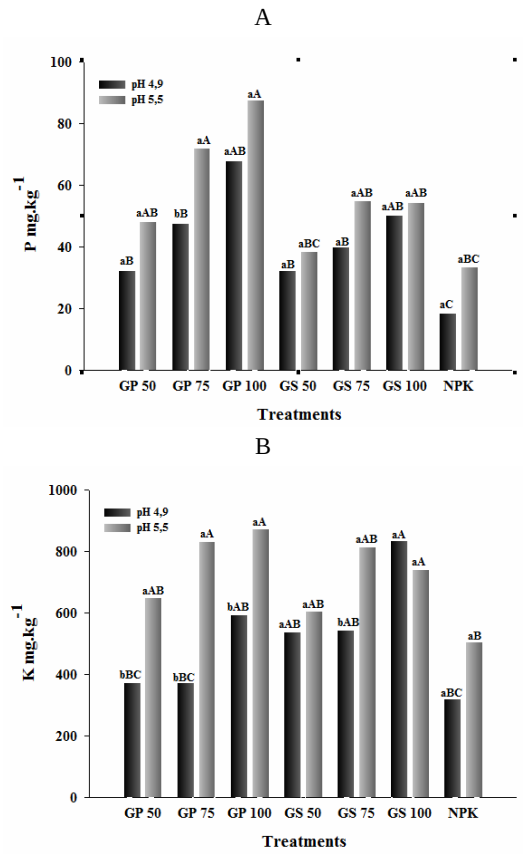



**Figure 1.** Average values of the fresh biomass of lettuce leaves at different pH levels (4.9 and 5.5) in the different treatments.



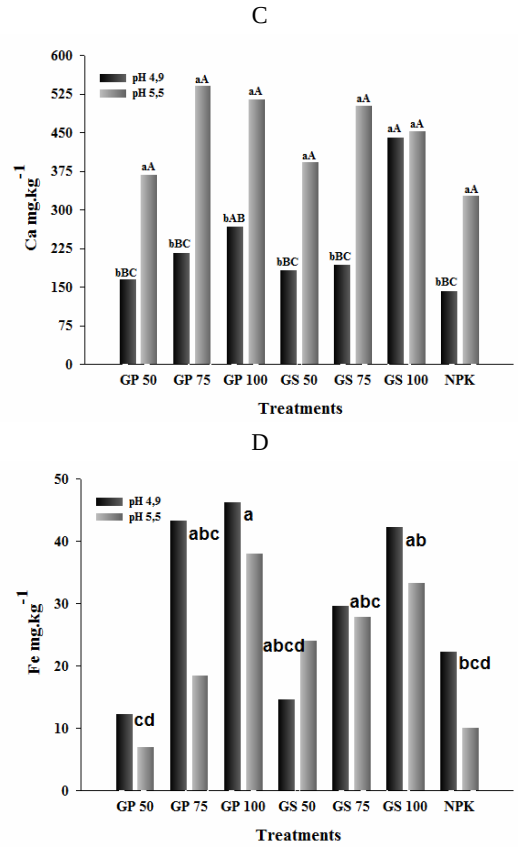




**Figure 2.** Contents of P (a), K (b), Ca (c), Mg (d) e Fe (e) in lettuce leaves at different doses of NPK+GP and NPK+GS. Lowercase letters differ between pH levels. Capital letters differ between treatments.

## Final Considerations

The use of HC plus 50% of NPK dose lead the same yield of lettuce plants as the 100% dose of mineral fertilizer.

The use of HC in soil with pH 4.9 increases nutrient uptake in relation to the NPK, resulting lettuce plants with higher nutritional content.

The effect of HC on lettuce productivity and nutrient uptake was also pronounced in the soil with pH 5.5. Probably with pH raise, the increase in nutrient uptake by lettuce plants resulted from a greater accessibility of the root system to the cations via complexation with HS.

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# Soil organic matter under different managements of eucalyptus harvest residues

D. DICK [(a)*], J. MARTINS [(b)], C. BAYER [(b)], J. SÃO JOSÉ [(b)], E. ARAÚJO [(c)]

(a) Institute of Chemistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.
(b) Soil Science Department, Faculty of Agronomy, UFRGS, Brazil.
(c) CMPC Celulose Riograndense, Guaíba, Brazil.
* E-mail: debby.dick@gmail.com

**Keywords:** *n*-alkanes, eucalyptus, carbon, sandy soil

**Abstract.** Soil organic matter (SOM) plays an important role in soil quality. In the eucalyptus harvest, residues are either kept on soil surface or removed for other uses. The aim of this work was to evaluate the effect of different harvest residue managements on SOM content and composition (plant and microbial derived) on soil profile. Three different residue management were adopted: no removal (NR), partial removal (PR) and total removal (TR). Harvest residue removal tended to reduce SOM, mainly in upper soil layers, when compared to residue maintenance, and impacted also on the microbial derived contribution.

## Introduction

Eucalyptus plantations are usually developed in soils of poor fertility that aren't adequate for other cultures, like sandy soils. In these areas, SOM plays an important role in soil quality. In the harvest process, residues could be kept on soil surface or could be removed for other uses, like thermal energy production [1]. In general, residue maintenance increases SOM, while its removal may cause SOM losses, due to soil exposition between plantations cycles. To define the better residue management strategy for a sandy soil, it is necessary to understand the fate of carbon inherited from residue within the profile.

Lipids are relatively stable during decomposition, and therefore can be related with its precursors material. Consequently, this substances are used as biomarkers to study SOM alterations [2]. The study of the fraction of n-alkanes from soil free lipids allows to evaluate the contribution to SOM of material derived directly from residue (chain lengths ≥25 carbon atoms) and that of microbial derived material (chain lengths < 25 carbon atoms) [2]. The aim of this work was to evaluate the effect of different harvest residue managements on SOM content and composition (plant or microbial derived) on soil profile.

## Materials and methods

**Experimental design.** The experiment was conducted in a sandy Entisol (4% clay), Southern Brazil, under humid subtropical climate that is being used for eucalyptus plantation in the last 30 years (3 culture cycles). Before the last cycle, three harvest residue treatments were established in randomized complete block design with four replicates blocks: no removal (NR) - maintenance of harvest residues (branches, leaves and litter layer from the previous cycle) and current plantation's litter on soil surface; partial removal (PR) - removal of previous harvest residues and maintenance of current plantation's litter; and total removal (TR) - removal of previous harvest residues and current plantation's litter, by means of a shade net above soil to prevent residues input into the soil.

**Sampling and analysis.** Six years after the last eucalyptus plantation, soil was sampled in seven layers up to 1 m depth, three litter samples were collected in each experimental unit and combined. In TR treatment the material over the shade net was collected. Carbon (C) content was determined by elemental analysis. Replicates samples was combined to perform free lipid extraction with a dichloromethane-methanol solution (3:1 v/v) for 24 h. *n-A*lkane fraction was separated by liquid chromatography and abundance was determined by gas chromatograph coupled to mass spectometer (GC-MS). Individual compounds were identified by comparing molecular ion at m/z 71 and published mass spectra libraries. From chromatograms, short/long chain ratio ($R_{S/L}$= [$\Sigma C_{18-24}/\Sigma C_{25-33}$]) was calculated [2].

## Results and Discussion

**Carbon.** C varied between 1.0 and 15.9 g.kg$^{-1}$ and decreased downwards in all sites, as expected. From 0 to 5 cm, C decreased in the order NR>PR>TR, indicating the depletion of SOM promoted by the residue removal mainly at the upper layers (Fig. 1).

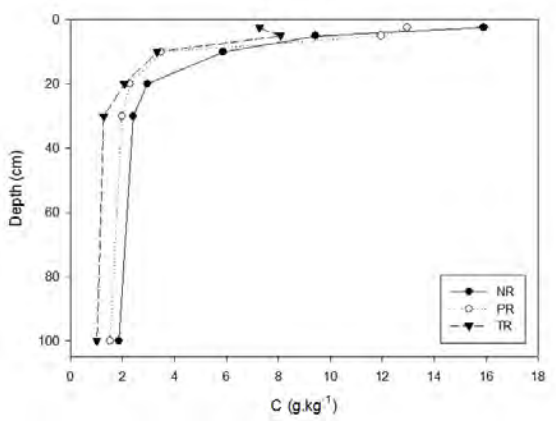



**Figure 1.** Soil carbon content (C) in depth in the different harvest residue management. No significative difference among treatments (ANOVA, p=0,05).

Despite the lack of difference among treatments, soil C content tends to decrease under TR, suggesting that in successives cycle plantations this is not a suitable strategy for keeping soil quality. The similarity between NT and PR treatments suggests that the input of current plantation's litter suffice to keep soil C content, possibly due to the low soil clay content to store SOM [3].

***n*-Alkanes.** $R_{S/L}$ ratio values (Table 1) indicates that microbial contribution increases with depth. Chromatograms demonstrate that contribution of vegetal derived lipids reache 1 m in NR treatment, while in PR this contribution occurs mainly till 30 cm, and is probably derived from current plantation's litter. The reduction in $R_{S/L}$ ratio in 75 – 100 cm in TR treatment can be attributed to the material inherited from residues from previous planting cycles, which were protected from microbial acttivity due to the depth. Alkanes length chains >30 C atoms are evidenced in deeper layers due to an attenuation effect of the other peaks, due to its decrease along the soil profile.

**Table 1.** *n*-alkanes short/long ratio ($R_{S/L}$) in the different treatments.

| Depth(cm) | $R_{S/L}$ | | |
|---|---|---|---|
| | NR | PR | TR |
| Litter | 0.5 | 0.2 | 0.1 |
| 0 – 2.5 | 1.1 | 0.7 | 1.5 |
| 2.5 – 5 | 1.8 | 1.8 | 2.8 |
| 20 – 30 | 2.5 | 1.7 | 4.3 |
| 75 – 100 | 2.5 | 3.3 | 1.6 |

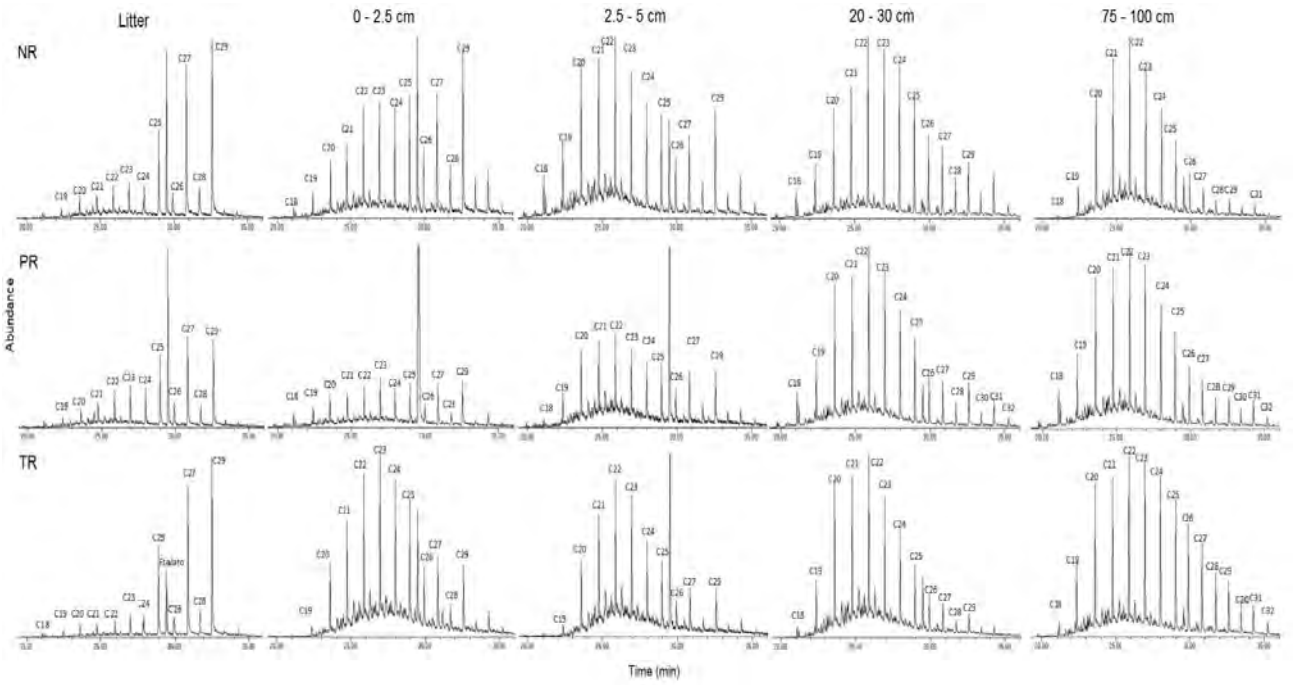



**Figure 2.** *n*-alkanes chromatograms profiles (m/z = 71) in the different treatments.

## Conclusion

In the study conditions, harvest residue removal tends to reduce SOM, mainly in the upper soil layers, when compared with harvest maintenance.

The relative contribution of microbial products to the lipid fraction of SOM increases in depth.

Harvest residue management affects plant derived lipids contribution to SOM in depth.

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# Improvement of ecological condition of soil by natural materials of sorption character

L. EPRIKASHVILI [(a)*], M. ZAUTASHVILI [(a)], T. KORDZAKHIA [(a)], M. DZAGANIA [(a)], N. PIRTSKHALAVA [(a)]

(a) Iv. Javakhishvili Tbilisi State University, Petre Melikishvili Institute of Physical and Organic Chemistry, 31 A. Politkovskaya str., 0186, Tbilisi, Georgia
* E-mail: luba.eprikashvili@tsu.ge

**Keywords:** natural zeolite, brown coal, organic-zeolite composite, improvement of ecological condition of soil.

**Abstract.** Economical effective complex fertilizers used for improving soil fertility can be produced in countries with suitable agricultural resources. The results of tests that were held on organo-mineral fertilizer, which consists of natural zeolite and brown coal of the Georgian origin are discussed in the article. The results obtained make it possible to consider the proposed substrate as an effective ameliorant providing long-term effect of the introduced fertilizer (prolongation effect), as well as preventing the washout of nutrients. The biometric indicators of plants grown on such a substrate are much higher than the control indexes.

## Introduction

One of the main issues being problematic as of to date is pollution of environment, maintenance and growth of soil fertility. Economic difficulties of recent years, as well as reduction of quantity of consumption of fertilizers has conditioned decrease of fertility and this process can be stopped by reasonable applying of non-traditional material, having soil restoring features. Natural zeolites are untraditional natural mineral fertilizers which enable significant increase in productivity of agricultural crops (up to 50%). Because of their high ion-exchange capacity and ability to retain moisture and useful components in soil for a long period, zeolites are used as long-term conditioners of water-salt regime of soil [1].

One of the most important functions of zeolite is its ability to reduce absorption of toxic and radionuclide substances from soil by a plant. The radionuclides and heavy metals existing in soil are adsorbed on zeolite and therefore are not washed away from soil or adsorbed by plants. Zeolites promote diminution of nitrogen nitration in soil and decrease amount of nitrates in product (up to 7-38%). They are considered to be the sources of microelements and thermo-regulators of soil [2].

Humin subsances are complex and biologically active ones which comprise of humin acids and humates. This is the balanced combination of micro-flora, macro- and microelements. Humins utilized in agricultre are easily soluble salts which represent active physiologic forms of humin acids. They act on the level of the cell, change penetrability of cellmemrances, increase activity of ferments, speed up the physiological and bio-chemical processes and stimulate synthesis of albumens and carbohydrates, breathing processes in the plant [3].

According to the above, the aim of the research is the investigation of the influence of a new organo-zeolite fertilizers (prepared on the basis of brown coal and zeolite) on the bio-productivity of agricultural crops. Such composition can perfectly perform its function: on the one hand to enrich soil with humus and increase its productivity; on the other hand, zeolite will ensure a prolonged giving of the above components to a plant; besides, it will still retain all the positive properties of natural zeolite.

## Materials and methods

In this work, brown coal (Akhaltsikha field of Georgia) and natural zeolite - clinoptilolite of sediment origin (Tedzami deposits, Khandaki Georgia section), modified with $K^+$ and $NH_4^+$ ions, were used as a source of organic matter for complex fertilizer production. X-ray fluorescence spectroscopic analysis of brown coal showed that the content of toxic elements does not exceed the permissible standards (MPC). Modification of zeolite with $NH_4^+$ and $K^+$ cations was carried out by treating the original natural clinoptilolite, 0.1N with $NH_4Cl$ and KCl solutions, respectively. As the second component of the fertilizer, is untreated brown coal. The meadow-brown soil with alkali reaction of its aqueous solution (pH=7.3-7.9) was used in the experiment. The soil is characterized by a low content of humus (1.93-2.90 %) and it belongs to the heavy loam by the granulometric composition [4]. The experiment was carried out in the vegetative vessels, in five versions, each in three repetitions. In the first variant, the soil (reference object) was used as a reference. In the second variant, the substrate was prepared by mixing finely divided following components: natural zeolite, unmodified + soil. The third option is soil + zeolite, modified with potassium and ammonium cations. The fourth variant of the substrate consisted of untreated brown coal and natural zeolite (without soil). The fifth option is brown coal + zeolite modified with potassium and ammonium cations. Before the beginning of the experiment, the sowing qualities of barley seeds were determined: germination and germination energy. The germination is the number of seeds that has germinated in the time period established for this culture (in this case, 7 days). It is expressed as a percentage of the total number of seeds taken for germination, and characterizes the ability to form normally developed sprouts under optimal germination conditions. The energy of germination characterizes the friendliness and speed of germination of seeds. It is determined in one analysis with germination, but the normal germination of seeds is counted earlier. As

a bed for germination of seeds, moistened quartz sand was used. The experiments confirmed the good sowing qualities of barley seeds (98.5%), which were subsequently used in the experiment variants described above [5].

**Results and Discussion**

The following biometric parameters have been determined: germination energy (GE), relative value of germination energy (RVGE), germination (G), relative value of seed germination (RVSG). Germination energy for the seeds barley was determined in three days after sowing but germination on the seven day. The greatest increase in GE and GC occurs when zeolites, modified with potassium and ammonium cations and brown coal are used during the third sowing. Enrichment of the soil with substrates affects the growth of both the above-ground part and the root system of the plant, although this difference on different substrates occurs differently. The root system develops more on the substrate: soil + zeolite and brown coal + zeolite, and the above ground part on the substrate – soil + modified zeolite. A large difference is also observed in case of brown coal + modified zeolite. Differences were also noticed in the dry mass of plant, both the aboveground part and the root system, which demonstrate an increase in the growth of plants grown on the studied substrates (Fig. 1). Further investigation in this direction is very actual from an ecological point of view. It contributes to the utilization of substandard coal industry wastes as organic components for increasing soil fertility.




**Figure 1.** Germination of plants on various substrates: a) Soil (object of comparison); b) The soil is a zeolite; c) Brown coal - zeolite; d) Soil - modified zeolite; e) Brown coal - modified zeolite.

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# Enhancement of the reductive Fe elution from $Fe_2O_3$ in a seawater-like medium using ascorbic acid with humic acids

H. IWAI [(a)]*

(a) Department of Marine System Engineering, Osaka Prefecture University, 599-8531 Sakai, Japan
* E-mail: h-iwai@marine.osakafu-u.ac.jp

**Keywords:** Fe elution, $Fe_2O_3$, ascorbic acid, restoration of seaweed-bed, electron shuttle

**Abstract.** A fertilizer composed of steelmaking slag and compost, which would be expected to supply dissolved Fe to the sea, was evaluated for use in a seaweed-bed restoration technique. In this study, the effect of HAs with added ascorbic acid (ASC) on the reductive elution of Fe from $Fe_2O_3$ into a saline medium was evaluated. The effect of reductive Fe elution was clearly enhanced by the co-presence of low concentrations of HA, due to the electron shuttling function of HA.

## Introduction

It is well known that Fe is an essential nutrient for organisms, and Fe is one of insoluble metal under sea condition. Brown macroalgal reproductive growth is regulated by dissolved Fe, their life cycle and biomass hence could be easily limited by a lack of dissolved Fe [1]. Seaweed bed depletion is regarded as one of the serious coastal problems, in particular, along with the coast of Japan. The restoration of seaweed beds has been examined by supplying Fe using a fertilizer composed of a mixture of steelmaking slag and compost. An increase in dissolved Fe concentration and the restoration of a seaweed bed were observed after applying such a fertilizer to a barren coastal area [2], and the dissolution of Fe and other minerals from steelmaking slag in seawater were investigated [3]. It also reported that the Fe bioavailability to brown macroalga was enhanced by seawater extractable organic matter derived from a compost [4]. However, the mechanism responsible for the elution of Fe has not been well-clarified. Fe(III)-oxides, such as hematite ($Fe_2O_3$) and magnetite, are found on the surface of steelmaking slag. It is known that the microbial reduction of Fe under anaerobic conditions is a relevant mechanism that explains why soluble Fe(II) can be produced from insoluble Fe(III)-oxides [5]. Quinone moieties in humic substances would likely play a role as electron acceptor/donor, which can assist the reductive Fe elution, in the natural sediment under aquatic condition [5]. Although the efficiency of humic substances on Fe elution is an essential factor for improving the extent of restoration, the role of humic substances has not been elucidated.

The present study reports on the effect of humic acid (HA) on the reductive elution of Fe into seawater-like conditions (pH 8, $I$ = 0.7). $Fe_2O_3$ was employed as a model of a Fe source, and ascorbic acid was employed as a reducing agent instead of the anaerobic bacteria which are able to reduce Fe. The relationship between the structural characteristics of HAs and the Fe elution efficiency was also investigated.

## Materials and methods

**Materials and chemicals.** To avoid side reactions with HAs and reagents, $Mg^{2+}$, $Ca^{2+}$ and $K^+$ were not used in the experiments. A saline medium, which contained 0.7 M NaCl, 0.05 M Tris-HCl (pH = 8.0) and 100 μM 1,10-phenanthroline (OP) in ultrapure water, was used as the base medium for the Fe elution assay. HAs were extracted from hardwood bark compost (HHA), softwood bark compost (SHA) and peat powder in Aberdeen Shire (PHA), and then purified according to IHSS procedures.

**Structural characteristics.** Structural features of HAs were evaluated by elemental compositions, acidic functional group contents, UV-vis absorptivities, FT-IR spectra and TMAH-py-GC/MS analyses.

**Fe elution assay.** A 12.0 – 12.5 mg sample of the $Fe_2O_3$ powder was added to a 15 mL poly-centrifuge tube. 9.6 mL of the saline medium and the HA solution then added, following which, the volume was adjusted to 12 mL with ultrapure water. After adding 0 – 120 μL of the 1 M ASC solution, the sample was then shaken for arbitrary incubation period in the dark at room temperature (20 ± 3°C). The sample was centrifuged (10000 rpm, 10 min and 5°C) after the incubation, a 10 mL portion of the supernatant was then transferred to a fresh 15 mL centrifuge tube. A 1 mL aliquot of 1 M aqueous $NaClO_4$ was added to the transferred samples, following which the Fe(II)-$OP_3$ complex was obtained by triplicate extractions with 1 mL of chloroform each time. To concentrate and adjust the volume of the sample, the solvent was removed under a stream of $N_2$ gas at 70 – 75°C, the dried sample was then dissolved in 1 mL of 50% EtOH. After centrifugation (3000 rpm, 3 min), the Fe(II)-$OP_3$ concentration in the supernatant was determined based on the absorbance at 510 nm by means of a V-630 spectrophotometer (JASCO, Japan) using 1-cm pass length quartz microcuvette.

## Results and Discussion

In our experimental condition, soluble Fe(II) species derived from $Fe_2O_3$ can be detected in the following equilibria:

$$\mathrm{ASC} \rightleftharpoons \mathrm{ASC}\bullet \quad (1),$$

$$\mathrm{ASC}\bullet + \mathrm{Fe(III)} \rightleftharpoons \mathrm{ASC} + \mathrm{Fe(II)} \quad (2),$$

$$\mathrm{Fe(II)} + 3\mathrm{OP} \rightleftharpoons \mathrm{Fe(II)\text{-}OP_3} \quad (3),$$

where ASC• represents the reduced form of ASC. Fe(III) can be reduced by the oxidation of ASC•, Fe(II) is then produced as a result (Eq. (2). The eluted Fe(II) is immediately complexed with OP, resulting in the Fe(II) being dissolved in the medium (Eq. (3). In the presence of 5 and 10 mmol of ASC, the amount of Fe eluted was increased with increasing of the incubation period. When the concentration of ASC was 1 mmol, only negligible amounts of Fe were eluted. To complex Fe(II) with OP immediately, the concentration of OP is preferably 10 times or more that of the target Fe(II) concentration. For this reason, an incubation period of 3 days and a concentration of ASC of 5 mmol was used in the Fe elution assay.

Figure 1 shows the effect of HA on the elution of Fe. Reductive Fe elution from $Fe_2O_3$ was stimulated by ASC in the presence of a low concentration of HAs, indicating that humic substances are capable of functioning as electron donors/acceptors. In this study, HA appears to contribute to the reduction of Fe, as described by the following equilibria:

$$ASC\bullet + HA \rightleftharpoons ASC + HA\bullet \quad (4),$$

$$HA\bullet + Fe(III) \rightleftharpoons HA + Fe(II) \quad (5),$$

where HA• represents the reduced form HA. The maximum eluted Fe concentration was found to be under the condition of 5 mg $L^{-1}$ HHA with ASC (ca. 2.5 μmol Fe(II)), however, the amount of Fe that was eluted drastically decreased with increasing HHA concentration. This electron shuttling function is largely induced by quinone moieties. However, a variety of side reactions could occur by radical accepted HA with a neighbouring HA, as described by the equations below:

$$HA\bullet + HA \rightleftharpoons HA + HA' \quad (6),$$

$$HA\bullet \rightleftharpoons HA' \quad (7),$$

where HA' represent a structurally altered HA by self-decomposition or condensation. The nucleophilic addition reaction, which causes the self-decomposition or condensation of HA, might also proceed along with Fe(III) reduction in the presence of high concentrations of HA, reductive Fe(II) elution would be thus inhibited with increasing the concentrations of HAs.

The electron shuttling function depends on the structural features of HA. A significantly large amount of aromatic compounds were detected as fragments of the HHA by TMAH-py-GC/MS. The fragmented structure of aromatic compounds is mainly derived from lignin-derivatives, hence the chemical structure would be expected to significant depending on the source of the HA. A di-phenolic structure is necessary to generate a quinone structure, an aromatic compound multi-substituted by O-containing functional groups, such as the sinapyl type, is a favourable structure. Radical capacity and its stability would be associated with aromaticity. It is known that an aromatic network increases with the degree of humification, the above structural analyses indicate that the HHA contains a highly poly-aromatic structure compared to the SHA and PHA. N-containing compounds, which were detected as pyrroles and indoles, may contribute to stabilizing free radicals due to their aromatic properties resulting from covalent N-H bonds. Therefore, the HHA had more favourable structural features for assisting in the Fe reduction process than the SHA and PHA. Based on the performance of reductive Fe elution, we conducted that hard-wood bark compost was a suitable humic acid source for use in the fertilizing technique to seawater containing limited amounts of dissolved Fe species.

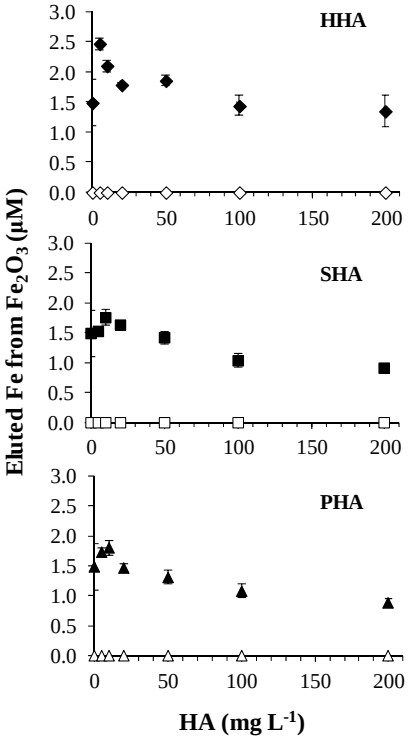



**Figure 1.** Effect of HAs on the reductive Fe elution with ASC. Filled and open plots (mean ± S.D., n = 3) represent the presence (5 mmol) and absence of ASC, respectively. The corresponding values of the absence of $Fe_2O_3$ were subtracted from the original values.

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# Trade-off of compost utilization on greenhouse gas emission and soil carbon sequestration in whole rice cropping system

S. T. JEONG [(a)], G. W. KIM [(b)], H. Y. HWANG [(a)], P. J. KIM [(a)*]

(a) Division of Applied Life Science (BK 21+ Program), Gyeongsang National University, Republic of Korea
(b) Institute of Agriculture and Life Sciences, Gyeongsang National University, Republic of Korea
* E-mail: pjkim@gnu.ac.kr

**Keywords:** life cycle assessment, manure composting, rice cropping, net global warming potential

**Abstract.** Effect of compost utilization on the net global warming potential (GWP) of a rice cropping system was determined using life cycle assessment. Fresh manure application significantly increased $CH_4$ and $N_2O$ emissions by 10% and 37% over the no-manure treatment in rice cropping system, respectively, and depleted SOC stock with 1.5 Mg C ha-1 year-1, due to priming effect. As a result, fresh manure application increased the net GWP by 76% over the no-manure treatment. In comparison, compost utilization decreased the net GWP by 30% over that of the fresh manure during the whole process. Manure composting increased the GWP of the industrial processes by 7%, but the 20% reduction of $CH_4$ flux and 0.2 Mg C ha-1 year-1 of SOC stock increase significantly decreased the net GWP during the whole rice cropping process. As a result, the GHG intensity which means the net GWP per gain yield was not different between the compost and the no-manure treatments.

## Introduction

Rice cropping system is the most important anthropogenic sources for methane ($CH_4$), which is a major greenhouse gas (GHG) and has 28 times higher the warming forces than carbon dioxide ($CO_2$) per unit of weight based on 100-yr global warming potential (GWP).

Soil organic carbon (SOC) is considered as the most key indicator of soil quality and agricultural sustainability, due to its important role on other biological, chemical and physical properties. Livestock manures as organic amendment have been utilized historically to improve soil quality and then increase crop productivity.

However, the application of organic amendments can tremendously increase $CH_4$ production under flooded rice fields. The release of labile OC compounds from amended OMs is an important factor to determine methanogen activity and $CH_4$ formation rates. Thus, the application of relatively stabilized organic amendments such as biochar and compost could be better option for reducing $CH_4$ emission in rice cropping fields.

However, because huge amounts of GHGs including $CO_2$, $CH_4$ and nitrous oxide ($N_2O$) may be emitted during the composting and transporting processes, the selection of compost as organic amendment for reducing GHG emission impacts in rice cropping system is still under debating. To evaluate more precisely the effect of compost utilization on GHG emission impact in rice cropping system, the net GWP which included GHG emissions and SOC stock change should be developed in the whole process.

## Materials and methods

**Life cycle assessment of greenhouse gas emission from composting to land application.** The fluxes of GHGs from composting and rice cropping processes were measured by direct measurement. Impact of GHG emissions during the whole process (from manure management to land application) were analyzed by the LCA method using the Gabi® 4 software (PE International, Stuttgart, Germany).

**Composting and sampling greenhouse gases during composting.** Cattle manure and sawdust mixture was composted using a conventional static chamber method for 50 days in a greenhouse. The emission rates of three biogenic GHGs ($CH_4$, $CO_2$ and $N_2O$) were periodically monitored using the closed chamber method [2].

**Compost application and rice cultivation.** To determine the impact of compost application on GHG emissions and SOC stock changes in rice cultivation system, two different manure plots (fresh and composted manures) were installed as main treatment in a typical temperate paddy field. In addition, no-manure application plots were arranged for comparison. Two Mg $ha^{-1}$ of composted and fresh manures were applied on dry weight basis in two manure treatments. In addition, the same levels of chemical fertilizers were applied in all three treatments, according to Korean recommendation rates for rice cropping.

**Determination of greenhouse gas fluxes in rice paddy.** Mineralized C loss and GHG fluxes were determined using the closed-chamber method [3] in rice field. The concentration of gases was analyzed using gas chromatography.

## Results and Discussion

### Greenhouse gas emissions in rice paddy field

Manure application significantly increased total $CH_4$ fluxes by 42-81% over 351 kg $ha^{-1}$ of $CH_4$ flux in no-manure application plot. Comparatively, total $CH_4$ flux was approximately 20% lower in the composted manure plots than 634 kg $ha^{-1}$ of seasonal $CH_4$ flux in the fresh manure treatment.

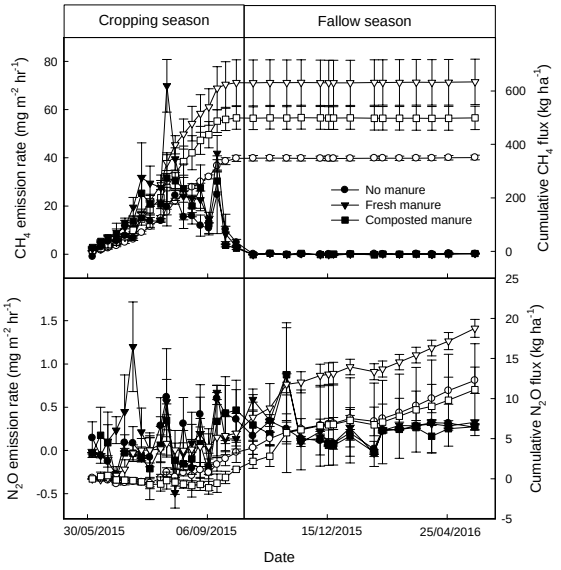



**Figure 2.** Changes of greenhouse gas emission in fresh and composted cattle manure applied paddy soil.

Comparing with $CH_4$ emission, very low levels of $N_2O$ were observed during the whole investigation period. The two different types of manure applications largely increased $N_2O$ emissions with similar patterns. The annual $N_2O$ flux was 14.3 kg $ha^{-1}$ in no-manure application, but manure applications increased this $N_2O$ fluxes by 10-37%. This increase was slightly higher in the fresh manure plot than 15.7 kg $ha^{-1}$ in the composted manure.

### Net ecosystem carbon budget in rice cropping system

**Table 1.** Chracteristics of net ecosystem carbon budget in fresh and composted cattle manure applied paddy soils

| Carbon budget | No manure | Fresh manure | Composted manure |
|---|---|---|---|
| Input (kg C $ha^{-1}$) | 7046 | 9045 | 8937 |
| $NPP_{Rice}$ | 6740 | 7989 | 7689 |
| $NPP_{Weeds}$ | 267 | 602 | 490 |
| Fertilizer | 39 | 39 | 39 |
| Manure | 0 | 776 | 718 |
| Output (kg C $ha^{-1}$) | 7699 | 10930 | 8696 |
| Harvest removal | 5170 | 6123 | 5894 |
| $CO_2$ | 2457 | 3992 | 2575 |
| $CH_4$ | 72 | 815 | 227 |
| NECB | -653 | -1525 | 241 |

The NECB which indicates SOC stock change was minus 653 kg C $ha^{-1}$ in the no-manure treatment. This implies this level of SOC stock might be annually depleted under no-manure application and rice straw removal. In comparison, 2 Mg $ha^{-1}$ of composted manure addition increased the NECB by around 241 kg C $ha^{-1}$, which means this level of SOC might be accumulated annually. Different with our general expectation, which manure application as organic amendment can increase SOC stock, 2 Mg $ha^{-1}$ of fresh manure application significantly decreased SOC stock with minus 1525 kg C $ha^{-1}$ of NECB, due to highly increased respired C loss.

### Net global warming potential and greenhouse gas intencity in whole rice cultivation system

In the no-manure treatment, the net GWP was approximately 16.4 Mg $CO_2$-eq. $ha^{-1}$. Composted manure increased the net GWP by approximately 24% over the no-manure treatment, due to additional GHG emissions during composting process. Manure composting additionally increased the net GWP by 2.6 Mg $CO_2$-eq. $ha^{-1}$, which covered approximately 13% of total GHG emission (20.3 Mg $CO_2$-eq. $ha^{-1}$) for the whole process. However, fresh manure application increased the net GWP by more than 76% over the no-manure treatment, due to increased GHG emissions, in particular $CH_4$, during rice cultivation. The GHGI was not big different between the no-manure and the composted manure application, but significantly higher in the fresh manure application than two treatments.

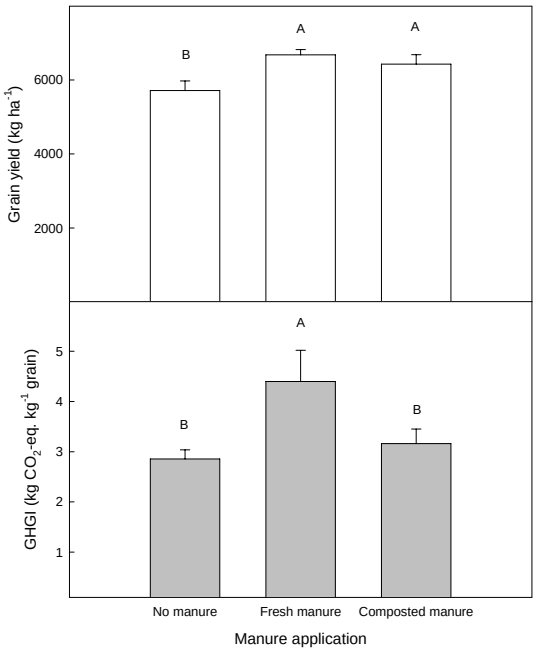



**Figure 3.** Grain yield and greenhouse gas intensity in fresh and composted cattle manure applied soils.

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# Changes of soil organic matter caused by EDTA soil washing treatment on PTM contaminated soils.

E. JEZ [(a)], D. LESTAN [(a)], C. BRAVO [(b,c)], M. DE NOBILI [(b)], M. CONTIN [(b)*]

(a) Centre for Soil and Environmental Science, Biotechnical Faculty, University of Ljubljana. Jamnikarjeva 101. 1000 Ljubljana. Slovenia
(b) Dipartimento di Scienze AgroAlimentari, Ambientali e Animali, University of Udine. via delle Scienze 206. I-33100 Udine, Italy
(c) Department of Life Sciences, University of Trieste, Via Licio Giorgieri 5, 34128 Trieste, Italy
* E-mail: marco.contin@uniud.it

**Keywords:** soil washing, EDTA, humic acids, fulvic acids, FT-IR spectroscopy, EEM fluorescence spectroscopy

**Abstract.** Two soils of contrasting pH and mineralogy (acid and calcareous) strongly polluted by Pb and Zn were remediated by soil washing with EDTA and cultivated after the treatment together with their respective non treated control soils. Humic and fulvic acids were extracted sequentially by NaOH (free fraction) and NaOH+$Na_2P_2O_7$ (bound fraction), before quantitative and spectroscopic characterization. Soil washing caused a significant reduction of the free humic fractions with minor changes in the bound humic fractions. The two cycles of cultivation were not able to restore the original composition of SOM. Structural changes in both FA and HA fractions were observed by UV-vis, FTIR and EEM fluorescence spectroscopies.

## Introduction

Soil polluted by potentially toxic metals (PTM) represents a widespread problem worldwide. When the concentration of PTM in polluted soils is several times the threshold values of soil washing may represent a valuable and efficient remedial technique. Several acids and chelates have been tested to improve its efficiency, but the use of EDTA was proved to be an environmentally sustainable and relatively economical strategy [1]. Nevertheless, the fertility of treated soils may be depressed. It is well known that EDTA has a strong affinity for Mn and other important plant micronutrients. Soil organic matter (SOM) is also strongly impacted by soil washing, therefore a strategy to restore the fertility of treated soils is needed.

The aim of our study was to evaluate the SOM changes induced by EDTA washing on two polluted soils of contrasting pH and mineralogy. In particular, we studied the changes caused on two fractions (free and bound) of humic and fulvic acids (HA and FA) by the EDTA washing treatment and tested whether two cycles of cultivation were able to restore the original SOM composition.

## Materials and methods

**Soil samples.** The acid soil (pH 5) was collected from an active farmland in Arnoldstein (Austria), whereas the calcareous soil was sampled from a vegetable garden in the Meza Valley (Slovenia). Lead mining and smelting for more than 500 years in Arnoldstein and more than 300 years in the Meza Valley caused environmental accumulation of potentially toxic metals (PTM) in the surrounding soils. Metals of major concern for Meza and Arnoldstein were Pb (4029±10 and 774±11 μg $g^{-1}$, respectively) and Zn (2219±16 and 339±5 μg $g^{-1}$, respectively).

**Soil washing with EDTA.** Remediation of contaminated soils was carried out in a pilot-scale chelate washing plant [2]: 50 kg of soil were washed in solutions containing 60 mmol EDTA $kg^{-1}$ soil (acid soil) and 100 mmol $kg^{-1}$ (calcareous soil). Then the soil was rinsed with 3 volumes of rinsing solution to remove EDTA-mobilized PTM. Process solutions were cleansed and recycled as described by Lestan [2]. The process was repeated three times and treated aliquots of the same soil were pooled together. The washing treatment removed 58 and 79% Pb and 10 and 33% Zn from respectively the calcareous and the acid soil.

**Soil cultivation.** Experimental plots were filled with original and remediated soil 30 days after remediation. Soils were fertilized with $MnSO_4$ (40 kg $ha^{-1}$) right before planting the selected vegetables. The first series of crops, in spring 2017, was spinach (*Spinacia oleracea*), parsley (*Petroselinum crispum*) and salad (*Lactuca sativa*) and the second series, in fall 2017, was spinach and Chinese cabbage (*Brassica rapa Pekinensis*). Sampling (1 kg of soil) was done in October when fall crops were still present.

**Free and bound humic (HA) and fulvic (FA) acid extraction.** The humic substances (HS) were extracted first with 0.5 M NaOH (free HS) and then with 0.1 M $Na_4P_2O_7$ plus 0.1 M NaOH (bound HS) under $N_2$. Free and bound humic acids (HA) were precipitated twice at pH 1.5 from the corresponding extracts and washed with acidified distilled water, then redissolved in 0.5 M NaOH and freeze-dried. Free and bound fulvic acids (FA) fractions were purified by adsorption chromatography on cross-linked polyvinylpyrrolidone (Aldrich) to eliminate carbohydrates, peptides and other no humic compounds (NH) and then freeze-dried [3].

**Humic fractions analysis.** Soil total organic carbon (SOC) was determined by automated flash combustion at 1080°C, whereas the organic C in humic solutions was determined by a Shimadzu TOC-VCPN. Spectra of humic

and fulvic acids were recorded by a Cary 1E (Varian) UV-visible spectrometer, a Spectrum 100 FT-IR (PerkinElmer) spectrometer, and by a Cary Eclipse Fluorescence spectrometer (Agilent).

## Results and Discussion

### Quantitative changes in SOC

Soil washing caused a slight decrease in the SOC of acid soil, that did not recover after two cycles of cultivation (Table 1). In particular, the free humic fractions (free FA+free HA) were more affected by the treatment, than the bound fractions, and cultivation did not restore the original quantities for both free and bound fractions.

**Table 1.** SOC, total extractable carbon (TEC), the sum of humic and fulvic C of free and bound fractions.

| Soil | Treatment | SOC | TEC | | FA+HA | |
|---|---|---|---|---|---|---|
| | | | | | Free | Bound |
| | | mg C $g^{-1}$ | mg C $g^{-1}$ | % | mg C $g^{-1}$ soil | |
| Acid | Original | 26.20 | 13.92 | 53.1 | 9.22 | 1.57 |
| | Remediated | 24.60 | 10.25 | 41.7 | 4.71 | 2.71 |
| | Orig. Veg. | 27.50 | 10.17 | 37.0 | 5.94 | 1.23 |
| | Rem. Veg. | 24.40 | 8.74 | 35.8 | 3.81 | 2.22 |
| Calcareous | Original | 38.40 | 15.16 | 39.5 | 5.32 | 7.08 |
| | Remediated | 39.80 | 11.57 | 29.1 | 2.92 | 6.77 |
| | Orig. Veg. | 42.70 | 12.56 | 29.4 | 4.20 | 6.21 |
| | Rem. Veg. | 35.70 | 11.76 | 32.9 | 2.70 | 7.01 |

Changes in the calcareous soil were much lower, but again the free fractions were more affected than the bound ones. The no humified fractions were also affected by the washing treatment.

### UV-Vis spectroscopy

The $E_4/E_6$ ratio of the free FA from the acid soil showed a remarkable decrease after treatment, indicating removal of low molecular weight FA. The opposite was observed for the bound FA. Changes were negligible for the HA fractions (Tab. 2) and were generally of lower magnitude in the extracts from the calcareous soil, and frequently in the opposite direction to the acid soil.

**Table 2.** The $E_{465}/E_{665}$ ratio of fulvic (FA) and humic (HA) fractions.

| Soil | Treatment | FA | | HA | |
|---|---|---|---|---|---|
| | | Free | Bound | Free | Bound |
| Acid | Original | 13.5 | 8.2 | 5.4 | 5.0 |
| | Remediated | 9.8 | 16.3 | 6.3 | 5.8 |
| | Orig. Veg. | 11.8 | 8.9 | 6.3 | 5.5 |
| | Rem. Veg. | 4.9 | 17.5 | 5.7 | 5.5 |
| Calcareous | Original | 17.0 | 13.4 | 4.7 | 6.2 |
| | Remediated | 17.2 | 11.6 | 7.2 | 5.8 |
| | Orig. Veg. | 21.4 | 14.8 | 6.3 | 6.4 |
| | Rem. Veg. | 13.4 | 15.1 | 6.7 | 5.2 |

The $SUVA_{254}$ (Tab. 3) showed a significant decrease in the free and bound FA fraction in both acid and calcareous soil, while it remained practically constant in the HA fractions.

### FTIR spectroscopy

In both the acid and calcareous soils a significant decrease in the aromatic, phenolic and quinones groups and a parallel relative increase in the carbohydrates were observed in the free HA extracted from remediated soils. Minor changes were observed in the bound HA, and generally involving the aliphatic structures. Regarding the FA fractions, a significant decrease was registered for 1120-1130 $cm^{-1}$ bands (guaiacyl CO-H and syringyl CO-H, C–OH of aliphatic OH–groups, sulfones) in the free FA fractions with a concomitant increase of 1650-1697 $cm^{-1}$ bands (C=C aromatics, C=O stretching) [4].

**Table 3.** E245/g Org.C ratio of fulvic (FA) and humic (HA) acid fractions.

| Soil | Treatment | FA | | HA | |
|---|---|---|---|---|---|
| | | Free | Bound | Free | Bound |
| Acid | Original | 195 | 40 | 366 | 353 |
| | Remediated | 85 | 77 | 362 | 420 |
| | Orig. Veg. | 141 | 45 | 398 | 357 |
| | Rem. Veg. | 124 | 75 | 407 | 472 |
| Calcareous | Original | 89 | 140 | 258 | 281 |
| | Remediated | 57 | 102 | 288 | 362 |
| | Orig. Veg. | 63 | 122 | 266 | 324 |
| | Rem. Veg. | 46 | 100 | 286 | 325 |

### EEM fluorescence

Excitation-Emission Matrix (EEMs) of FA and HA were in agreement to the structural changes observed by FTIR.

## Conclusions

EDTA-soil washing caused a strong perturbation in the quality and quantity of humified organic matter. The acid soil was more affected, in particular in its free FA fraction than the calcareous soil, although the free HA fraction was structurally altered in both soils. Two cycles of cultivation did not restore either the quantity or the original composition of SOC in both soils. These results suggest that the addition of humic extracts may support the revitalization of EDTA-washed soils.

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# The dynamic of change some physical and physico-chemical properties during composting of municipal solid wastes and biomass of energetic plants

A. KAŁUŻA-HAŁADYN [(a)]*, E. JAMROZ [(a)], J. BEKIER [(a)]

(a) Wroclaw University of Environmental and Life Sciences, Institutes of Soil Science and Environmental Protection, ul. Grunwaldzka 53; 50-357 Wroclaw, Poland
* E-mail: andrea.kaluza@upwr.edu.pl

**Keywords:** MSW compost, CEC, compost maturity

**Abstract.** The aim of the research was to analyze the composting process, in the aspect of changes of some physical and physicochemical properties of the organic part of municipal solid waste and biomass of willow and hay mixture. The dynamics of changes in CEC values in relation to the base value of the initial materils of both tested composts was higher for MSW compost than for compost from willow biomass. The dynamics of pH changes was similar in the tested types of compost and was rather small during the process of composting, regardless of the type of organic residues and initial reaction.

## Introduction

Composting is a part of the oxidative processes of biological and biochemical transformations that occur under strictly defined temperature, humidity and pH conditions, as well as C/N ratio. The most important aspect in the production of compost is to ensure the proper course of the organic matter transformation process, so that the product obtained does not pose a threat when it is introduced into the soil environment and brings tangible benefits in the form of a positive impact on plant growth and development [1].

Accurate knowledge and understanding of the transformation of organic matter, which takes place in the composting process, as well as a proper assessment of the stability and maturity of compost, are necessary for the effective use of composts. The direction of organic matter transformation during the process depends on the type of composted material.

Composting belongs to dynamic processes during which many biochemical changes occur. The physical properties of the waste processed play an important role at every stage of the composting process as well as during the use of the final product [2]. Knowledge of the physical properties of substrates and mixtures and their relations occurring during the composting process allows for effective planning and execution of the process, as well as the selection of appropriate composting methods and technologies.

The aim of the research was to analyze the composting process, in the aspect of changes of some physical and physicochemical properties of the organic part of municipal solid waste and biomass of willow and hay mixture.

## Materials and methods

The objects of the research were composts produced from: mixtures of biomass of hay and energy willow (BWC) and organic part of municipal solid waste (MSWC). The initial material of variant 2 was obtained according DANO technology [3]. In both variants, the temperature and humidity measurements of composted piles were carried out on a daily basis at regular intervals. If necessary, irrigation and material mixing was carried out to maintain optimal water and air conditions.

For laboratory tests mixed samples were collected in different phases of compost maturity, which represented the initial material of both composts and after: 1, 37, 54, 71, 130 and 167 days of composting, corresponding to different stages of their maturity. On the basis of the collected material, moisture was determined (by weight-drying method), the pH of the water extract (in the ratio 1:10), potentiometrically, the exchange capacity (CEC) by the Harada and Inoko method [4].

The results were statistically analyzed using Statistica 13. Correlation coefficients between individual parameters were calculated as well as indexes of dynamics of changes in the tested parameters.

## Results and Discussion

One of the important factors for the correct course of composting and also one of the most important physical criteria for assessing compost maturity and at the same time is a measurable indicator of the intensity of biotransformation processes occurring during composting process [5]. In the compost produced from biomass of willow and hay mixture, the thermophilic phase was reached after 13 days of composting and lasted for 14 days (Fig. 1).

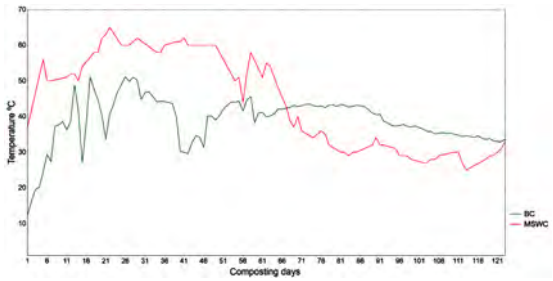



**Figure 1.** Temperature changes during composting of MSW and BW composts.

In the compost from municipal waste, the thermophilic phase was reached after only 5 days and lasted much longer, for as much as 40 days. Experiments carried out so far have allowed us to conclude that there is

a strict negative correlation between temperature and oxygen concentration [6].

Studies carried out by Chen et al. [7] and Drozd et al. [5] indicate pH changes during the composting process. These changes result from different intensities of biochemical processes occurring during composting, which are depended mainly from the temperature, oxygen content and humidity of maturing compost.

The analyzes carried out showed that there was a difference in pH of the aqueous extracts between the tested composts. BWC was characterized by an acid reaction (pH 5.3-6.0), while the MSWC pH exceeded 7.0 (Table 1). The increase of pH during composting indicates the course of ammonification processes, especially in the thermophilic phase of composting, when especially in compost (MSWC) there is a marked change in pH in the alkaline direction (from 6.9 to 7.9). After the thermophilic phase, both composts still show a small increase in pH (Table 1).

The cation exchange capacity (CEC) and degree of organic residues decomposition according many authors [4] are significantly correlated. Therefore this parameter seems to be a good index of compost maturity and indicates compost's ability to retain nutrients. CEC usually increases during composting but many authors suggest different values for CEC in matured compost, depending on type of organic residues. The difference of CEC values observed between tested composts at various stages of maturation is related to the type of organic residues (Table 1). Obtained results, much higher for municipal waste in comparison to those from biomass of energetic willow, confirm that this parameter cannot be treated as the only and objective criterion of compost maturity.

Interesting results have been obtained by calculating the dynamics of changes in CEC values in relation to the base value of the initial materials of both tested composts (Table 1).

Table 1. Single based dynamic indexes for BC and MSWC

| Composting days | Single based dynamics indexes for MSWC | | Single based dynamics indexes for MSWC | |
|---|---|---|---|---|
| | pH | CEC | pH | CEC |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 |
| 37 | 1.02 | 0.71 | 1.08 | 1.45 |
| 54 | 1.04 | 1.09 | 1.15 | 1.65 |
| 71 | 1.06 | 1.48 | 1.12 | 1.58 |
| 130 | 1.09 | 1.29 | 1.21 | 1.80 |
| 167 | 1.12 | 1.39 | 1.11 | 1.94 |

The dynamics (an increase in both cases) was higher for MSW compost than for compost from willow biomass although in both experiments a temporary decrease of dynamic intensity was observed, after 54 days in MSW compost and after 71 days in willow biomass compost. Linear increase of pH dynamics was observed for energy willow compost while for MSW compost there was a small decrease in the intensity after 71 days of composting. Both compost from willow and municipal wastes showed similar values for single based dynamics indexes - 1.12 and 1.11 respectively at the end of composting process. It can be concluded that transformation of organic matter and mineral components during composting process expressively slowed down after reaching a certain stage of maturity. This stage is related to stabilization phase according to the temperature changes.

Correlation coefficients of the relationships forming during the composting process of both types of biomasses.Particularly noteworthy is the relationship between individual parameters and the composting days. On the basis of the obtained results, a significant increase of pH can be observed with the composting time of both tested composts. Composting time significantly influenced an increase of CEC in compost produced from municipal solid waste.

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# Composite materials for controlled release of nutrients

M. KLUCAKOVA [(a)*], R. KRATOCHVILOVA [(a)], P. SEDLACEK [(a)]

(a) Materials Research Centre, Faculty of Chemistry, Brno University of Technology, Purkynova 118, 612 00 Brno, Czech Republic
* E-mail: klucakova@fch.vutbr.cz

**Keywords:** superabsorbent, nutrient, lignihumate, release

**Abstract.** Controlled release fertilizers based on superabsorbent polymers containing lignohumate and conventional NPK were prepared. Their swelling behavior as well as releasing of nutrients were investigated by means of several different methods in first phase of experiments. After that, an effect of prepared materials incorporated in soil on growth of corn was studied.

## Introduction

Humic substances are the principal part of organic soil matter. They affect biological uptake and bioaccumulation of toxic chemicals in plants as well as the pollution of the underground water supplies. They play important roles in the mobility and bioavailability of nutrients and contaminants in the environment [1].

Humic substances play irreplaceable role in soil from agronomical point of view. Therefore, they are used as additives in fertilizing products [2]. They have a beneficial effect on soil quality and plant growth. On the other hand, humic substances are suitable for agricultural application only if care is taken to ensure of limited dosing. It means that humic substances should have a certain concentration which cannot be exceed [3]. This can be accomplished by using of fertilizer systems that are capable of providing controlled release of nutrients.

Our study deals with the development of multifunctional superabsorbent composite materials containing commercial NPK fertilizer as a source of mineral constituents and lignohumate as organic functional materials in combination with poly (acrylic acid-co-acrylamide) as polymer with high water retention. In comparison with composite materials combining humic substance with mineral fertilizers and fertilizers coated by polymers, our systems should combine advantages of controlled release of nutrients (organic and mineral) with swelling ability (water conservation). The effect on corn growth was investigated in growing box.

## Materials and methods

**Synthesis of Superabsorbents.** Eight different samples of superabsorbent polymers were synthetized through the rapid solution polymerization of partially neutralized acrylic acid (AA; Sigma Aldrich) eventually acrylamide (AM; Sigma Aldrich) under normal atmospheric conditions. Potassium peroxydisulfate (KPS; Sigma Aldrich) was used as an initiator and N,N-methylenebisacrylamide (MBA, Sigma Aldrich) as a crosslinking agent. Weight quantity of AA (57 g) was dissolved in distilled water (100 $cm^3$). AA solution (25 $cm^3$) was neutralized by 10 $cm^3$ of 8.5 M potassium hydroxide solution (KOH, Penta). Powders of potassium lignohumate (LH; Amagro company) and NPK (Lovofert NPK, Lovochemie a.s.) were dispersed in this mixture and crosslinked by MBA (0.016 g). Then the initiator KPS was added (0.5 g). The mixture was continuously heated and stirred until reaching of cca 85°C then highly viscous mixture was removed from the beaker and replaced to an oven for 24 hours which was settled up on 80°C. Dried product was crushed by hammer into small pieces [4]. The contents of NPK and LH are showed in Table 1.

**Table 1.** Nutrients of superabsorbent hydrogels

| Sample | NPK (g) | LH (g) |
|---|---|---|
| A | 0.66 | 0 |
| B | 6.60 | 0 |
| C | 0.66 | 1 |
| D | 6.60 | 1 |
| H | 0 | 1 |

**Swelling Experiments.** Samples in form of xerogel were mixed with distilled water in the ratio 50 mg:100 $cm^3$. The swelling degree was determined on the basis of weight increase after 24 hours.

**Releasing Experiments.** Samples in form of xerogel were mixed with distilled water in the ratio 50 mg:100 $cm^3$. Releasing of active substances was monitored in time. The time development of pH and conductivity was measured using Mettler Toledo sevenmulti. The release of K and P was determined by means of ICP-OES (Horiba Ultima 2). The release of LH was measured by means of UV/VIS spectrometer Hitachi U-3900H.

**Pot experiments.** The model soil was prepared by mixing of peat (200 g), kaolin (120 g), silica sand (1660 g) and $CaCO_3$ (20 g). Seeds of corn were leached in 0.05 M NaClO (5 min) in order to remove pickling agent and washed by distilled water. After 24 hours, the seeds were placed separately into curled up paper towels and covered by distilled water (5 days at 28 ± 2°C). The germinated seeds were placed into the 250 g of model soil mixed with 1 g of xerogel and 40 $cm^3$ of water (3 seeds into 1 pot). The pots were placed in the growing box at 26 ± 2°C with periodical watering. Shoot and root were separated and individually measured.

## Results and Discussion

### Swelling Experiments

Swelling degree $Q$ was calculated as the amount of absorbed water normalized on the weight of xerogel: $Q$ =

($m_{hydrogel} - m_{xerogel}$)/$m_{xerogel}$. As can be seen, swelling of all prepared samples is relatively high. Although, its depression caused by the addition of NPK was observed. The swelling degree decreased with increasing NPK content. On the other hand, the presence of LH supported swelling (Fig. 1).

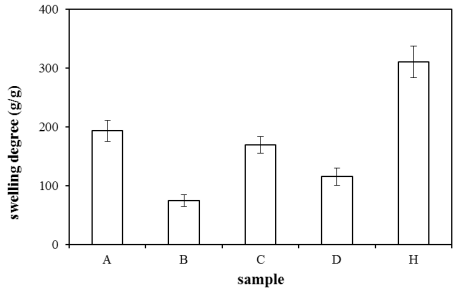



**Figure 1.** Swelling degree of prepared superabsorbents.

### Releasing experiments

Figure 2 displays releasing of K and LH from prepared superabsorbents. We can see, that the presence of LH did not influence of K release from samples with higher NPK content (B and D). The release of K from other samples enriched by LH was higher comparing with samples without LH. According to this observation, the release of K from LH was higher or comparable with K releasing from NPK. In contrast, LH leaching was depressed by presence of NPK mainly in the case of its higher content. Results obtained for P had a similar character excepting sample H without NPK (no P was contained in hydrogel).

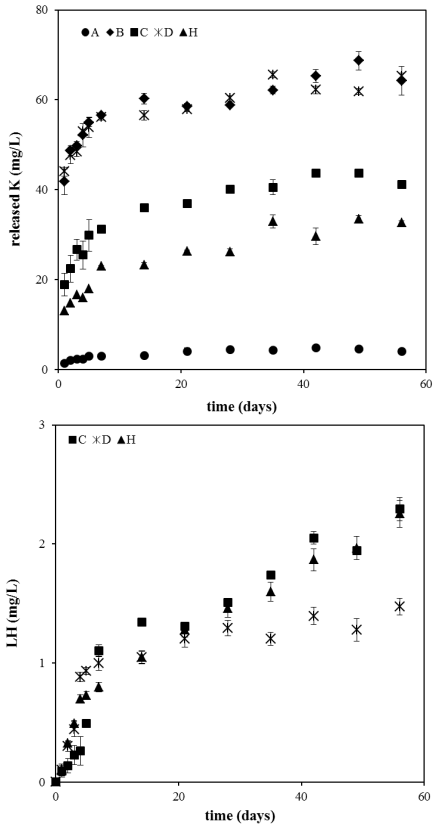



**Figure 2.** Releasing of K and LH from superabsorbents.

The influence of superabsorbents application on corn growth is shown in Fig. 3. The positive effect on growth was observed for all prepared samples. The presence of superabsorbents supported not only growth of above-ground part of plants but also their roots (not shown). This effect was partly caused by better management with water and partly by slow supplying by nutrients from NPK and LH. Our results showed that heightened content of NPK in superabsorbent without LH (sample B) had slightly inhibiting character. In contrast, the combination of LH and higher amount of NPK had synergic effect on corn growth. The material containing only LH (sample H) affected corn growth positively in comparison with plant growing without application of superabsorbent but its effect was lower. In general, the application of superabsorbents supported growth of corn. The better water management and slow supplying by nutrients had positive effect on the height of plants as well as the length of roots.

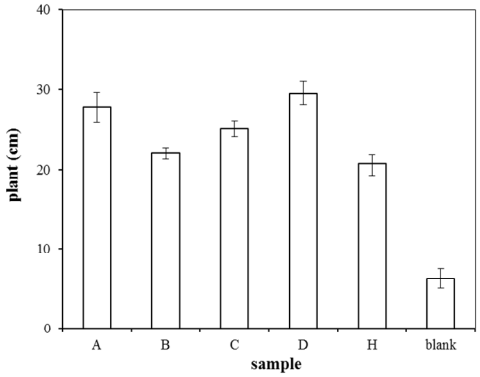



**Figure 3.** The influence of composites on corn growth.

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# Application of humic acid in a Malaysian supper large scale field experiment

O. KOSTOV [(a)*], N.T. YE [(a)], V. VASILEVA [(b)]

(a) Nexus Technology Consultancy, Malaysia
(b) Forest Research Institute, Bulgarian Academy of Science
*E-mail: ognyan.kostov@gmail.com

**Key words**: humic acid, tropical soils, organic matter, yield, oil yielding plants

**Abstract.** The effect of live earth humates (humic acid 45%, iron (Fe) 1.5%, sulfur (S) 1.5%) on the yield of fresh fruit bunches (fruits of palm oil trees) in weathered tropical soils have been investigated. The obtained results showed long lasting positive effect on the yield. Next to this physiological status of the palm oil trees and structure of the weathered soils have been improved. The full positive effect need being assessed in a longer chain.

## Introduction

Application of humic acid in a number of soils types and plants are very well known to have positive effect on soil fertility, increasing root mass, improving and balance soil microbial populations, increasing efficiency of mineral fertilizers, stimulate clay-humus complexes and conserved nutrients, improving retention and plant uptake of nutrients and many others improving's of agro eco systems. Weathered tropical soils have very low humus content, low water holding capacity, Also tropical soils are poor in NPK and need heavy supply of nutrients to ensure good yields to meet the need of growing population. The knowledge of application of humic acid to increase the yield from Palm Oil Plantation in tropical soils is very limited [1, 2].

## Materials and Methods

Lahad Datu Plantation soils are typical weathered tropical soils. They are poor of NPK with strong deficiency of P and have low pH values (pH = 4.3). They are situated in a hilly areas and have strong water erosion in December and January. The experimented product was bought from Utah, USA, Live Earth Products Ltd. The chemical composition was: humic acid 45%, iron (Fe), 10% and sulfur (S) 1.5%. The application rate was 300 g/tree/first year. The amount of humic acid (135 g humic acid /tree) was put in the middle distance between trees rows, on the surface (12 cm depth) where the young roots of palm oil trees was situated. The humic acid was put in the March, 2005. The number of palm oil trees were 136 plants/ha equivalent to 245 ha experimented area. Observations on the surface root growth, number of leaves, size and number of fresh fruit bunches (FFB), green color and chloroses were done the first year of application. The mineral fertilizers ($kg.ha^{-1}$) in amounts of N, 150 kg; P, 95 kg; K, 170 kg; Mg, 50; B, 10 were in ratios of: N,1: P,0.63: K, 1.13: Mg, 0.33: B, 0.06. They were applied as: N as Urea, P ($P_2O_5$), as rock phosphate, Mg as MgO and B as borax.

The tonnage of the FFB were measured at nearby palm oil mill. Also it was observed reduction of amount of Al, As, Cu and Zn in the harvested FFB. The aerobic composting of empty fruit bunches (next product after fresh fruit bunches) resulted approximately to "fresh formed" 1.4 kg humic acid per 20 kg compost (data not shown). Therefore, effect of application of live earth product have to be better assessed in the following chain and not for the first treatment only.

## Results and Methods

Weathered tropical soils are considered as low fertility soils [1] and needs increase of applied amount NPK, micronutrients and improving the ratios of NPK according to requirements of weathered tropical soils. Tropical soils next to strong deficiency of NPK they need as oil yielding plants much more K. Province Sabah, having many palm oil plantations with different NPK rates and ratios showed steady low yields of 22 t/ha for the whole province for more than 10 years (Figure 1). By improving the amount and ratios among mineral fertilizers in Lahad Datu Plantation the yield increased from 22 to 33 $t.ha.year^{-1}$ which is equivalent to 50% increase. The increase was so high because the usual amount of N applied in Sabah Province was 75 $kg.ha^{-1}$, and the increase rate to 150 $kg.ha^{-1}$ is 100%. This high increase of the yield is due also to the increase of amount of P and K as compare to the fertilization rates of Sabah Province. The obtained results showed also that further increase of the yield can be expected by additional organic fertilization. The application of live earth product in a rate of 300 g per palm tree resulted in the increase of the yield. It was statistically significant.

The long lasting positive effect on yield increase were assessed as very positive. For the whole treated area the yield was 35,525.0 t from 245 ha. The tonnage from applied product only was 3,185 t for 245 ha. As whole, the yield increase as compare to the yield of the whole province Sabah was very high of 78.7% in the first year. The profit coming from the yield of fresh fruit bunches (FFB) was high enough to compensate relatively high price considering the far distance to Malaysia of live earth product. Also after first treatment with live earth product the reduction of the yield regarding the time is very slow (37.0, 36.5, 36.0, 35.5 $t.ha^{-1}$). In the first year it was observed also an increase of new surface root mass, appearance of the new leaves, better color of the leaves, number of the FFB, size of the FFB and better soil structure around the place of application of live earth product.

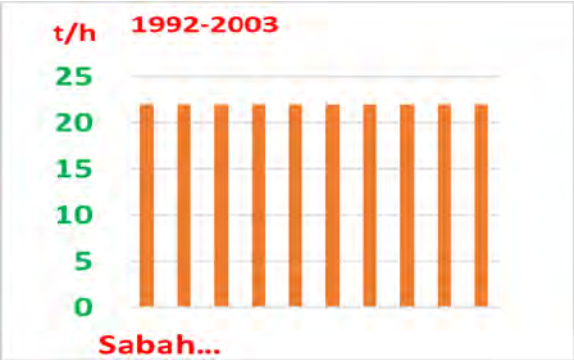



**Figure 1.** Dynamic of yield for 12 years in the Sabah Province.

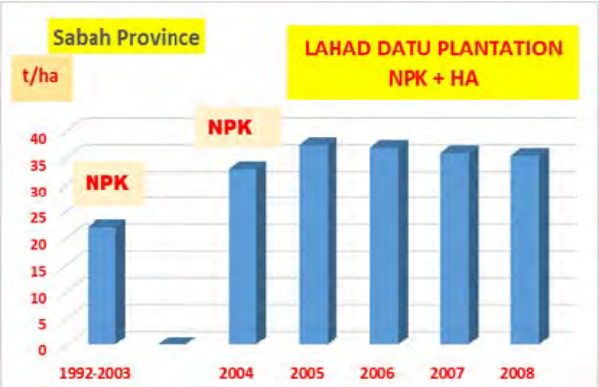



**Figure 2.** Lahad Datu Plantation Yield Dynamics.

## Conclusion

Improving (amount and ratios) of mineral fertilizers application to Palm Oil Plantation resulted in a significant increase of the yield of Fresh Fruit Bunches. Application of Live Earth Product has very long lasting positive effects to the yield of Fresh Fruit Bunches. Also it improves physiological status of the Palm Oil Trees and stabilizes the increase of root mass.

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# Effect of K-Humat (Tki- Hümas) and Phosphorus Application on Maize Growth

A. MOHAMEDELNOUR [(a)*,] E. GÜNERİ [(a)]

(a) Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Ankara University, Turkey
* E-mail: soils_star39@hotmail.com / mohamedali@ankara.edu.tr

**Keywords:** maize, humus, phosphorus, growth parameter, yield

**Abstract.** A pot experiment was carried out to evaluate the effect of humat and phosphorus on maize growth. Two factors were studied using three levels of K-humat (TKİ) – (6, 12 and 18 L/da) and three levels of phosphorus (80, 120 and 160 mg/kg). Humat was applied at sowing time. The experiment was laid out in complete randomized design having three replications. Parameters recorded were plant height, a number of leaves, stem diameter and dry matter yield per plant. According to results the humus levels and phosphorus significantly effect on maize growth and dry matter compared to the control.

## Introduction

One of the greatest problems facing most developing countries is the inability to grow enough food for her ever-increasing population. Maize (*Zea mays L.*) is one of the most important staple food cereals in West and Central Africa after wheat and rice [1]. The contribution of developing countries in the share of world maize cultivated land is about 67% but in maize production, their share is only 46 percent whereas China and USA collectively produce 60 percent of world's corn [3]. Maize is used as a food for the human diet, feed poultry, and animals. Maize has higher nutritional value and is a rich source of raw material for industrially produced value-added stuff. Humic acid applications led to significant increase in soil organic matter content and improve plant growth and crop yield [2, 4]. The chemical properties of HS are known to vary considerably from fulvic acid (FA) to humic acid (HA) and their fractions. Soil application of humic acid had a significant increase in plant growth characters, photosynthetic pigments, total and marketable yield [5].

## Materials and methods

**Plant materials and pots layout:** The pots experiment of maize was conducted at the Department of Soil Science and Plant Nutrition, Ankara University, Turkey. Plants were grown in 48 pots filled with 2 kg of soil. Three replications per treatment were involved in a complete randomized design. Each experimental pot included 4 plants/pot. The pots were irrigated every three days depending on the soil field capacity (all pots were irrigated with 80% of the field capacity). Nutrient solution was prepared with distilled water and the concentrations of nutrients were in all treatments as follows (mg/kg): potassium (K), 100; nitrogen (N),150; phosphorus (P), 40 (as recommended dose of maize was set in all treatments). Humat (humic+fulvic acid) was obtained from Turkish coal enterprises (TKİ) as commercial product (Table 1). Treatments were as follows: 1, P1H1 (P1= 40 mg/kg + H1=0 L/da); control 2, P1H2 (P1= 40 mg/kg + H2=6 L/da); 3, P1H3 (P1= 40 mg/kg + H3=12 L/da); 4, P1H4 (P1= 40 mg/kg + H4=18 L/da); 5, P2H1 (P2= 80 mg/kg + H1=0 L/da); 6, P2H2 (P2= 80 mg/kg + H2=6 L/da); 7, P2H3 (P2= 80 mg/kg + H3=12 L/da); 8, P2H4 (P2= 80 mg/kg + H4=18 L/da); 9, P3H1 (P3= 120 mg/kg + H1=0 L/da); 10, P3H2 (P3= 120 mg/kg + H2=6 L/da); 11, P3H3 (P3= 120 mg/kg + H3=12 L/da); 12, P3H4 (P3= 120 mg/kg + H4=18 L/da); 13, P4H1 (P4= 160 mg/kg + H1=0 L/da); 14, P4H2 (P4= 160 mg/kg + H2=6 L/da); 15, P4H3 (P4= 160 mg/kg + H3=12 L/da); 16, P4H4 (P4= 160 mg/kg + H4=18 L/da).

**Measurement of Growth Characteristics:** Data collected include: plant height (cm) and stem diameter (cm) were measured weekly, after four weeks of planting.

**Fresh and Dry matter (g plant$^{-1}$):** After harvest, the leaves and stem were collected, washed thoroughly with tap water and then deionized water, dried at 65˚C to constant weight.

## Results and Discussion

The data presented in Table 1 showed some chemical properties of humat was obtained from Turkish coal enterprises (TKI) as a commercial product, and Table 2 showed some physicochemical properties of soil used in this study.

**Table 1.** Humat from TKI content

| Humat main components | Content |
|---|---|
| Total organic matter, % (W/W) | 5.0 |
| Total (Humic + Fulvic acid), % (W/W) | 12.0 |
| Water-soluble potassium oxide, % (W/W) | 2.0 |
| pH | 11-13 |

**Table 2.** Physicochemical properties of soil used

| pH 1:2.5 | EC 1:2.5 | N % | P (mg kg$^{-1}$) | K (mg kg$^{-1}$) | SOM | Texture |
|---|---|---|---|---|---|---|
| 7.98 | 0.29 dSm$^{-1}$ | 0.088 | 3.48 | 37.2 | 0.89 | Clay Loam |

**Effect of humus and phosphorus on maize** ***(Zea maize)*** **growth parameters:** The results of this study showed that the application of different rates of humus and phosphorus significantly improved the growth and yield of maize. All treatments gave significant increase compared with control (Table 3).

**Table 3. Biometry of plants (average values)**

| No | Treatment | Plant height | Stem diameter (cm) | Fresh weight (g plant$^{-1}$) | Dry weight (g plant$^{-1}$) |
|---|---|---|---|---|---|
| 1 | P1H1 | 78.1 | 0.65 | 20.7 | 3.2 |
| 2 | P1H2 | 80.1 | 0.67 | 20.8 | 3.2 |
| 3 | P1H3 | 83.0 | 0.65 | 21.9 | 2.9 |
| 4 | P1H4 | 84.6 | 0.70 | 23.0 | 3.3 |
| 5 | P2H1 | 86.7 | 0.70 | 28.0 | 3.6 |
| 6 | P2H2 | 84.2 | 0.70 | 26.7 | 3.3 |
| 7 | P2H3 | 88.5 | 0.72 | 27.0 | 3.1 |
| 8 | P2H4 | 84.9 | 0.67 | 26.9 | 3.3 |
| 9 | P3H1 | 83.1 | 0.70 | 31.4 | 3.9 |
| 10 | P3H2 | 85.3 | 0.72 | 26.2 | 3.8 |
| 11 | P3H3 | 84.4 | 0.73 | 27.2 | 4.2 |
| 12 | P3H4 | 81.8 | 0.78 | 25.1 | 4.0 |
| 13 | P4H1 | 85.6 | 0.68 | 27.5 | 3.9 |
| 14 | P4H2 | 89.0 | 0.70 | 27.2 | 4.2 |
| 15 | P4H3 | 86.6 | 0.75 | 27.2 | 3.9 |
| 16 | P4H4 | 87.0 | 0.73 | 25.8 | 3.9 |




Figure 1. Picture of K-humat (Tki- Hümas).

**The plant height:** The maximum plant height (89 cm) was recorded in P4H2 treatment ((P4= 160 mg/kg + H2= 6 L/da). that is because they improve crop growth by improving the chemical and physical condition of the soil by the uses of organic materials (humat).

**Stem diameter (cm):** The increase in stem diameter (thickness) might be due to the improvement in translocation of assimilates. The increase in C uptake appeared to stimulate plant growth as elevated $CO_2$ increased stem diameters.

**Fresh and Dry Weights of Plant:** The data related to fresh and dry weights of Plant is presented in Table 3. which showed that Humat, P, and their interaction had a significant effect on maize growth. The maximum fresh and dry weights of Plant were recorded as 31.4 and 4.2 respectively.

**Conclusion**

In conclusion, the findings suggest a great influence of humate and phosphorus on improving characteristics of maize growth.



Figure 2. Plants growth and status.

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# Three dimensional excitation emission matrix (3D-EEM) fluorescence spectroscopy for soil powder samples without extraction

Y. NAKAYA [(a)*], S. NAKASHIMA [(a)], M. MORIIZUMI [(b)], M. OGUCHI[(c)], S. KASHIWAGI [(c)], N. NAKA[(c)]

(a) Department of Earth and Space Science, Graduate School of Science, Osaka University, 1-1 Machikaneyama-cho, Toyonaka-shi, Osaka 560-0043, Japan
(b) Department of Bioresource Sciences, Faculty of Agriculture, Ryukoku University, 1-5 Yokotani, Seta Oe-cho, Otsu, Shiga 520-2194, Japan
(c) HORIBA, Ltd., 2 Miyanohigashi, Kisshoin, Minami-ku, Kyoto 601-8510, Japan
*E-mail: ynakaya@ess.sci.osaka-u.ac.jp

**Keywords:** 3D-EEM fluorescence spectroscopy, soil organic.

**Abstract**. 3D-EEM fluorescence spectroscopy for Japanese typical soils in solid states is reported, and reduction of fluorescence quenching is examined by using diluting agent. 3D-EEM spectra for 100 wt% soil sample showed fluorescence maxima at Ex. 450 nm/ Em. 545-555 nm. Intensities at fluorescence maxima were increased for diluted Typic Paleudult and Typic Hydraquent. These results can be explained by decreases of absolute amount of fluorescence components for the diluted samples. For diluted Typic Hapludand, intensities at fluorescence maxima decreased, suggesting fluorescence quenching possibly due to blackish color of the soil.

## Introduction

Fluorescence spectroscopy has been widely used for examining quality and quantity of humic substances extracted from soils and sediments by using alkaline soluition. Recently, it has been applied to humic substances in soils without any extraction procedures based on Laser-Induced Fluorescence (LIF) spectroscopy of soil samples by Milori et al. [1] However, fluorescence quenching by sample itself can prevent quantitative treatment of fluorescence spectrum [2, 3]. In this study, three dimensional excitation emission matrix (3D-EEM) fluorescence spectroscopy for three Japanese typical soils, Typic Paleudult, Typic Hydraquent and Typic Hapludand in solid states is reported for the first time without using lasers. In order to examine fluorescence quenching, 3D-EEM spectra for the soils diluted by alumina ($Al_2O_3$) powder were also measured.

## Materials and Methods

Representative soil samples (Typic Paleudult, Typic Hydraquent and Typic Hapludand) were taken from artificial soil fields in Central Region Agricultural Research Center (National Agriculture and Food Research Organization, Tsukuba, Ibaraki prefecture, Japan) and air-dried at 40 $^oC$. They were sieved through a 2 mm meshed sieve and powdered in a mortar. Their total carbon, nitrogen and water contents are shown in Table 1. 0.8 g of the sample was preserved as a non-diluted sample, other was mixed with alumina powder ($Al_2O_3$, Wako) to obtain 0.8 g of diluted soil samples with 50 and 10 wt% of the soils.

3D-EEM spectra for the non-diluted (100 wt%) and diluted (50 and 10 wt%) soil samples in solid states were measured by a 3D-EEM spectrometer, Fluorolog-3 (HORIBA, Ltd., Japan) with a soil sample holder (fluorescence of 90$^o$ direction with respect to incidence). The samples were hand-pressed in the holder by a glass plate and the measurement surface was roughly flat. Excitation wavelength (Ex.) was 250-800 nm (excitation slit: 5 nm band-pass) and emission wavelength (Em.) was 300-850 nm (emission slit: 5 nm band-pass). Signal accumulative time was 0.1 sec. at 5 nm intervals.

**Table 1.** Total carbon (TC), nitrogen (TN) and water (wat) contents (%) for Typic Paleudult, Typic Hydraquent and Typic Hapludand soils.

| Soil | TC (%) | TN (%) | Wat (%) |
|---|---|---|---|
| Typic Paleudult | 0.71 | 0.06 | 2.2 |
| Typic Hydraquent | 1.18 | 0.10 | 3.4 |
| Typic Hapludand | 7.29 | 0.43 | 10.2 |

## Results and Discussion

3D-EEM spectra for the non-diluted (100 wt%) soils of (a) Typic Paleudult, (b) Typic Hydraquent and (c) Typic Hapludand are shown in Figure 1. All soil samples in solid states showed fluorescence. Fluorescence maxima can be recognized at Ex. 450 nm for each sample (Figure 1). Fluorescence emission wavelength maxima were 555 nm for (a) Typic Hapludand, 545 nm for (b) Typic Hydraquent and 550 nm for (c) Typic Paleudult.

Fluorescence intensities at the fluorescence maxima for the non-diluted (100 wt%) and diluted (50 and 10 wt%) soil samples were plotted against dilution ratios in Figure 2(a), (b) and (c). For (a) Typic Hydraquent and (b) Typic Paleudult, the sample with higher dilution ratio of soil showed the stronger fluorescence intensity. These results can be explained by decreases of absolute amount of fluorescence components for the diluted samples.

On the other hand, (c) Typic Hapludand showed weaker fluorescence intensities for the more diluted soils. In fact, (c) Typic Hapludand contains more carbon and nitrogen than the others (Table 1) with darker color. In fluorescence spectroscopy for dissolved humic substances in solution state, it is known that fluorescence quenching can occur for concentrated humic substance solutions

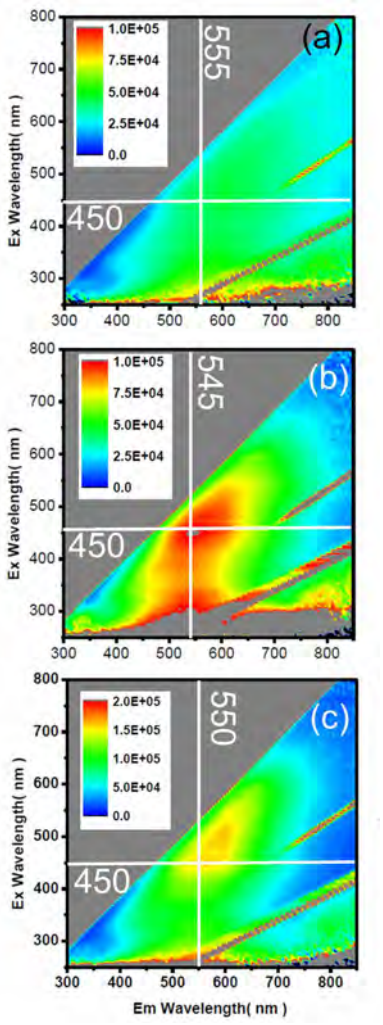



**Figure 1.** 3D-EEM spectra for the non-diluted (100 wt%) soils of (a) Typic Paleudult, (b) Typic Hydraquent and (c) Typic Hapludand. Positions of fluorescence maxima are indicated.

because of self-absorption of emission. In the present study, blackish color of (c) Typic Hapludand might be possibly responsible for fluorescence quenching. Dilution by alumina (white powder) might reduce the quenching despite decreases of absolute amounts of fluorescence components. Additional dilution measurements (0, 2, 5, 10, 20, 50, 100 wt%) are underway and will be analyzed.

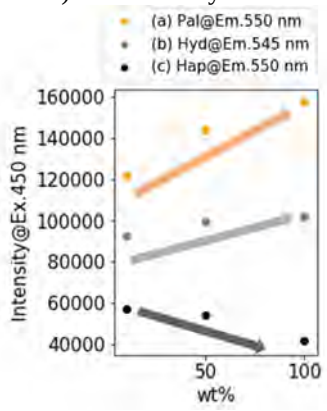



**Figure 2.** Fluorescence intensities by 450 nm excitation for (a) Typic Paleudult (Emission at 550 nm), (b) Typic Hydraquent (Emission at 545 nm) and (c) Typic Hapludand (Emission at 550 nm) at the fluorescence maxima for the non-diluted (100 wt%) and diluted (50 and 10 wt%) soil samples.

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# Changes in the soil fertility down the profile of Haplic Chernozems as a result from 50-years mineral fertilization

M. NANKOVA [(a)*]

(a) Dobroudja Agricultural Institute, 9520 General Toshevo, Bulgaria
* E-mail: nankova_margo@abv.bg

**Keywords:** long-term mineral fertilization, norms, soil fertility properties

**Abstract.** The systematic introduction of macro elements at different norms and ratios during a 50 years period of cultivation of Haplic Chernozems leads to formation of different levels of soil acid complex indices, available N forms of total N and C at depth up to 100 cm with well expressed differentiation. The long-term two-field agricultural use of the trial field without mineral fertilization was characterized with lowest soil fertility potential. According to the 40$^{th}$ year of investigation total N concentration at the end of 50$^{th}$ year increased with 17.24%.

## Introduction

Soil is among the nature's most important resources and are the basis for existence for humans, animals and plants. It is a physical medium of socioeconomic structures, source of raw materials and protects geogenic and cultural heritage of human [1]. Maintaining and increasing soil organic matter (SOM) trough modern agricultural practices is a combination of technological activities of soil tillage, hydromeliorative practices, balanced fertilization with mineral (substances)q organic and microbial fertilizers, economically utilization of pesticides and returning of plant residues to soil, mechanically treated or preliminary composted [2]. Soil organic carbon is the main component of organic matter and it is directly associated with nutrient availability, soil physical properties, and biological soil health and buffer actions over various toxic substances. Its level determined the abundance of nutrient and equilibrium of various nutrient elements [3]. The equilibrium level of SOC in the soil is the function of climate, and the nature of soil and vegetation [4]. Increasing level of soil organic carbon into soil not only enhance soil quality but also help to reduce GHGs emission; lowers climate change. The systematic mineral fertilization for a period of 40 years with different norms and ratios between nitrogen, phosphorus and potassium had high effect on the agrochemical condition of slightly leached Chernozem (Haplic Chernozems) down the soil profile [5].

The aim of this investigation is to follow the effect of the long-term mineral fertilization on the nutrition regim of Haplic Chernozems after 50 years application of fertilizers differentiating in norms and combinations between nitrogen, phosphorus and potassium.

## Materials and methods

A long-term fertilizer experiment, which was established in 1967, is still running. Four nitrogen and phosphorus and three potassium norms in two field crop rotations (wheat-maize) were tested – 0, 60, 120, 180 and 0, 60, 120 kg/ha, respectively. The experiment was designed according to the method of the "net square", applying the full version of the design in four replications (4 x 4 x 3 = 48). On the 50$^{th}$ year from the beginning of the trial (2017) after wheat harvest, soil samples were taken every 20 cm down the soil profile to depth 100 cm. The changes of some agrochemical characteristics were determined in selected variants with high average 50$^{th}$ year productivity.

## Results and Discussion

Acidification is one of the main degenerative processes in the soil that cause decreasing of SOM. Acid complex are formed from soil formation processes and human activity. Soils also possess buffer systems to counteract the acidification, which differ by their capacity. Soil acidity forms for the 0-100 cm profile were significantly affected by the type of fertilizer combination after 50-years mineral fertilization (Table 1).

**Table 1.** Exchangeable cations and degree of saturation with bases, average for the 0-100 cm profile

| Fertilizer variants | Exchangeable $cmol_c kg^{-1}$ soil | | | | | V % |
|---|---|---|---|---|---|---|
| | $H_{8.2}$ | Al | Ca | Mg | Ca+Mg | |
| $N_0P_0K_0$ | 3.19 | 0.00 | 27.03 | 1.60 | 28.63 | 90.21 |
| $N_{60}P_0K_0$ | 3.43 | 0.00 | 26.43 | 3.17 | 29.60 | 89.59 |
| $N_{120}P_0K_0$ | 2.66 | 0.00 | 28.29 | 1.38 | 29.67 | 91.91 |
| $N_{180}P_0K_0$ | 3.14 | 0.18 | 26.70 | 2.17 | 28.87 | 90.48 |
| $N_0P_{180}K_0$ | 2.55 | 0.00 | 29.03 | 2.22 | 31.25 | 92.49 |
| $N_{60}P_{180}K_0$ | 3.79 | 0.00 | 25.49 | 2.69 | 28.18 | 88.24 |
| $N_{120}P_{120}K_{120}$ | 3.29 | 0.00 | 25.31 | 3.13 | 28.44 | 89.70 |
| $N_{180}P_{60}K_{60}$ | 4.13 | 0.22 | 25.31 | 3.09 | 28.40 | 87.68 |

Long-term mineral fertilization with various has the strongest effect on the exchangeable $Al^{3+}$ in the soil absorption complex. It was detected only in the surface layers (0-20 cm) in the variants $N_{180}P_0K_0$ and $N_{180}P_{60}K_{60}$ and does not present in the soil absorption complex further down the profile to 100 cm. The lowest values of residual hydrolytic acidity ($H_{8.2}$) in 0-100 cm layer were registered in the variant with independent fertilization with 180 kg $P_2O_5$/ha (2.55 $cmol_c kg^{-1}$ soil). We established weaker average change in exchangeable cations values between 40$^{th}$ and 50$^{th}$ years of investigation. It is evidence from average value for sorption capacity ($T_{8.2}$) in 0-100 cm layer. The highest value of this index was obtained in long-term fertilization with $N_0P_{180}K_0$ and $N_{60}P_0K_0$ (Fig. 1)

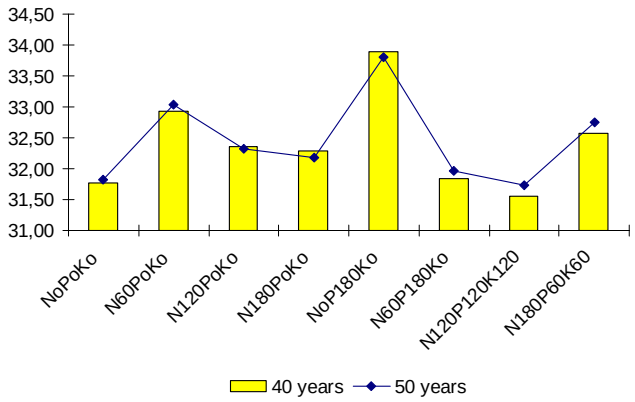



**Figure 1**. Sorption capacity ($T_{8.2}$) average for 0-100 cm depth after 40thy and 50thy years of mineral fertilization.

The size of the nitrogen norm is significant influenced not only for agricultural production in different climatic conditions, but and for agrochemical soil properties. The data obtained show the following: increasing the N norms affected the available N forms (Fig. 2). Average for the 0-100 cm depth sum of N-forms at 50th year (2017) decreased to 51.04% comparing to the 40th year (2007), mainly due to the decreasing of $NO_3$-N.

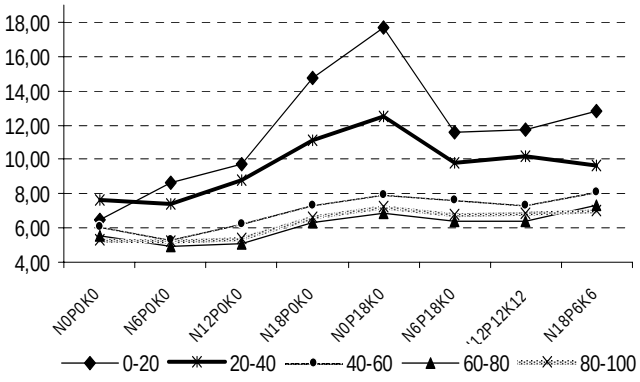



**Figure 2**. Nitrogen mineral forms by depth layers after 50thy years of mineral fertilization, mg/1000 g.

At the end of the studied period, averaged for the depth of the 0-100 cm profile, a significant effect was established to a maximum degree both under the independent influence of the investigated factors and their interaction (Table 2). The depth of the investigated profile was the factor with decisive effect on soil nitrogen concentration. Total concentration of soil N was subjected to dynamic changes averaged for the entire 1 m depth. Its amount was lowest in the control variant (Fig. 3). The variant with systematic $N_{120}P_{120}K_{120}$ fertilization characterized with higher total N content averaged for the entire profile than the variants with independent N fertilization.

**Table 2.** Variance analysis of the total nitrogen concentration for a 50-year period of investigation

| Source | df | Mean Square | F | Sig. |
|---|---|---|---|---|
| Fert. Var (A) | 7 | 243.613 | 104.973 | 0.000 |
| Soil depth (B) | 4 | 19171.455 | 8260.990 | 0.000 |
| A x B | 28 | 48.777 | 21.018 | 0.000 |
| R Sguared=0.999 (Adgasted R Squared=0.998) | | | | |

At the end of the 50th year (2017) averaged for the experiment total N concentration increased with 17.24% comparing to the 40th year (2007).

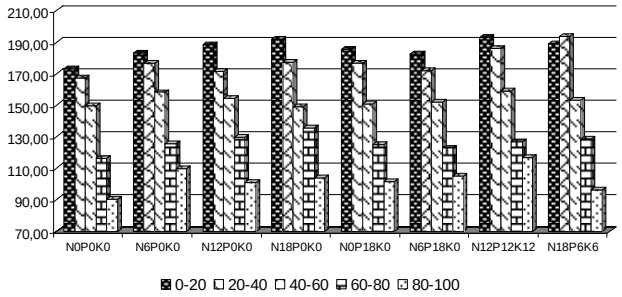



**Figure 3.** Total N concentration by depth layers after 50thy years of mineral fertilization, %.

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# Influence of fulvic acid-containing products on the mineral nutrition and yield of sunflower plants

T. POPOVA [(a)], R. VASSYLEVSKA[(b)], K. CHAKALOV [(a)*], V. SAVOV [(a)]

(a) Balkan Plant Science Ltd., 25A, Tsar Osvoboditel Bld, Sofia, Bulgaria
(b) Institute of Plant Physiology and Genetics, Acad. G., Bonchev Str., Bldg 21, 1113 Sofia, Bulgaria
*E-mail: romb_ltd@abv.bg; konstantinchakalov@yahoo.com

**Keywords:** sunflower, mineral nutrition, fulvic acids

**Abstract.** In our previous work we proved significantly increasing of mineral nutrition of maize hybrids with N, P, K treated with bio-humates. Bio-solubilizatioin increase content of fulvic acid in products. The aim of this study is to examine the effect of fulvic acids on the mineral nutrition and reproductive manifestations of sunflower plants. Single treatment of sunflower plants with a combination of fulvic acids and wheat germ extract or yeast isolate results in a slight increase of yield and has a moderate effect on the mineral nutrition.

## Introduction

The influence of humus substances on plants is well known. In our previous studies we prove the effect of biohumates derived from biodegraded lignite or compost on the reproductive manifestations of maize plants [1, 2]. The characteristic of the obtained substances indicates that it is possible to produce larger quantities of fulvic acids, which are popular on the market [1, 2, 3, 4]. For example, the Canadian company Advanced Nutrients offers the product Golden Goddess, made on the base of fulvic acids and mineral fertilizers.

The aim of this study is to examine the effect of fulvic acids combined with wheat germ extracts and/or yeast isolate on the mineral nutrition and reproductive manifestations of sunflower plants.

## Material and methods

Seeds of *Helianthus annuus L.* variety "1114" were sown in the beginning of May 2016 on the territory of the experimental base of the Institute of Plant Physiology and Genetics, BAS, Sofia (42º50´ N, 23º00´ E, altitude of 595 m). The soil in the field (0-15 см) is of sandy-loam type. The seeds were sown in 5 m-long rows spaced 0.50 m apart with 3 replications. The applied practices were standard for growing sunflower in the climatic conditions of Sofia Valley. Along with the sowing, fertilization with 25 kg/dka was also applied. Each variant had 3 replications; each replication was on 10 $m^2$.

The fulvic acids were obtained from biosolubilized lignite from Stanyantsi mine after KOH extraction and precipitation of humic acids with phosphoric acid. Advanced Nutrients' Golden Goddess fulvic acid-containing product has total organic C – 0.31%; $P_2O_5$ - 1.48%; $K_2O$ - 1.05% and N-1.55%. The other tested modifications contained the same fulvic acids together with wheat germ extracts (WG) or yeast isolate (Y).

The leaf diagnostics was done 1 week after the button formation of the 5th leaf from the bottom up. The treatment was carried out by spraying according to the following scheme:

- Control
- Golden Goddess (GG) AN - 500 ml/ha
- Golden Goddess (GG-ROMB) WG+FA=1:1 – 500 ml/ha
- Golden Goddess (GG-ROMB) WG+FA=1:1 – 1st treatment - 21.06.2016; 2nd treatment – button formation
- Golden Goddess (GG-ROMB) –FA+ WG - 2nd treatment – button formation
- Golden Goddess (GG-ROMB) –FA+Y - 2nd treatment – button formation
- WG – wheat germ extract; FA – fulvic acids; Y – yeast isolate

## Results and Discussion

The data in Table 1(A) and Table 1(B) show that treatment of plants only with fulvic acids (FA) does not have any positive effect on the mineral nutrition, yield and protein content; the oil content increases slightly without a significant difference. Combining FA with WG improves nitrogen assimilation with almost 6% and K assimilation – with 8%. The yield is increased with 27%, but the oil and protein content is considerably reduced. The product with FA and yeast isolate, applied once, has no considerable effect on the mineral nutrition, slightly increases the yield (4%) and slightly decreases the oil content. When applied twice, the combinations considerably increase the N assimilation – with 12 and 11% and K content (especially the FA+Y combination – with 19%). These two variants are the only ones which increase Mg assimilation - with 9.5 and 7%. Yield, however, is positively affected (12.78% increase) only after two-fold treatment with FA together with WG. Unfortunately, this negatively affects the oil content of the grain.

**Table 1(A).** Mineral nutrition, yield and quality of treated sunflower plants, in %

| Variant | N | P | K | Ca | Mg |
|---|---|---|---|---|---|
| 1. Control | 2.67 | 0.23 | 3.15 | 2.62 | 0.80 |
| 2.GG+AN | 2.54 | 0.23 | 3.29 | 2.58 | 0.72 |
| 3.GGR+WG +FA(1) | 2.81 | 0.22 | 3.40 | 2.63 | 0.81 |
| 4.GG+Y(1) | 2.70 | 0.22 | 3.15 | 2.69 | 0.87 |
| 5.GGR+WG +FA(2) | 2.98 | 0.22 | 3.20 | 2.71 | 0.88 |
| 6.GG+Y(2) | 2.95 | 0.22 | 3.75 | 2.72 | 0.86 |

**Table 1(B).** Yield and quality of treated sunflower plants

| Variant | Proteins {%} | Oil {%} | Yield kg/ha |
|---|---|---|---|
| 1. Control | 17.53 | 32.79 | 4101 |
| 2. GG+AN | 16.59 | 33.75 | 4096 |
| 3. GGR+WG+FA(1) | 15.47 | 28.22 | 4271 |
| 4. GG+Y(1) | 18.84 | 30.50 | 4360 |
| 5. GGR+WG+FA(2) | 18.94 | 21.36 | 4625 |
| 6. GG+Y(2) | 17.82 | 22.39 | 3954 |

## Conclusions

Single treatment of sunflower plants with a combination of fulvic acids and wheat germ extract or yeast isolate results in a slight increase of yield and has a moderate effect on the mineral nutrition. The two-fold treatment obviously alters the balance between the main macro elements and the fertilization rates should be adjusted in order to preserve the quality of this industrial crop.

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# Effect of humified organo-mineral fertilisers on nitrogen in a soil-plant agroecosystem-A $^{15}N$ study

B. K. SAHA[(a)], M. T. ROSE [(b)], V. WONG [(c)], T. R. CAVAGNARO [(d)], A. F. PATTI [(a)*]

(a) School of Chemistry, Monash University, Clayton, Victoria, 3800, Australia.
(b) NSW Department of Primary Industries, Wollongbar Primary Industries Institute, Wollongbar, NSW 2477, Australia.
(c) School of Earth, Atmosphere & Environment, Monash University, Clayton, Victoria, 3800, Australia.
(d) School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, South Australia, 5064, Australia.
* E-mail: tony.patti@monash.edu

**Keywords**: nitrogen fertiliser, brown coal-urea, humic, mineralisation of nitrogen

**Abstract.** The ability to inhibit the rate of release of nitrogen (N) from urea fertiliser will reduce N losses to the environment and enhance N use efficiency. A $^{15}N$ microcosm experiment was conducted to study the efficacy of brown coal-urea (BCU) granules on the N losses and crop yield of sweet corn. Application of N fertiliser as BCU considerably influenced the gaseous losses, microbial immobilisation and plant recovery of $^{15}N$ in a corn-soil system. Granulation of BC with urea significantly reduced the fertiliser derived gaseous losses of $^{15}N$ as $N_2O$ and $NH_3$ compared to the sole application of urea. The decreased gaseous $^{15}N$ losses and increased fertiliser-$^{15}N$ retention by BC and soil microbial biomass were mainly responsible for increased yield and N use efficiency of the sweet corn.

## Introduction

Urea is the dominant and most widely used nitrogen (N) fertiliser for agricultural crop production world-wide. However, one major drawback is that less than 50% of the added fertiliser-N is utilised by crop plants due to its very poor use efficiency [1]. The gaseous and leaching losses of N are largely responsible for low N use efficiency (NUE), leading to a variety of adverse environmental effects such as ground water contamination and greenhouse gas accumulation in the atmosphere [2]. From economic and environmental perspectives, it is necessary to improve the fertiliser N use efficiency and reduce the losses to the environment. Granulation of humified organic material with inorganic fertiliser has been shown to reduce gaseous and leaching losses of fertiliser-N by decreasing the nutrient release due to increased adsorption by organic matter which prolonged the availability of nutrients in the soil [3]. Therefore, the granulation of humic acid rich brown coal (lignite) with urea was studied to evaluate the dynamics, turnover, fate and recovery of fertiliser-N in the soil-plant system. The granulation of brown coal with urea (BCU) to give fertilizer industry standard compliant granules was undertaken. This study used $^{15}N$ labelled BCU fertiliser to evaluate the fertiliser N use efficiency by measuring $^{15}N$ uptake by sweet corn and to quantify soil N dynamics by determining $^{15}N$ immobilisation in microbial biomass and soil organic matter, inorganic N accumulation in soil and N losses through leaching and gaseous emissions.

## Materials and methods

The microcosm experiment was carried out in the field at the Department of Primary Industries (DPI), Wollongbar Research Institute (28°90'95"S and 153°41'54"E), NSW, Australia. The soil is acidic with pH 4.7, containing high concentrations of iron and is classified as a Ferrosol. Polyvinyl chloride (PVC) rings were used to prepare the microcosms where the $^{15}N$ labelled BCU granules were applied to sweet corn. The PVC ring was 50 cm height with 30 cm diameter which were installed in the field blocks to a depth of 50 cm. The microcosm experiment was conducted simultaneously with the corn trial having similar treatments. BCU granules (BCU having 20% N enriched with 10 atom percent $^{15}N$) were applied at two rates of N (equivalent to 180 and 90 kg $ha^{-1}$) in this trial. Ten atom percent $^{15}N$ enriched urea with the same N application rate was applied to compare with the BCU blended material. All the fertilisers were mixed within the top (0-15 cm) of the soil in the granular form. The experiment was laid out following a completely randomised block design with four replicates of each treatment. Only one seedling was allowed to grow in each microcosm. To each microcosm, a static gas chamber was installed to collect the greenhouse gases (GHG) and volatilised $NH_3$ samples. The GHG flux was calculated according to the method and equations detailed by van Zwieten et al. [4]. Ammonia emissions were measured using acid treated polyurethane foam absorbers [5] and the flux was calculated using the equation of Singh et al. [6]. At the end of the experiment, soil samples were collected from each microcosm to a depth of 45 cm with a hand auger for $^{15}N$ and mineral N measurement. The concentration of $^{15}N$ of $N_2O$ in the gas mixtures was determined at the Stable Isotope Facility at the University of California, Davis. The $^{15}N$ from soil, plant and volatilised gas samples were measured using an elemental analyser (Carlo Erba/Fisons/Interscience) coupled via a ConFlo II interface to an isotope ratio mass spectrometer (EA-IRMS, Finnigan Delta S). A one-way ANOVA including factor fertiliser type were run. The multiple comparisons among the different treatments were undertaken using a Tukey test.

## Results and Discussion

Addition of BCU fertiliser to soil exhibited considerable effects on the dynamics, mineralisation, fate and recovery of applied $^{15}N$ in soil-plant agroecosystem.

### $N_2O$ and $NH_3$ emissions

Application of $^{15}N$ enriched fertiliser as BCU granules resulted in a significant decrease in $^{15}N_2O$ and $^{15}NH_3$ emissions derived from added fertiliser-N for both high and low N amended soil over commercial urea (Figure 1). The loss of fertiliser-N as $N_2O$ and $NH_3$ was decreased by around 50% and 60%, respectively due to addition of BCU compared with the sole application of urea.

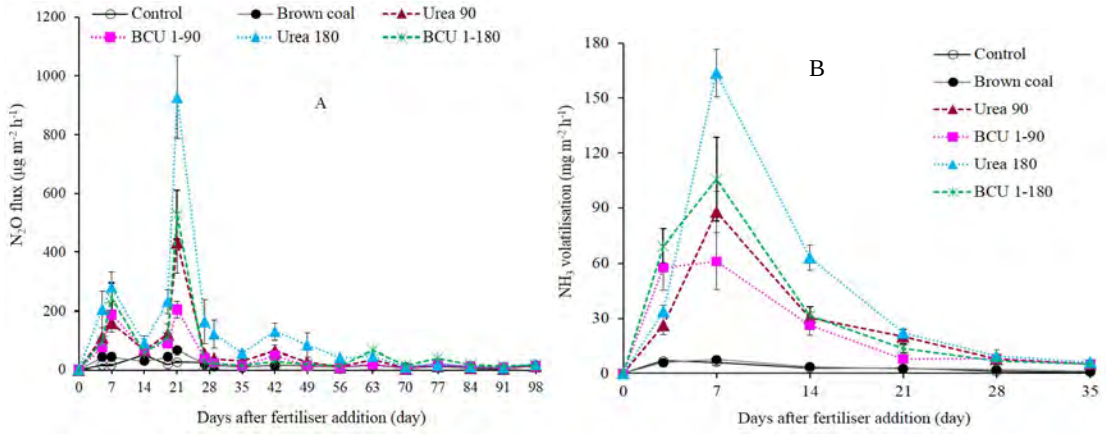



**Figure 1.** Effect of BCU granules and urea on the $N_2O$ (A) and $NH_3$ (B) emissions from soil.

### Cob yield and N concentration of sweet corn

A significantly higher cob yield was obtained from the plants receiving 180 kg N $ha^{-1}$ as BCU compared to the plants receiving the equivalent amount of N as urea. Importantly, statistically similar amounts of cob yield were obtained from the plants receiving 90 kg N $ha^{-1}$ as BCU and the plants receiving 180 kg N $ha^{-1}$ as urea (Figure 2 A). Compared to urea, the addition of BCU granules to soil increased cob yield of sweet corn by 23%. The cob $^{15}N$ derived from applied fertiliser was significantly higher in plants receiving 180 kg N $ha^{-1}$ as BCU compared to plants receiving the equivalent amount of N as urea only and other treatments including control (Figure 2 B). Nevertheless, the cob $^{15}N$ concentration derived from the applied fertiliser was considerably higher in plants receiving the higher rate of fertiliser-N than the low rate of fertiliser-N application. The results from this $^{15}N$ study clearly demonstrate the advantage of applying the urea in combination with a humic acid rich material such as brown coal on nitrogen. Further work is need to confirm the range of humic acid rich materials that could be used in this way to increase nitrogen use efficiency.

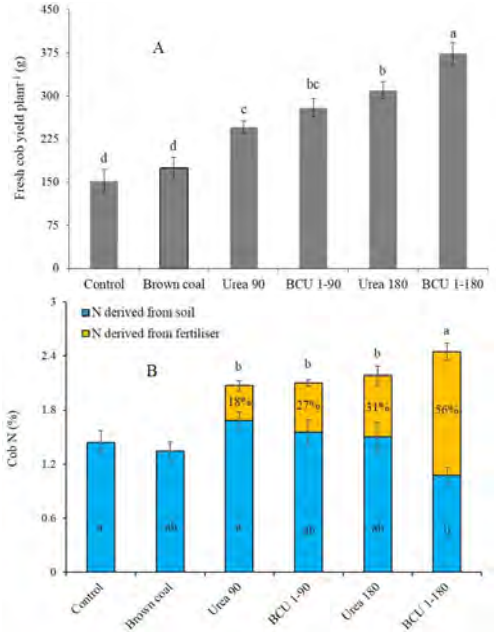



**Figure 2.** Effect of BCU granules and urea on the cob yield (A) and cob N (B) concentration of sweet corn.

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# Effect of humic substances on bacterial growth of rhizosphere *Pseudomonas* strains and their impact on plants

V. SAVOV, T. GEORGIEVA*, Y. EVSTATIEVA, D. NIKOLOVA

Faculty of Biology, Sofia University "St. Kliment Ohridski", 1116 Sofia, Bulgaria
* E-mail: teodora.vg@abv.bg

**Keywords:** *Pseudomona*s, Humic substances, growth characteristic

**Abstract.** Growth characteristics of three rhizosphere *Pseudomona* strains were studied using a different fraction of humic substances as media compaunds. Supplemented humic fractions were demonstrated a positive effect on the biomass density during batch cultivation. Humic acids and fulvic acids stimulate the growth of the soil bacterial strains acting as a regulator of the cell metabolism. The biological effects of humic substances with combination of studied rhisospheric *Pseudomonas* strains were tested for their effect of the development of plants by determination of biometric characteristics of *Euphorbia pulcherrima* (*Poinsettia),* cultivar „Mirat red".

## Introduction

Many strains from different species of genus *Pseudomonas* are proven as important plant growth promoting rhizobacteria (PGPR) and are able to enhance crop yield by direct or indirect performance in plants. *Pseudomonas putida* group are aerobic, gram-negative bacteria, general in agricultural soils, and are well adapted for growing in the rhizosphere. [6] They possess many traits that make them well suited as biocontrol and growth-promoting agents. These include the ability to 1) grow rapidly in vitro and to be mass produced; 2) rapidly utilize seed and root exudates; 3) colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant; 4) produce a wide spectrum of bioactive metabolites (i.e., antibiotics, siderophores, volatiles, and growth-promoting substances); 5) compete aggressively with other microorganisms; and 6) adapt to environmental stresses [1]. Object of the following studies will be to monitor their effect on the amount of synthesized secondary metabolites and having an appropriate concentrations for their application to the plant.

Interacting with living organisms, humic substances even in small quantities affect their growth by inhibiting or stimulating it. Even though HS belong to the most stable components of the soil organic fraction, their decomposition, brought about by action of microorganisms, constitutes a significant part of the turnover of organic carbon in soil [2]. Microbes not only degrade and transform HS but they also mediate their formation. HS on the other hand affect the survival and efficiency of microbes [3, 5].

## Materials and methods

**Bacterial strain.** Three strains from the genus *Pseudomonas*, *Ps. putida* BTCC1046 and rhizospheric isolates Or2 and Or5, were investigated in this study. The *Pseudomonas putida* BTCC1046 strain was selected from the collection of NBIMCC. The other two strains are rhizospheric isolates from the collections of the Department of Biotechnology (Faculty of Biology, Sofia University "St. Kliment Ohridski") and were primarily characterized based on their 16S ribosomal gene sequencing as *Pseudomonas taiwanensis* Or2 and *Pseudomonas putida* Or5.

**Culture Media and Growth Characteristics.** For all the experiments the microbial strains were batch cultivated on Nutrient broth (Bul-Bio ltd). The cell suspension of *Pseudomonas* strains were standardized with 0.9% NaCl at $10^8$ CFU/ml at optical density 590 nm by McFarland standard (Cat. № 3414). The bacterial growth was monitored by measuring the optical density to every 2 hours for the $24^{th}$ hour batch cultivation at 590 nm.

**Influence of humic substances.** Humic substaces of potassium humate (K-humate) isolated from a high quality product of "Humate Sakhalinsky" were used. This product has a stable content of active ingredients – salts of humic and fulvic acids and contains useful minerals and trace elements - Si, Fe, Mg, Zn, Co, Cu, Mn. The raw material for humate production is Leonardite - highly-oxidized lignite (www.humate-sakhalin.ru). To prepare an extraction solutions of humic acids, fulvic acids and mixture of both we used a method with some modifications from CDFA- California Department of Food and Agriculture [4].

**Effect of the tested strains with humic substances on plants**.

Humic substances prepared by extraction from different sources of organic materials were tested for their effects on growth of Poinsettia - humic substances from "Sachalin" leonardite, humic substances from "Sachalin" leonardite inoculated with *Pseudomonas* strains and fulvic and humic substances extracted from "Staniantsy" lignite inoculated with *Pseudomonas* strains. For control was used Nutrient solution.

Poinsettia plants are grown in peat-perlite mixture at pH 5.8-6.0. The plants were treated each week with nutrient solution – 2 ml/l and twice with a 2 ml/l of the test solutions in the branching and active growth phase. Two weeks after that they were treated again. At the end of the experiment, total biomass, total leaf surface, relative and total surface area of red leaves were estimated.

## Results and Discussion

**Dynamics of accumulation of biomass.** At Figure 1 are presented results of 24th hour batch cultivation of *Pseudomonas* strains and NB media in presence of humic substances. The extraction solutions of humic acids, fulvic acids and mixture of humic/fulvic acids were diluted of 100 x than added to the media. The amount of added humic substances to the NB media is 1:4. The presence of humic substances in culture media did not affect the development of the tested *Pseudomonas* strains. The extraction solutions of fulvic acid and mixture of humic/fulvic acids increase the optical density up to 10% for *Ps. putida* Or5 strain.

**Study of the effect of the selected strains solubilized with humic substances on the growth of *Poinsettia*.**

The data for the biometric measurements from the experiment with test plants are presented in fig. 2 and 3. The application of all tested substances increase the red leaves area of treated plants compared to the control plants. The ratio between humic and fulvic acids determines the effect on the treated plants. The results from the experiment show that humic substances, being biologically active, are capable of regulating the growth of microorganisms. A combination of bacterial and humic compositions applied to poinsettia plants has a positive effect on their development.

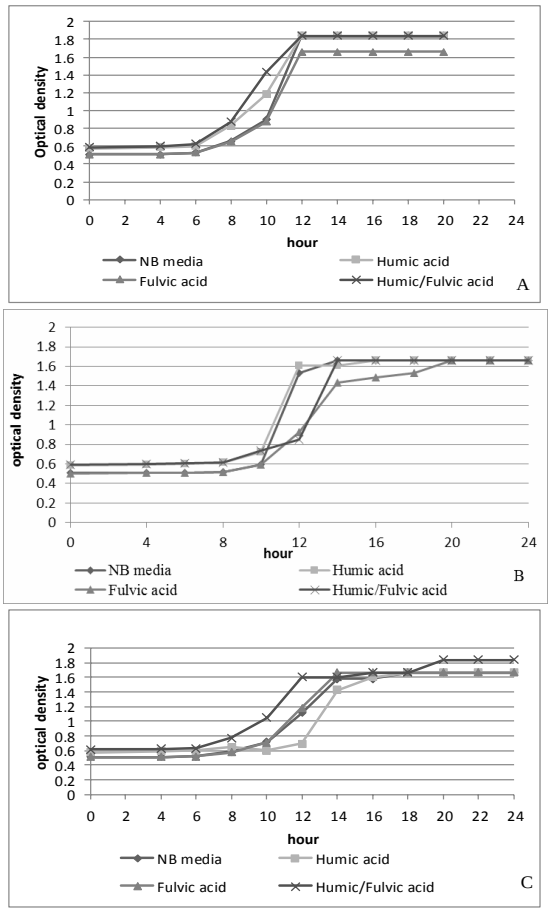



**Figure 1.** Biomass accumulation (OU) in presence of Humic substances: A*) Ps. putida 1046* B) *Ps. taiwanensis* Or2, C) *Ps. putida* Or5.

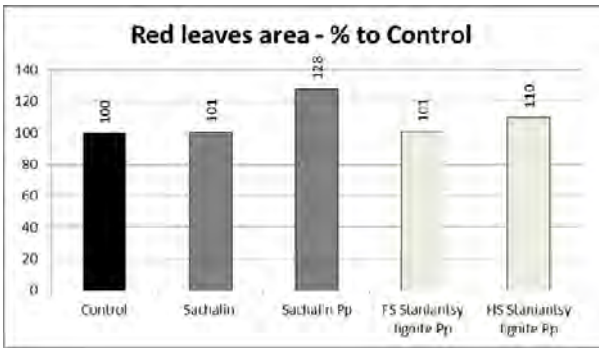



**Figure 2.** Effect of the tested variants on the development of red leaves area of *Poinsettia.*

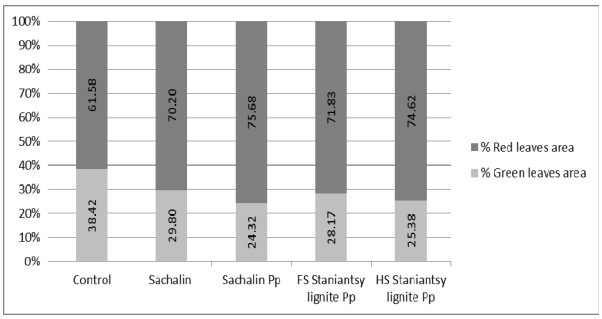



**Figure 3.** Comparison between red and green leaves area of the tested Humate variants.

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# Reuse of fish farming water for the irrigation of plants with economic interest: a project to recycling organic matter in favor of social development in the Brazilian Northeast

A. V. T. SILVA [(a)], H. B. MAIA [(a)], R. A. S. MENDES [(a)], L. P. C. ROMÃO [(b)], M. O. O. REZENDE [(c)], R. R. NUNES [(a)*]

(a) Academic Unity of Serra Talhada, Federal Rural University of Pernambuco, Brazil
(b) Department of Chemistry, Federal University of Sergipe, Brazil
(c) São Carlos Institute of Chemistry, University of São Paulo, Brazil
* E-mail: ramom.rachide@ufrpe.br

**Keywords:** fish farming, *Oreochromis niloticus*, aquatic humic substances, dissolved organic matter

**Abstract.** In this study, the reuse of fish farming water is suggested for the irrigation of plants with economic interest, aiming to recycle its organic matter and nutrients in favor of social development in the Brazilian Northeast region. Historically, the countryside of the Northeast region, particularly in the backwoods agriculture is practiced as a limited economic activity, restricted to local and familiar farms, due mainly to low rainfall, long periods of drought and high temperatures. On the other hand, fish farming is an economic activity in development throughout the region, since this practice demands less water to produce commercial protein when compared to cattle in confinement or other large ruminants. Therefore, this study is a project that aims to combine both practices, proposing the reuse of the fish farming water for the irrigation of plants with economic interest, in favor of social development in Brazilian Northeast. For that, samples of water will be collected and characterized, the organic matter fractioned (DOM and AHS: HAs and HFs), and hormone-like activity (auxin-like effect) will be evaluated. Then, agronomic assays will be performed in the cultivation of test-plants: cherry tomato, maize, and bean. As expected results, the combination of these practices through the reuse of the fish farming water should increase the productivity of the plants, due to the recycling of organic matter and nutrients and the hormone-like activity of compounds present in the water. That should result in more profit and, consequently, an improvement in the quality of life and social welfare in the countryside.

## Introduction

The Brazilian fish farming plays an important role in the local development and generation of income in all the country. However, in the Northeast Region, this practice stands out since the low rainfall and periods of prolonged drought have precluded large ruminant farming for the production of commercial protein [1].

Although it is a curious fact, according to the Water Footprint Network, that fish farms demand much less water for meat production if compared to the cattle confinement, i.e., 0.8 ML of water is required to produce 1 kg of fish protein, against 17 ML per kg of beef, approximately [2].

Regarding the water from the fish farming tanks, its quality is well known for how rich is it in nutrients and organic matter. Therefore, the reuse of this water in the irrigation of plants/crops with economic interest can provide, among other environmental benefits, the recycling of nutrients and the incorporation of organic matter into the soil, increasing its fertility and agricultural potential [1, 3].

In addition, known as hormone-like active, the molecules which form the organic matter – present in the water tanks – may support an important role in the regulation of many metabolic routes in the vegetal cells and tissues, leading to the growth of stronger, healthier and more productive plants, just as the vegetal hormones act [4].

Therefore, with the development of this project, we intend to reuse the fish farming water for the irrigation of plants with economic interest, and to evaluate the hormone-like activity (auxin-like effect), aiming to promote the recycling of the organic matter and nutrients in favor of social development in the Brazilian Northeast region.

## Materials and methods

Water samples will be collected from fish ponds at the COOPVALE fishermen association (Cooperativa dos Productores do Vale do Itaparica), located in the municipality of Itacuruba, state of Pernambuco, Brazil, where *Oreochromis niloticus* (Nile tilapia) are farmed. This association has the participation of approximately 30 associated families and aims to promote the quality of life by increasing the income of the rural worker through fish farming.

**Characterization of the fish farming water.** Samples of water will be analyzed via chemical, physical, biological and spectroscopic methods, to learn the characteristics and composition of their different fractions and water quality:

(1) On the organic fraction: total organic matter (OM), dissolved organic matter (DOM), total organic carbon (TOC), aquatic humic substances (AHS), humic acids (HAs), and fulvic acids (FAs);

(2) On the inorganic fraction: macro and micronutrients, cation exchange capacity (CEC), base saturation (V%), suspended solids, and particle size distribution (PSD); and

(3) Water quality: pH, turbidity, and general microbiological analyses.

**Evaluation of hormone-like activity.** The hormone-like activity (auxin-like effect) will be evaluated in the fish farming water and in their organic fractions: dissolved organic matter (DOM) and aquatic humic substances (AHS), fractioned into humic acids (HAs) and fulvic acids (HFs).

The DOM will be extracted according to the methodology of D'Imporzano and Adani [5] and the AHS (HAs and HFs) according to Chin et al. [6]

Germination tests will be assessed through bioassays with biologically certified lettuce seeds, *Lactuca sativa* in different solutions (water, HAs, HFs and DOM). Additionally, tests will be performed using a sample control (W) and with a sample reference (indole-3-acetic acid, AIA). To perform the bioassays, Petri dishes covered with filter paper (Whatman 91) will be moistened with 1.4 mL of solution and 10 seeds of *L. sativa* will be placed. Seeds will be germinated in a darkroom at 25° C for 48 h. Then, the Petri dishes will be opened, the seeds removed and the roots measured [4].

The bioactive samples will be characterized by spectroscopic techniques, $^{13}C$ CPMAS-NMR and GC/MS. The use of these techniques will support the understanding about the effects observed in the biological tests and elucidate, in biochemical terms, the pathways through which the bioactivity occurs.

**Agronomic assays.** Samples of yellow oxisol will be collected at the Federal Rural University of Pernambuco (UFRPE) (7°56'40.4"S 38°17'49.6"W). To characterize the soil, the following attributes will be determined: pH, organic matter (OM), total organic carbon (TOC), cation exchange capacity (CEC), base saturation (V%), macro and micronutrients and soil texture.

In order to determine the capacity of water and OM retention, 20 mL of static soil will be percolated by 50 mL of samples of water at a rate of ~0.5 mL $min^{-1}$. The content of organic carbon and moisture will be quantified in predetermined periods.

To test the agronomic efficiency, different plants of economic interest will be cultivated in a greenhouse: cherry tomato, maize, and bean. The experimental design will be completely randomized, testing conventional irrigation (tap water) and comparing it to alternative irrigation (water from fish farming tanks), with 10 replications per plant species. Each experimental unit (vessel) will have a capacity of 4.0 L and will be filled with 3.5 kg of soil.

The following biometric indexes will be determined during the experiments: plant size, leaf size, number of leaves, fruit appearance, and other events related to plant development (pests, invasive plants, anomalies, etc.). Additionally, the fruits will be counted and will have their weight and biometric data analyzed (size and circumference, when applicable).

**Technical-economic evaluation of the applied technology.** To evaluate the suitability of the fish farms for water collection, transportation, and application in irrigating economically interesting plants, it is mandatory to know the costs of attached to the irrigation technology suggested in this project.

Also, it is important to learn about the profitability of this practice, i.e., if the application of water from fish tanks would increase the agricultural production of the farmer, resulting in more profit and, consequently, an improvement in the quality of life and social welfare in the countryside.

**Expected results**

The proposed irrigation is expected to present results and agronomic efficiency superior to those of conventional irrigation, intensifying the production, thus increasing the profit to the farmers, resulting in more income and better quality of life in the countryside of the Brazilian Northeast region. In addition, it is expected that the increase in productivity should be directly related to the hormone-like activity of the organic molecules present in the fish farming water.

After completing a study of technical-economic simulation, it is expected to evaluate the impact of the suggested technology in the agricultural production and consequent increase in the income of rural workers in areas of social degradation, which would increase income, thus improving their quality of life.

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# Evolution of Maize Residues into SOM in a study of two years incubation

G. X. SONG [(a)], E. H. NOVOTNY[(c)], M. H. B. HAYES [(b)*],

(a) Institute of Marine Science & Technology, Shandong University, Qingdao 266237, China
(b) Chemical Sciences, University of Limerick, Ireland
(c) Embrapa Solos, Rua Jardim Botânico, 1024 CEP 22460-000 Rio de Janeiro-RJ, Brazil
* E-mail: michael.h.hayes@ul.ie

**Keywords:** Soil organic matter, crop residues, humification, humic substances, humin

**Abstract.** Maize (*Zea mays*, a C4 plant) stover was incubated in a long-term cultivated soil for 24 months in order to better understand the transformation of crop residues into soil organic matter. Humic fractions, including humin, were isolated using an exhaustive sequential extraction procedure based on the charge density and polarity differences of the humic substances: Repetitive aqueous NaOH extraction was carried out at pH 7, 10.6, 12.6, and with 0.5 M NaOH. Then, an exhaustive 0.1 M NaOH + 6 M urea solution was applied to the soil residue to isolate the soluble 'urea humin' fraction. All isolates were further fractionated by the XAD-8 and XAD-4 resin-in-tandem procedure. Finally a concentrated suphuric acid plus DMSO solvent was employed to the fine clay to isolate the most recalcitrant humin material. The samples were characterized by elemental and $\delta^{13}C$ analyses, FTIR and solid-state $^{13}C$ NMR spectroscopy with CSA-filter and DD spectral editing. The data indicated that ca 47%~60% and 28%~40% of tranformed organic carbon was in the HAs and FAs fractions. The urea FA fractions and DMSO/acid FA fractions showed less $^{13}C$ enrichment compared to the fulvic fractions isolated in alkaline only solutions. The refractory humin was predominantly aliphatic and with low aromaticity.

## Introduction

The return to soil of crop residues to maintain soil structure and increase soil fertility is common practise. There is now increased focus on its role in increasing soil carbon sequestration and in mitigating greenhouse gas emissions. The sequential extraction procedure can be be used to study the compositions of components of soil humic substances (HSs).

In order to better undstand the transformation process of the organic matter, maize stover was incubated for 24 months in a soil that had been under long-term cultivartion to wheat for over 30 years. An exhaustive sequential extraction procedure was used to isolate a series of humic, and also humin fractions from the incubated soil [1, 2, 3, 4]. The humic fractions and humin were characterized using elemental and $\delta^{13}C$ analyses, FTIR and solid state $^{13}C$ NMR spectrometry. In some cases, the data are also compared with those for humic samples isolated from a 12 month incubation study under the same condition and with the control (unadmended) soil.

## Materials and methods

**Soil and experiment design.** A cultivated soil [3] sample (32.5 kg) from the Teagasc, Carlow, Ireland, Oak Park site, was mixed thoroughly with chopped and ground maize powder (24.5 kg), and some NPK fertilizer (400 g) was added. The soil mixture was incubated in a plastic barrel in the laboratory, and stirred and watered regularly. The soil sample studied was taken after incubation for 24 months.

**Extraction of HSs** After $H^+$ exchanging and washing with water, the soil was exhaustively extracted in aqueous solutions (with pH adjustments using aqueous NaOH) at pH values of 7, 10.6, and 12.6, then with 0.5 M NaOH, and finally with a urea enhanced alkaline solution (0.1 M NaOH + 6 M urea) [1, 3]. Finally the organic matter-enriched fine clay was extracted with DMSO+6% conc. $H_2SO_4$ (v/v). For more details please refer to [1, 2, 3].

## Results and Discussion

### Yields, elemental and $\delta^{13}C$ analyses

The yields of humic fractions isolated were significantly different from those of the 1st year (Table 1; [3]). In the 1st year, the fractions isolated at pH 7 could be attributed mainly to the native soil. In the 2nd year, yields significantly increased due to transformations of maize to SOM. Yields of highly oxidized, well humified material at pH 7 were low compared to those for the less humified fractions isolated at pH 10.6 and pH 12.6 (which accounted for 47.2 and 38.7% of the yields, respectively). Compared to the significant increases for fractions isolated at pH 10.6 and 12.6, the urea isolates dropped to 1.5%. The maize material contributed to the major proportion of urea HAs in the 1st year, as revealed by high isotopic $^{13}C$ values. The yield of DMSO humin was about 64%.

A $\delta^{13}C$ enrichment in the urea isolates compared to the control soil was observed in the 1st year [3]. However, the urea FAs and DMSO/acid FAs fractions showed less $^{13}C$ enrichment compared to the fulvic fractions isolated in alkaline only solutions in the 2nd year. That was to be expected because the smaller molecule FA fractions isolated from "humin" were considered to be trapped in the humin matrix. From the results of $\delta^{13}C$ enrichment in the fine clay, and in humin (urea HAs, DMSO humin) (supported by another study), it may be considered that the accumulation of $^{13}C$-enriched compounds resulted from the selective preservation of transformed plant residues following incorporation into the SOM, from the selective utilization of plant residues by microbes, as well as from some material directly derived from plant (maize) tissues. These results favour the in situ formation of SOM either by re-condensation of small molecules, or by selective preservation or utilization of biopolymers from soil microorganisms.

**Table 1.** Yields, C and N contents* and $\delta^{13}C$ values of the samples (*on a dry and free ash basis)

| Fraction | Yield | C* | N* | $\delta^{13}C$ |
|---|---|---|---|---|
| | g kg$^{-1}$ | % | % | ‰ |
| **HA pH 7** | 1.16 | 50.2 | 4.74 | -20.66 |
| **HA pH 10.6** | 13.44 | 63.86 | 5.83 | -19.63 |
| **HA pH 12.6** | 10.86 | 58.42 | 5.59 | -19.11 |
| **HA 0.5 M NaOH** | 0.52 | 57.63 | 5.97 | -18.63 |
| **Urea HA** | 0.35 | - | - | - |
| **FA pH 7** | 1.04 | 55.1 | 4.94 | -22.51 |
| **FA pH 10.6** | 0.91 | 50.09 | 4.42 | -22.67 |
| **FA pH 12.6** | 0.9 | 49.11 | 3.59 | -21.52 |
| **FA 0.5 M NaOH** | 0.12 | 52.96 | 4.45 | -21.62 |
| **Urea FA** | 0.11 | 54.02 | 13.44 | -23.4 |
| **XAD-4 acids pH 7** | 0.4 | 43.47 | 4.49 | -21.99 |
| **XAD-4 acids pH** | 0.19 | 50.35 | 5.29 | -21.69 |
| **XAD-4 acids pH** | 0.18 | 51.34 | 4.99 | -21.51 |
| **DMSO FA*** | - | 42.12 | 5.45 | -24.29 |

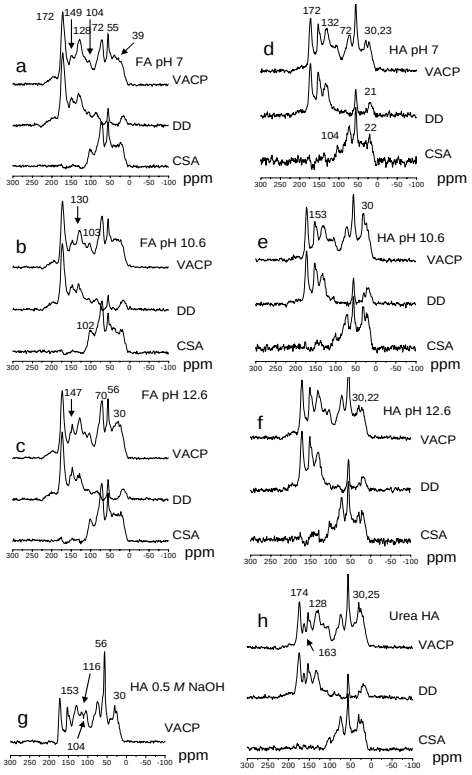



**Figure 1.** Full VACP-TOSS $^{13}C$ NMR spectra of FAs isolated from the incubated soil at pH7 (a), 10.6 (b), 12.6 (c), and of HAs isolated at pH7 (d), 10.6 (e), 12.6 (f), and 0.5M NaOH (g), and urea HAs (h) and corresponding DD and CSA spectra**.**

After two years of incubation, it is clear that lignin residues were still evident, especially in HAs, as revealed by sharp resonances for methoxyl at 55 ppm, and O-aryl C at 153 ppm (Fig. 1). The spectra of the HAs and FAs are relatively similar, though the FAs, are dominated by strong resonances for carboxyl, O-alkyl C, a sharp methoxyl, and relatively low aromatic C (110-140 ppm). The integrated areas data indicate that aliphatic C, methoxyl C from lignin units, and O-alkyl C (mainly from carbohydrate) gradually increased and the O-aryl C, the aryl and carboxyl groups progressively decreased as the pH values of the extracts increased. The unprotonated aryl carbons in FAs varied between 73~79%, and these were greater than for the corresponding HAs fractions (DD spectra).

The spectral shapes for the HAs and urea HAs, are also similar. Significant sharp resonances are observed for: methoxyl, O-aryl C, and carboxyl groups, a strong aryl C resonance indicate significant contributions from lignin-type units, and the O-alkyl C resonance for carbohydrate is confirmed by the distinct anomeric C at 102 ppm in the CSA spectra. The percent of unprotonated C in the aryl C region decreased progressively as the extractant pH values increased, and varied from 64.6~53.8%. The urea HAs contained greater enrichment of aliphatic groups, and less methoxyl, O-alkyl C, and aryl C.

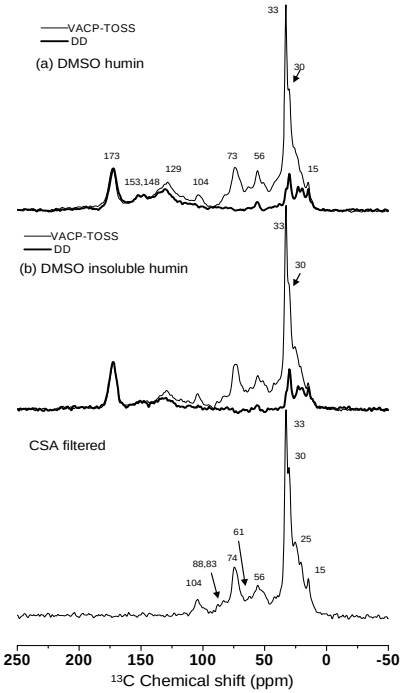



**Figure 2**. VACP-TOSS $^{13}C$ NMR spectra (thin line) of DMSO humin (a), DMSO insoluble humin (DIHU) (b) and the corresponding dipolar dephasing spectra (thick line), and chemical shift anisotropy (CSA) filtered- TOSS spectrum of DMSO insoluble humin (c).

The spectra (Fig. 2) for DMSO/acid humin are significantly similar, and show broad, but clearly resolved peaks. The VACP spectra have major peaks at 15, 30 (shoulder), 33, 56, 73, 104, 129 and 173 ppm. The largest peaks are in the aliphatic carbon region (0-46 ppm), indicating that both humin fractions are predominately aliphatic in nature) [1,4].

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# Humic products in agriculture and remediation technologies: chemical properties, biological activity and affectivity in laboratory and field experiments

O. YAKIMENKO [(a)*], V. TEREKHOVA [(a,b)], M. PUKALCHIK [(c)], A. STEPANOV [(a)]

(a) Department of Soil Science, Moscow State University, 119992 Moscow, Russia
(b) Ecology and Evolution Institute RAS, Moscow 119071 Russia
(c) Skolkovo Institute of Science and Technology, 143025 Moscow region, Russia
* E-mail: iakim@soil.msu.ru

**Keywords:** humic substances, crop production, soil remediation, environmental quality assessment

**Abstract.** The chemical composition of a wide number of commercial humic products (CHP), produced by industrial companies from lignite, peat, bottom sediments and organic wastes have been investigated. The biological activity of CHP was studied in laboratory tests using various endpoints: plant biometry, soil chemical and biochemical parameters, bioassays with the battery of test-cultures. The effectiveness of CHP as plant growth promoters and conditioners for degraded soils was evaluated in pot experiments. Examples of "environmental quality" integral assessment of CHP calculated basing on sets of soil chemical, ecotoxicological and biological are discussed.

## Introduction

Commercial humic products (CHP) produced by industrial companies from various organic raw materials are being increasingly used in agriculture. Depending on organic source material (mostly lignite, peat and composts), humic acids (HA), which are considered to be the active ingredient in CHP, are different in chemical structure and, consequently, in their efficiency as plant growth promotors and soil conditioners.

The objective of the study was to evaluate in a number of laboratories, pot and field experiment the potential of CHP as plant growth stimulators for agriculture and as remediating agents for contaminated soils.

## Materials and methods

Chemical properties of a number of lignite-, peat- and lignosulphonate- derived CHP (elemental content, IR-spectra, absorption and fluorescence spectra) are analysed. The biological activity of the same CHPs is examined in laboratory and field experiments using various endpoints: plant biometry, soil chemical and biochemical parameters, bioassays with the battery of test-cultures, effect on yield and crop quality in field trials. Potential of CHP to decrease the content of available species of heavy metals in contaminated soils is evaluated in case studies. Several approaches to assess the effectivity and "integrated environmental quality" of CHP are tested and compared using detoxication coefficient, Triad method, Harrington desirability function.

## Results and Discussion

### Chemical Characterization

Results of the chemical set of experiments showed, that organic matter genesis is the key factor influencing composition and structure of HA in CHP. HA from fossil coal is more aromatic, hydrophobic, contain fewer functional groups and mobile low-molecular components comparable to ones from sub-bituminous coal. Peat-derived HA is the most aliphatic, hydrophilic and enriched with amino acids and carbohydrates [1]. Besides, using absorption and fluorescence spectra one can discriminate organic matter source for CHPs from lignite, peat and lignosulphonate [2]. Some examples are given in Fig. 1 and 2.

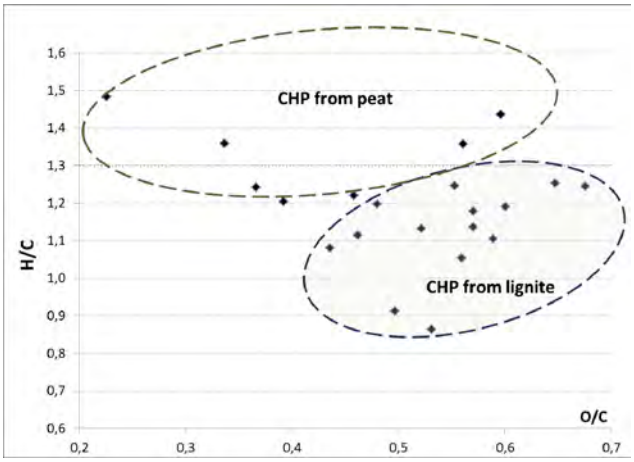



**Figure 1.** Atomic ratio diagram for CHPs from peat and lignite.

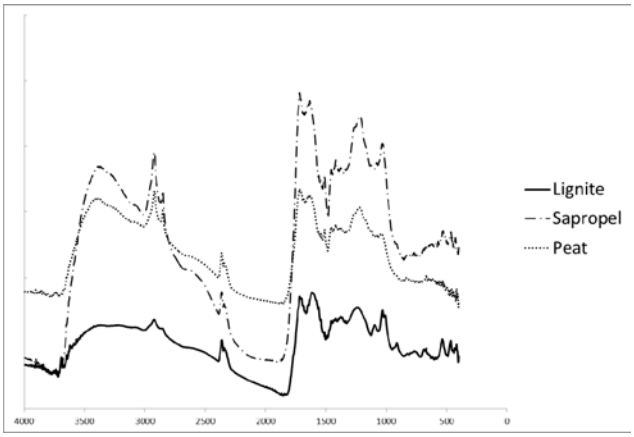



**Figure 2.** FTIR-spectra of HA extracted from typical CHPs from lignite, peat and sapropel.

**Bioassays and field trials**

Bioassays showed, that CHP can provide both stimulating and inhibiting effect, depending on the sensitivity of test-culture, experimental design, nature and properties of CHP. In laboratory tests most of analyzed CHP promoted the development of roots and shoots, stimulated fluorescence and growth of algae and affected the activity of some soil enzymes (urease, catalase and phosphatase) at concentrations 10-100 mg $L^{-1}$ [1, 3].

In field trials, pre-plant seed treatment or foliar spray with CHP promoted 4-8% increase of crop production. In heavy metal contaminated soils application of CHP decreased the toxic effects of Pb and Cd on plants.

**Integrated environmental quality assessment**

Integrated "environmental quality indicators", calculated using various endpoints and a number of statistical approaches have been calculated for a number of experiments and showed that CHP is capable to decrease negative effects of toxicants in the soil ecosystem.

The integral index of environmental risk on contaminated and background urban soils was calculated using TRIAD method, based on sets of soil chemical, ecotoxicological and biological data. The obtained environmental risk index showed that CHP applied at rates 0.0025 and 0.01% are able to reduce ecological toxicity in urban soils [3].

In another experiment, CHP at rate 0.1 g $kg^{-1}$were incubated with soil spiked with copper and then the soil extracts were tested in bioassays using the battery of tests: root and shoot length of *Sinapis alba* and *Hordeum vulgare*, fluorescence and growth of *Scenedesmus quadricauda*, $CO_2$ emission and nitrogen fixation. To assess integrated effects on soil ecosystem Harrington desirability function was used.

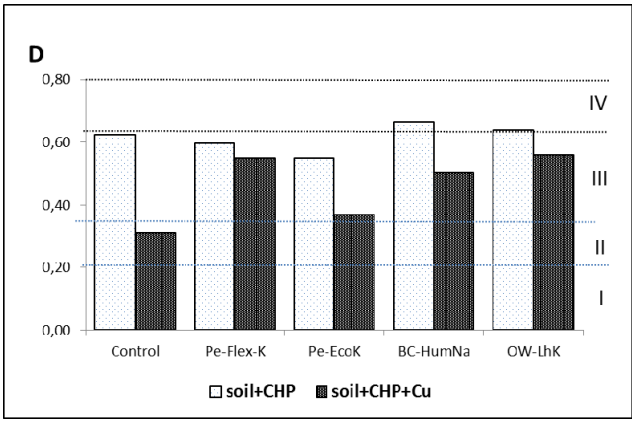



**Figure 3.** Integrated assessment of a number of CHPs' effects based on Harrington desirability function for incubated soils and soils spiked with Cu. CHP from peat (Pe-Flex and Pe-Eco), brown coal (BC-Hum) and lignohumate (OW-LhK) at rate 0.1 g $kg^{-1}$ ; ranges of the desirability scale D are as following: (I) - "very bad" [<0.2]; (II) - "bad" [0.2-0.37]; (III) - "satisfactory" [0.37-0.63]; (IV) - "good" [0.63-0.8].

Figure 3 shows the location of each treatment within the range of desirability scale. Two of the five CHP used (BC-HumNa and OW-LhK) characterized by a high physiological activity and transferred intact system without the addition of toxicant into the category of "good". Copper ions had a negative effect on soil biota (category "bad" in control). In spiked treatments, all the CHP showed detoxifying effect (D value falls into the category of "satisfactory"). Hence one should bear in mind that the mechanisms of action of various CHP are not clear and requires further focused research.

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# Effect of aluminum complexation on degradation of humic acid by humus-decolorizing fungus and $H_2O_2$

Y. YANAGI [(a)]*, K. YOSHIDA [(b)], N. MATSUO [(b)]

(a) Graduate School of Sciences and Technology for Innovation, Yamaguchi University, 7538515 Yamaguchi, Japan
(b) Faculty of Agriculture, Yamaguchi University, 7538515 Yamaguchi, Japan
* E-mail: yyanagi@yamaguchi-u.ac.jp

**Keywords:** decolorization, carbon accumulation, Andosols, Al–humus complex

**Abstract.** Formation of the aluminum (Al) complexes with soil organic matter is considered to be one of the major factors for higher carbon accumulation and carbon stabilization in Andosols. However, little has been reported on the stabilization of humic substances by complexing with Al. To evaluate the stability of humic acid complexed with Al (Al–HA complex), the decolorization of synthesized Al–HA complex by a fungus and $H_2O_2$ were examined. The percent decolorization of Al–HA complex showed lower values than HA showed by fungus and $H_2O_2$ at pH 4.0. These results suggest that the Al complexation improve the stability of HA.

## Introduction

Formation of the complexes of aluminum (Al) with soil organic matter (Al–humus complex) is considered to be one of the major factors for higher carbon accumulation and carbon stabilization in Andosols [1]. A number of the studies have shown the correlation between the organic carbon content and the amount of Al complexed with organic matter (e.g. Al extracted with sodium pyrophosphate) and the negative correlation between the organic carbon content and the carbon mineralization rate. Several researchers reported that the mineralizations of low molecular weight organic acids were suppressed by complexation with Al [2]. Consequently, these studies suggested that the soil organic matter is stabilized by the formation of Al-humus complex. Several mechanisms of soil organic matter protection by Al–humus complexes have been proposed (e.g. making functional groups more condensed, less assessable to enzymatic attack, and formation of stable aggregation) [3]. Since highly toxic $Al^{3+}$ is released from Al–humus complex under the low pH conditions, Al–humus complexes are also assumed to protect soil organic matter by decreasing microorganisms activity in non-allophanic Andosols with lower pH [1]. However, the mechanisms of soil organic matter stabilization by Al–humus complex is still incompletely understood. In particular, little has been reported on the stabilization of humic substances, that is the main component of soil organic matter, by complexing with Al.

The purpose of this study is to evaluate the stability of humic acid (HA) complexed with Al (Al–HA complex). We examined the degradation properties of synthesized Al–HA complex by the white-rot fungus, *Coriolus consors*, which have been reported to be active decolorizer of HAs [4] and $H_2O_2$ using the decolorization as the indicator of degradation.

## Materials and Methods

**Fungal strain.** The fungus *Coriolus consors* NBRC 9078, known to be an active decolorizer of humic acids, was used in this study as inocula.

**Preparation of humic acid and synthesize Al–HA complex.** Powdered HA sample was prepared from A horizon of non-allophanic Andosols (Mutsumi, Yamaguchi, Japan) according to the IHSS method. Powdered Al–HA complex sample was synthesized from the HA according to the previously reported method [5]. The prepared HA was dissolved in 0.1 mol $L^{-1}$ NaOH, passed through a $H^+$-saturated cation exchange resin, and adjusted to pH 4.0. The hydroxyl Al solution (0.02 mol $L^{-1}$, pH 4.0) was added to the HA solution until it was completely flocculated. The flocculated Al–HA complex was recovered, dialyzed with distilled water, and freeze-dried.

**Incubation conditions and detection of decolorization.** The decolorization of Al–HA complex was monitored on Martin liquid medium (10 ml, pH 6.8 and 4.0) suspending 0.01 g of powdered Al–HA complex. The medium inoculated with *C. consors* or added $H_2O_2$ (final concentration of 1.0 mol $L^{-1}$) was incubated at 25 ºC under the dark and shaking culture conditions. After the given incubation periods, the culture medium was filtered. The filtrate residue (residual HA) was dissolved in 0.1 mol $L^{-1}$ NaOH and the absorbance of this solution at 600 nm ($A_{600}$) was measured. Non-inoculated culture (control) and inoculated culture without HA (bio-blank) were also prepared and $A_{600}$ of these cultures were measured according to above-described procedures. The decolorization of humic acid was detected using the same procedure. The percent decolorization was calculated by the following formula: decolorization (%) = {$A_{600}$ of control – ($A_{600}$ of sample – $A_{600}$ of bio-blank)} / $A_{600}$ of control × 100.

## Results and Discussion

### Decolorization of Al–HA Complex by *C. consors*

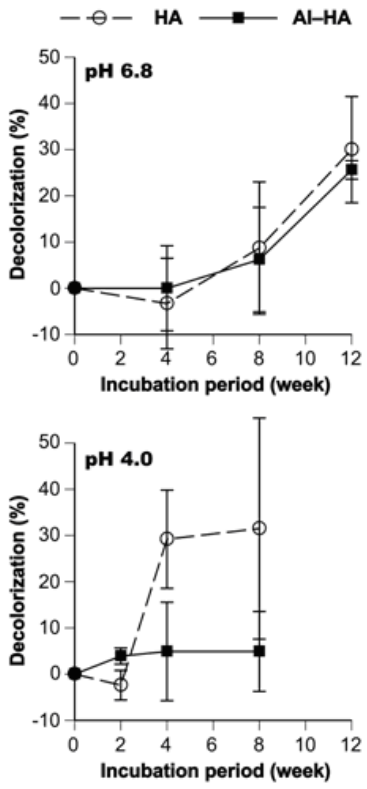



**Figure 1.** Decolorization of Al–HA complex and HA by *C. consors*. Upper is incubated at pH 6.8 and lower is incubated at pH 4.0. Bars indicate standard deviation.

The percent decolorization of Al–HA complex and HA by *C. consors* were shown in Figure 1. The percent decolorization of both Al–HA complex and HA increased with incubation periods at pH 6.8. There is no significant difference between HA and Al–HA complex. In contrast, there was no appreciable increase in percent decolorization of Al–HA complex with an increase in incubation periods at pH 4.0, although rapid increase in percent decolorization of HA was detected at 4 weeks incubation. Therefore, Al–HA complex showed significantly lower values than HA showed at 4 weeks onward. These results suggest that the formation of Al–HA complex improve the stability of HA against microbial degradation under the lower pH condition. The suppression of microbial activity by $Al^{3+}$ toxicity may be involved in this improvement of the stability. However, Al released from Al–HA complex might be changed to non-toxic hydroxyl Al at pH 6.8 because of the long-term shaking incubation.

### Decolorization of Al–HA Complex by $H_2O_2$

Figure 2 displays percent decolorization of Al–HA complex and HA by $H_2O_2$. Al–HA complex showed the lower value than HA showed at both pH conditions. These results suggest that Al–HA complex is resistant to inorganic oxidation than HA is. Therefore, it may be considered that Al complexation also improves chemical stabilization of HA structure. In addition, it was reported that the antioxidant activity of some flavonoids changed by complexation with Al [6]. Therefore, our results may be related to these activity change. Because the difference in percent decolorization between Al–HA complex and HA at pH 4.0 was larger than that at pH 6.8, however, the conformational structure of HA also might be related.

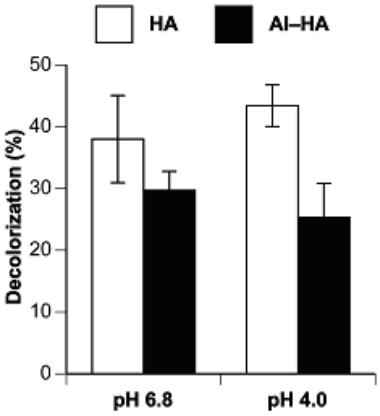



**Figure 2.** Percent decolorization of Al–HA complex and HA by $H_2O_2$ (1.0 $molL^{-1}$, 1 week incubation ). Bars indicate standard deviation.

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# Extraction of fulvic-acid-like compounds from mushroom (*Lentinus Edodes*) bed waste and their effect on plant growth

S. YOSHIZAWA [a]*, Y. HASHIKAWA [a], H. YAJIMA [b], R. TOMOI [c], Y. HIDAWA [d]

(a) Department of Environmental Science, Meisei University, Hodokubo, Hino, Tokyo 191-8506, Japan
(b) Department of Applied Chemistry, Tokyo University of Science, Kagurazaka, Shinjuku, Tokyo 162-8601, Japan
(c) Entrex Inc., BYGS 7F, Shinjuku, Shinjuku, Tokyo 160-0022, Japan
(d) Nihon Sofken Co., Ltd., Takatanobaba, Shinjuku, Tokyo 169-0075, Japan
* E-mail: yoshizaw@es.meisei-u.ac.jp

**Keywords:** mushroom bed waste, extraction, plant growth

**Abstract.** A huge amount of mushroom bed waste (BW) is generated in Japan. For the purpose of effectively utilizing the BW, we report on extraction of fulvic-acid-like compounds (FALC) from the BW according to the IHSS method. Characteristic spectral shapes from ultraviolet-visible (UV-vis) spectra and three dimensional (3D)-fluorescence spectra of the purified FALC were compared with those of the standard fulvic acid from the Dando soil in Japan. It was found that the BW contains high concentration of FALC. And it was also found that cultivation period of mushrooms is an important factor for humic substances forming.

## Introduction

After cultivation of mushrooms, the waste medium (the mushroom bed waste, BW) is generated, the quantity of which, in the whole country of Japan, are reported to be 300,000 tons a year. Mushroom bed is mainly composed of sawdust of broadleaf tree and corncob as cultivation medium and wheat bran, bean curd and rice bran are added as nutrients. The bed waste has been usually used as an auxiliary material for composting biomass. In order to use the BW as compost raw material, there are many technical problems, because the BW ferments anaerobically by which contains much water (moisture content of 80-90%) and, therefore, it is necessary to add auxiliary low moisture content materials to the BW. Further the BW requires mixing with livestock manure because the nutrient is biased. Therefore, a large quantity of the BW has been processed by the landfill and incineration.

There are few reports on extraction of the valuable ingredients contained in BW for the purpose of effectively utilizing the BW and for the purpose of developing a method for recycling the BW. In this study, we report on extraction of fulvic-acid-like compounds (FALC) from the BW and on the addition effect for cultivation soil of lettus growing.

## Materials and methods

### Mushroom BW

The BW after cultivation of commercial mushroom (*Lentinus edodes*) in the Ueno Village, Gunma, Japan was used in the experiment of this study. The mushroom bed was composed of broadleaf tree sawdust, wheat bran and corn bran. After three months for growing mushroom mycelium, mushrooms were cultivated. Two kinds of BW were used; one is after onetime harvest for about one month and another is after multiple harvests, which are abbreviated as BW (A) and BW (B), respectively.

### Procedure

After drying and fracturing the BW, which passed through a 1 mm mesh was used as a sample. In order to analyze the FALC, the colored components were recovered from FALC fraction with DAX-8 resin according to the International Humic Substances Society (IHSS) method. Characteristic spectral shapes from ultraviolet-visible (UV-vis) spectra and three dimensional (3D)-fluorescence spectra of the purified FALC were compared with those of the standard fulvic acid from the Dando soil in Japan designated from the Japanese Humic Substances Society (JHSS). The FALC concentration in the BW was calculated by preparing a calibration curve of the fluorescence intensity based on a concentration of the standard fulvic acid.

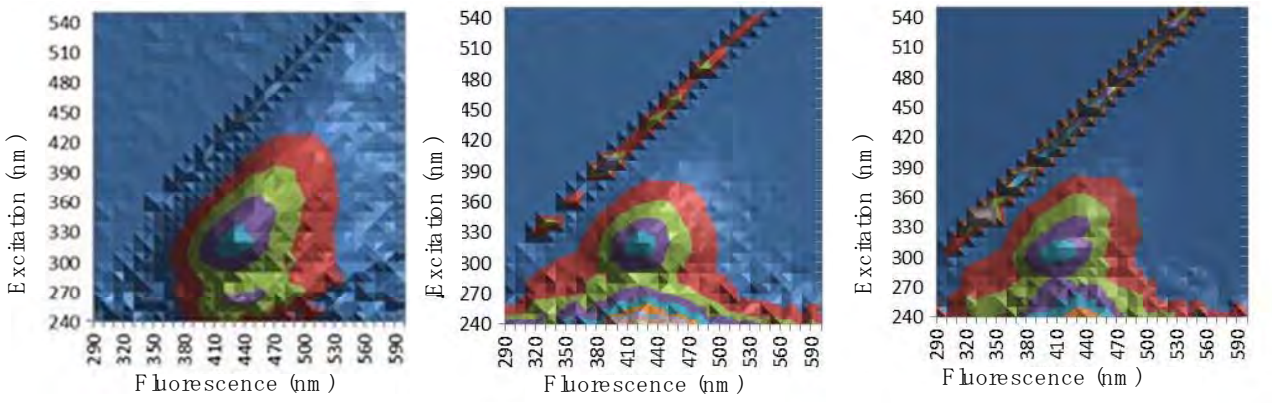
## Results and Discussion

### C and N Content

Table 1 shows comparison of ash content, TOC, TN and C/N ratio of the mushroom bed before growing mucelium and after the harvesting. The C/N ratio of the mushroom bed before growing mycelium is 59.7, which is higher than that of BW (A) of 35.2. In growing the mycelium, it absorbs both carbon and nitrogen from the bed to form any nitrogen compounds. When edible mushroom starts to grow, most of them disapperes in the mycelium. As the compounds which did not disappear remain in the mycelium, the TN, therefore, increased in the bed from before growing mycelium to after the harvesting to introduce the C/N ratio decreasing. [1].

**Table 1**.Comparison of each component and C/N ratio of the mushroom bed and the BW (A).

| | Bed before growing mycelium | BW (A) |
|---|---|---|
| ash (%) | 2.87 | 4.85 |
| TOC (%) | 41.2 | 39.4 |
| TN (%) | 0.69 | 1.12 |
| C/N ratio | 59.7 | 35.2 |




(a) DFA (310/420) (b) BW (A) (320/440) (c) BW (B) (310/410)

**Figure 1.** Comparison of 3D-fluorescence spectra between DFA and FALC from the bed waste (In parentheses, excitation / fluorescence wave length).

### 3D-Fluorescence and UV-vis Spectra

Figure 1 shows the comparison of 3D -fluorescence spectra between DFA and FALC extracted and purified from the mushroom (*Lentinus edodes*) bed waste according to the IHSS method. It showed a peak of FALC from the bed waste at almost the same position as the fluorescence peak 310 nm/410 nm (excitation/fluorescence) characteristic of DFA.

Figure 2 shows UV-vis spectra of FDA and FALC, which shows that there a peak around 230 nm and a gentle soulder from the short wavelength side to the long wavelength side in both DFA and fulvic-acid-like compounds.

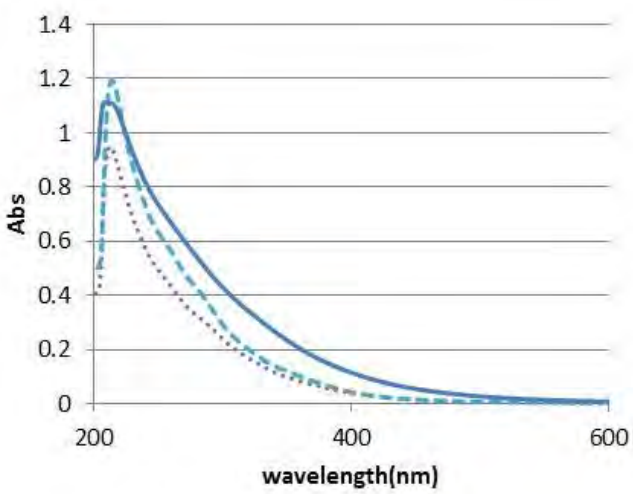



**Figure 2.** UV-vis spectra of FDA and FALC.
DFA; solid line, FALC from WA (A); dotted line and WA (B); dashed line.

### FALC Content in BW

Table 2 shows content of FALC in the BW (A) and (B). It is found that the BW contains high concentration of FALC. The FALC of the waste BW (B) was about twice that of (A). As BW (B) was after multiple harvest, it was found that cultivation period of mushrooms is an important factor for humic substances forming. It was found that the BW (B) was used as a waste bed after harvesting mushrooms several times, and the cultivation period of the mushrooms is an important factor for humic bed decay. White-rot fungus *Lentinus edodes* have ability to biodegrade cellulose and lignin. A manganese peroxidase depolymerized high-molecular-mass humic acids by forming FALC. [2]

**Table 2.** Comparison of FALC content in BW (A) and BW (B)

| | BW (A) | BW (B) |
|---|---|---|
| FALC content (mg/g) | 10.6 | 23.8 |

Finally, the additional effect on cultivation soil of lettus will be reported in the conference.

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# WINNERS OF TRAVEL AWARDS

# Molecular evolution of ageing dissolved organic matter in forest soils

S. BENK[(a)*], Y. LI[(a)], V. ROTH [(a,b)] , G. GLEIXNER[(a)]

(a) Max Planck Institute for Biogeochemistry, Molecular Biogeochemistry, 07745 Jena, Germany
(b)Thüringer Landesanstalt für Umwelt und Geologie, 07745 Jena, Germany
* E-mail: sbenk@bgc-jena.mpg.de

**Keywords:** FT-ICR-MS, radiocarbon, $^{14}C$, DOM, lignin, carbon cycle

**Abstract.** We correlated the calibrated radiocarbon ($^{14}C$) ages of forest soil dissolved organic matter (DOM) with the molecular composition of the samples in order to identify markers for DOM transport in the Critical Zone. We found that molecular entities with low $^{14}C$ ages were of lower molecular weight, more unsaturated, less oxygenized and less heteroatom-containing than those associated with higher $^{14}C$ ages. A chemical library search suggested that lignin dimers are prominent markers with low $^{14}C$ age that can be used as fast transport markers.

## Introduction

Time dependent evolution of DOM integrates the complex balance of biotic and abiotic processes that control DOM composition. Therefore, the search for compositional marker molecules to identify ecosystem constitution and source characteristics is gaining increasing interest [2]. The integration of electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FT-ICR-MS) allows studying the molecular composition of DOM in unprecedented detail, providing chemical information about thousands of mass-resolved peaks due to accurate determination of their exact mass [3]. Correlating the molecular composition of DOM to $^{14}C$ ages of the respective samples has been demonstrated to be a promising approach for identifying degradation states of marine DOM on the basis of individual sum formulae [4].

This study encompasses forest soil water DOM evolution in an effort to identify possible marker compounds for DOM in the Critical Zone. We employed $^{14}C$-AMS and in a correlation approach linked calibrated SPE-DOM $^{14}C$ ages to molecular composition data from ultrahigh-resolution mass spectrometry [1]. We hypothesize that (1) DOM formation and processing patterns are differing from marine DOM ageing trends (2) leaching from plant biomass governs the molecular composition of $^{14}C$-young carbon inputs (3) bioutilization of the plant leachates determines the composition of aged DOM.

## Materials and methods

Soil water samples were collected in March, May and November of 2005 at three different long term monitoring sites in Germany. The locations include a beech-dominated old-growth forest in the Hainich National Park, Thuringia, a maple and spruce mixed forest stand near the village of Thann, Bavaria and the Wetzstein spruce forest in Thuringia. The water samples were taken during a regular biweekly sampling scheme using permanently installed glass ceramic suction plates at 5 and 10 cm depth. DOM was extracted from soil water samples using PPL solid phase extraction [5] with an average extraction efficiency of 63%. FT-ICR-MS analysis was performed at the University of Oldenburg [1]. Aliquots of SPE-DOM in methanol were dried in tin capsules and radiocarbon analysis was carried out on a 3 MV Tandetron $^{14}C$-AMS at the MPI-BGC, Jena (Germany). Possible effects of residual ester-bound organic solvent on the $^{14}C$ activity were tested via comparison of bulk DO$^{14}C$ vs SPE-DO$^{14}C$ and multiple subsequent hydrolysis steps on sample material from the study sites. Age calibration was performed using OxCal and the NH1 bomb 2013 curve [6]. Spearman rank correlation was employed on relative intensities of mass peaks with respect to the $^{14}C$ calibrated ages of the samples. Rank correlation was used to account for known competitive ionization effects that affect the ion intensities of the individual masses [7]. Only mass peaks present in at least 8 out of 10 samples were considered for correlation in order to identify bulk trends. Statistical data analysis and correlation was done in R 3.3.1 and Canoco 5. For characterization of molecular entities matching of sum formulae with the ChEBI database was performed using the web interface at http://www.ebi.ac.uk/chebi/.

## Results and Discussion

The radiocarbon activity of the samples ranged from 102 to 117 percent Modern Carbon (pMC). All samples were “modern” and the calibrated age range encompassed ~50 years.

Molecular formulae that were either significantly positively or negatively correlated ($n = 10$, $p = 0.05$) with cal. $^{14}C$ age showed distinct differences in their m/z ranges as well as H/C and O/C elemental ratios (Fig. 1). Negative correlation of a molecular formula with age herein means the relative intensity of this sum formula was higher in SPE-DOM with low calibrated $^{14}C$-age than in samples with higher $^{14}C$-age. The masses of negatively correlated formulae ranged from 250 to 450 m/z while the positively correlated formulae ranged from 450 to 650 m/z. For heteroatom-containing sum formulae, only positive correlation significance levels were reached for some cases.

Many of the $^{14}C$-“young” correlated sum formulae can be estimated to represent lignin-type structures from their elemental composition (H/C: 0.6 - 1.7, O/C: 0.1 - 0.6; [8]). While there is no direct way of receiving

structural information from FT-ICR-MS data, matching with the ChEBI library revealed that the group of negatively $^{14}$C-age correlated formulae could possibly be mostly comprised of methoxylated phenylpropanoid-derivatives. Lignin dimers were prominent suggestions among flavonoids, coumarins and stilbenes, all of which result from the phenylpropanoid pathway [9]. This suggests that the group of negatively correlated compounds represents inputs of plant-derived molecules that feature recently assimilated carbon.

Positively $^{14}$C-age correlated sum formulae were more saturated and more highly oxygenized, their molecular weight was higher and in some cases they contained nitrogen or sulfur as heteroatoms. From the elemental composition in the Van Krevelen (VK) space the positively correlated sum formulae could theoretically represent more tannin-like structures (H/C: 0.5 - 1.5, O/C: 0.6 – 1.2; [8]. Database search did not yield tannin-type structures for positively correlated sum formulae even though tannin structures are annotated in the ChEBI library and contrastingly structures of phenylpropanoid-derivatives that were glycosylated were suggested. The tannin region in the VK space lies between the lignin and carbohydrate areas (H/C: 1.5 - 2.2, O/C: 0.6 – 1.2) [8] and consequently the structure suggestions are very likely.

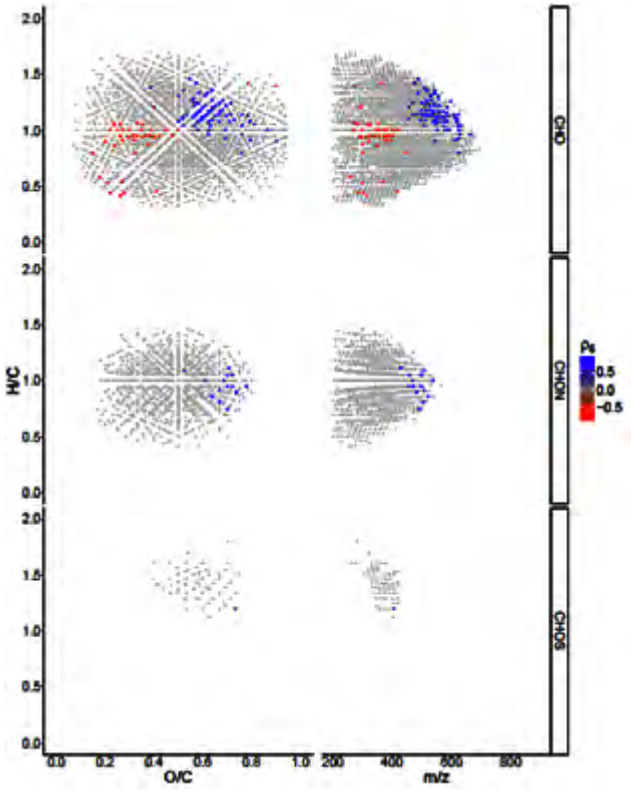



**Figure 1.** Van Krevelen and H/C vs m/z plots separated according to elemental composition showing the molecular correlation pattern with calibrated $^{14}$C ages of SPE-DOM (p = 0.05, n = 10). Orange formulae are negatively ($^{14}$C-"young") correlated. Blue formulae are positively ($^{14}$C-"old") correlated.

It has recently been established that glycosylation of phenylpropanoids greatly affects their bioavailability [10]. The glycosylation of phenylpropanoids increases their stability decreases the toxicity. Consequently, the suite of positively $^{14}$C-age correlated compounds could represent detoxified plant leachate degradation products.

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# Evaluation of humic substances from hydrochar and of Anthrosols profile (Amazonian Black Earth) by spectroscopic techniques

L. R. BENTO [(a)*], C. A. MELO [(a)], A. B. MOREIRA [(a)], O. P. FERREIRA [(b)], M. C. BISINOTI [(a)]

(a) Instituto de Biociências, Letras e Ciências Exatas, UNESP, Univ Estadual Paulista, Campus São José do Rio Preto, Departamento de Química e Ciências Ambientais, Laboratório de Estudos em Ciências Ambientais. Cristóvão Colombo, 2265, São José do Rio Preto, São Paulo State, Brazil, 15054-000.
(b) LaMFA- Laboratório de Materiais Funcionais Avançados, Departamento de Física, Universidade Federal do Ceará, P.O. Box 6030, Zip Code 60455-900. Fortaleza - CE, Brazil.
* E-mail: bento_rai@hotmail.com

**Keywords:** hydrothermal carbonization, humic substances hydrochar, Terra Preta de índio.

**Abstract:** The aim of this study was to evaluate humic substances (HS) using UV-Vis, Molecular Fluorescence and InfraRed along an anthropogenic profile of "Terra preta de índio" soils of Amazonian and also the HS of a hydrochar produced with sugarcane industry by-products. Based the results was observed that the 0-20 cm fraction showed the highest aromaticity and the fraction 80-100 cm showed the lowest aromaticity. Hydrochar HS presented similar characteristics as HS of TPI in the 0-20 cm fraction, with a more intense C=C band and lower fluorescent intensity.

## Introduction

In the Amazon is present land strips, called Terra Preta de Indio (TPI), which are soils known for their high fertility. These soils were formed by anthropogenic action, in which the Indians burned their wastes and threw them into the soil [1]. This ancient practice has inspired the production of biochar in order to produce materials that can be used as soil conditioners to improve fertility or stock carbon. Some authors have demonstrated that it is possible to produce hydrochar rich in carbon and nutrients by hydrothermal carbonization of vinasse and sugarcane bagasse [2, 3].

Hydrochar has around 70% of organic matter in its composition and the characteristics of the HS is unknown. Thus, the objective of this study was to evaluate HS extracted along a TPI profile, as a reference of fertile soil to compare it with the HS extracted from hydrochar.

## Materials and methods

**Hydrothermal Carbonization.** Hydrochar was prepared using 19.2 g of sugarcane bagasse, 384 mL of vinasse and 16 mL of concentrated $H_3PO_4$ according to Melo et al. [2].

**Soil sampling.** The soil TPI was collected in Maués – AM in-depth profile of 1 meter. Samples were fractionated in: 0-20 cm, 20-40 cm, 40-60 cm, 60-80 cm, 80-100 cm.

**Extraction and characterization of HS.** HS of hydrochar and soil samples were extracted following the protocol of IHSS. Functional groups in the HS were analyzed using Attenuate Reflectance (ATR) with Fourier Transform Infrared Spectroscopy (FTIR) (Perkin Elmer, Spectrum Two UATR, USA). UV-Vis spectra of an HS solution containing 10 mg $L^{-1}$ of total organic carbon (TOC) (pH 8, 0.05 mol $L^{-1}$ $NaHCO_3$) were obtained with UV-VIS spectrophotometer (UV-2600, Shimadzu, Kyoto, Japan) and the $E_4/E_6$ ratio was evaluated. The same sample was evaluated by molecular fluorescence (Cary Elipse, Varian, Australia) and the degree of humification ($H_{465}$) was obtained from the emission spectrum [4]. Total luminescence (TL) spectra, in the form of excitation-emission matrices (EEM), were recorded over the emission wavelength range from 300 to 600 nm and the excitation wavelength from 250 to 500 nm [5].

## Results and Discussion

The $E_4/E_6$ ratio varied from 3.70 (fraction 0-20 cm) to 4.23 (fraction 80-100 cm) as shown in Table 1, and the ratio obtained for hydrochar was 4.8. The low ratio $E_4/E_6$ is correlated with aromaticity and a high ratio indicates smaller molecules or more oxygenated groups [6]. Thus, the HS of the hydrochar appears to have more content oxygenated groups in relation to HS of TPI.

TL spectra of the HS soil (Figure 1) show that the fluorescent intensity (FI) increases with depth and was observed the presence of the same two fluorophores at an excitation/emission wavelength: 305–308 ex/497–520 em and about 458–460 ex/530–562 em. The two peaks can be respectively ascribed to chromone derivatives, such as flavonoids, and extensively conjugated quinones and phenols with an elevated polycondensation degree [5, 7]. The index $H_{465}$ obtained from emission spectrum indicated that the HS surface had a lower degree of humification ($21.06 \times 10^3$) than the deeper fractions ($46.6 \times 10^3$) as shown in Table 1. The fractions of profile 20-40 cm, 40-60 cm, 60-80 cm presented similar $E_4/E_6$ ratio, fluorescent intensity (FI) and humification degree. The results showed that the HS of the 0-20 cm fraction has a higher degree of aromaticity compared to HS in deeper fractions, may be ascribed to a greater association between surface HS and metal ions. The fraction 80-100 cm is a transition fraction between the anthropogenic horizon and the characteristic soil of the region (yellow oxisol). This fraction presented higher FI, $H_{465}$ and ratio $E_4/E_6$, that indicates lower aromaticity and higher index humification.

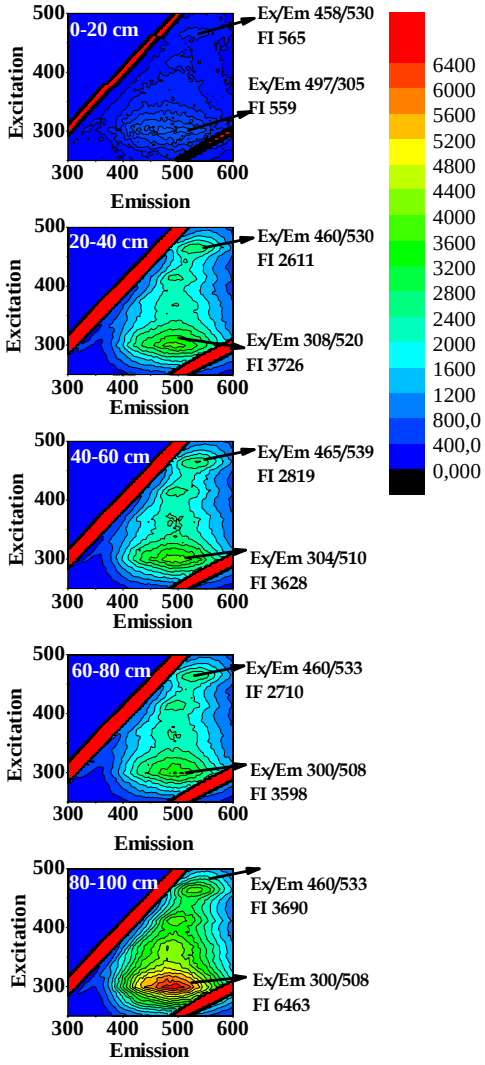



**Figure 1.** Total luminescence (TL) spectra, in the form of excitation-emission matrices (EEM).

**Table 1.** Indexes obtained by UV-Vis and fluorescence.

| Sample | $E_4/E_6$ | $H_{465}(x10^3)$ |
|---|---|---|
| 0-20 cm | 3.70 | 21.06 |
| 20-40 cm | 3.76 | 34.50 |
| 40-60 cm | 3.85 | 34.39 |
| 60-80 cm | 3.89 | 34.50 |
| 80-100 cm | 4.23 | 46.63 |
| Hydrochar | 4.80 | 71.74 |

Figure 2 shows that HS from hydrochar featuring smaller FI like 0-20 cm fraction. The $H_{465}$ index of HS from hydrochar presented is greater than fractions of soil. HS hydrochar showed a more intense C=C band in FTIR spectrum in relation to HS of the soil indicating higher aromaticity.

With the data obtained the HS of the hydrochar seems to have greater similarity with the fraction 0-20 cm, although presented with a high degree of humification.

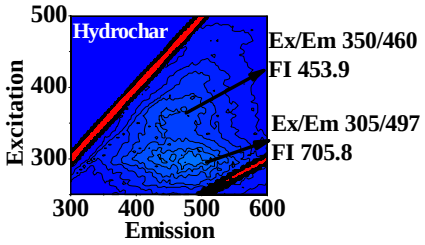



**Figure 2.** Fluorescence excitation-emission matrices (EEMs) from hydrochar.

The FTIR analysis allowed to infer the presence of O-H, C-H, C=O and C=C in the HS of the soil. The 0-20 cm fraction presented a more intense band of C=C in relation to the deeper fractions, that corroborates the data the fluorescence and UV-Vis.

## Conclusion

It was concluded that the aromaticity decreased with increasing depth for the soil profile. The presence of higher intensity in the C = C band corroborates with the UV-VIS and molecular fluorescence. Results showed that the HS of the hydrochar studied presented comparative characteristics of HS of TPI soils of the superficial fraction (0-20 cm).

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# Effect of temperature and humic substances on initial bacterial adhesion to reverse osmosis membranes

S. BINAHMED [(a)], S. R. CASTRILLÓN [(a)*], R. HOZALSKI [(a)*]

(a) Department of Civil, Environmental, and Geo-Engineering, University of Minnesota, 500 Pillsbury Dr SE, Minneapolis, United States
* E-mail: sromerov@umn.edu; hozal001@umn.edu

**Keywords:** organic fouling, single cell force spectroscopy, reverse osmosis, humic acid

**Abstract.** Membrane separation processes are increasingly being used in water treatment. Reverse osmosis (RO) membranes are used for brackish and seawater desalination as well as wastewater reclamation. Biological and organic fouling are major technical problems affecting the performance of membrane processes. To formulate strategies for biofouling control, it is critically important to understand the mechanism of initial bacterial adhesion on membrane surfaces, and how it is affected by different environmental parameters. In this work, we study the effect of temperature and the presence of a humic conditioning film on the bacterial adhesion to RO membrane surfaces.

## Introduction

Reverse osmosis (RO) membranes are used for potable water production from brackish and seawater [1] and water reclamation from secondary wastewater effluents [2]. Natural waters contain dissolved organic matter (DOM) while dissolved effluent organic matter (EfOM) is a major constituent of secondary effluent in wastewater treatment plants. [3] EfOM comprises humic substances from the drinking water supply together with microbial products resulting from the biodegradation of human and industrial wastes [4]. Accordingly, RO feeds in both potable water and water recovery operations contain different molecular weight DOM composed partly or mainly of humic substances [2, 5]. Humic acids (HA) and fulvic acids (FA) are the components of humic substances that are soluble at pH > 2, with humin making up the remainder. [4]

The presence of DOM contributes to the formation of a conditioning or fouling layer on the surface of the membrane which can provide nutrients to bacteria and facilitate biofilm formation by promoting bacterial adhesion to the surface [6, 7]. The resulting colonization of the membrane surface and subsequent formation of a biofilm (i.e., biofouling) is a major technical problem in membrane processes, leading to decreased peremance and selectivity [8–10].

RO sytem performance is affected by several parameters such as membrane and feed properties as well as temperature. It has been well established that temperature affects the performance of RO membranes such that water permeability increases and salt rejection decreases with increasing temperatures [11]. Additionally, higher temperatures increases the rate of biofilm formation but yield thinner biofilms compared to biofilms formed at lower temperatures that cause similar pressure drop increase [1]. However, little is known about the effect of temperature on the initial bacterial adhesion especially in the presence of a conditioning layer.

In this study, we investigated the effect of temperature on intial bacterial adhesion to the surface of RO membranes. Our goal is to study the effect of HA or FA conditioning layers on the initial stages of RO membrane biofouling as a function of temperature.

## Materials and methods

**Fouling and Characterization of RO membrane**. RO membranes will be fouled with Suwannee River HA or FA using a crossflow system. In addition to water permability and salt rejection, surface properties (hydrophilicity, charge, and roughness) of the RO membrane will be compared before and after fouling. Also, the formation of the fouling layer on the surface of the RO membrane will be confirmed by Fourier Transform Infrared Spectroscopy (FTIR).

**Single-cell force spectroscopy experiments**. Atomic force microscopy (AFM) will be used to determine the adhesion forces of bacterial cells on the surface of clean and HA or FA-coated RO membranes. The technique, known as single-cell force spectroscopy (SCFS), involves immobilization of a single *Pseudomonas fluorescens* bacterium to the tip of an AFM cantilever (SCFS) [12]. Experiments were carried in a temperature-controlled fluid cell at 25 ºC, 30 °C and 40°C. Viability of the cell will be verified in situ using fluorescent microscopy.

## Results and Discussion

**Single-cell force spectroscopy experiments**. Figure 1 shows a representative force curve reflecting the interaction of a single bacterial cell with a membrane interface. The force curve consists of an approach curve collected as the cantilever fucntionalized with the cell is engaged on the surface, and a pull-off force curve that demonstrates adhesiveinteractions between the bacterium and the surface as the cantilever retracts from the surface. Data inferred from the force curve include the maximum adhesion force andrupture separation (distance at which interactions between the cell and the membrane surface vanish). In addition, the pull-off force curve exhibits a series of consecutive spring-like instabilities that are due to the stretching and force unfolding of biomacro-molecules on the cell surface. Unfolding events such as

those shown in Figure 1 demonstrate that surface proteins or proteinaceous structures mediate bacterial adhesion on membrane surfaces. Because temperatures above ambient induce heat unfolding of protein, we expect to observe stronger adhesion forces as the temperature is increased.

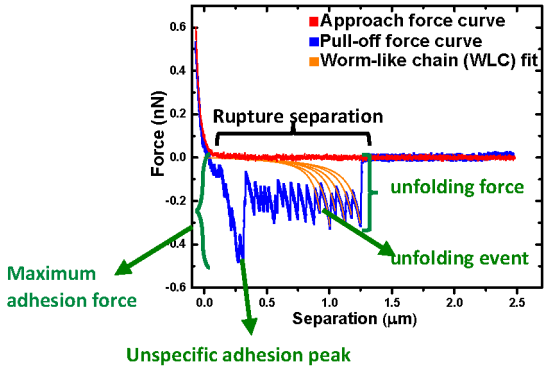



**Figure 1.** Representative force curve from SCFS experiments showing approach and retraction curves. Unfolding events (sawtooth pattern) are fitted to the WLC model [12].

Our previous study investigated the interaction between a *P. fluorescens* bacterium with pristine and Upper Mississippi River NOM-fouled ultrafiltration (UF) membranes. [12] The presence of NOM fouling layer was confirmed by FTIR as shown in Figure 2. SCFS results showed weaker interaction forces between the cell and the NOM (Upper Mississippi River) fouled membranes which was attributed to the hydrophilic nature of NOM.

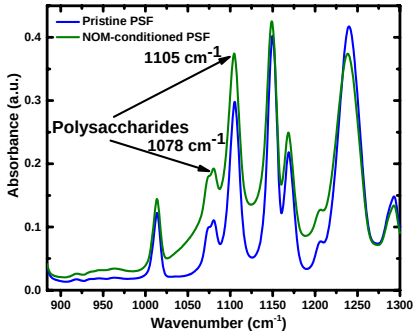



**Figure 2.** FTIR spectra of pristine and NOM-conditioned UF membranes.[12]

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# Lability and spectroscopic characteristics of dissolved organic matter from Amazonian rivers

I. C. CONSTANTINO [(a)*], A. M. TADINI [(b)], S. MOUNIER [(c)], A. B. MOREIRA [(a)], M. C. BISINOTI [(a)]

(a) Instituto de Biociências, Letras e Ciências Exatas, UNESP - Univ Estadual Paulista "Júlio de Mesquita Filho", São José do Rio Preto-SP, Brazil.
(b) Instituto de Química de São Carlos, Universidade de São Paulo, P.O. Box 780, 13560-970 São Carlos-SP, Brazil
(c) Laboratoire PROTEE, Université de Toulon - CS 60584, TOULON, CEDEX 9, France
* E-mail: isabela.carreira@gmail.com

**Keywords:** Amazon, freshwater, labile, recalcitrant, organic matter, fluorescence and CP/PARAFAC.

**Abstract.** Black (BW) and white (WW) waters showed distinct characteristic, including the Labile (LOM) and recalcitrant OM (ROM) content. Spectroscopic parameters and fluorophores were determined. In the drought season, WW rivers content most ROM, according to less decreasing of fluorophores during kinetic experiment and higher $SUVA_{254}$ values. For the flooded period, a distinct behaviour was observed. BW had around three times less LOM content than WW samples, despite the highest dissolved organic carbon (DOC) concentration due to the intense runoff process observed during the rainy season.

## Introduction

It is widely known the importance of dissolved organic matter (DOM) in aquatic systems and its play a role in dynamics of other species given information on biogeochemical cycles, transport, complexation and toxicity of pollutants. A recent pool of organic matter is considered a more reactive and labile (LOM) fraction, being transformed by chemical, biological or photochemical process in recalcitrant (ROM) organic matter. An approach to quantify LOM and ROM in freshwater samples using $H_2O_2$ kinetic consumption was used [1]. The Amazonian region is one of the most relevant biomes of the world, its rivers show different characteristics in order of NOM content and pH. Assessment of temporal DOM quality is an important factor to understand biogeochemical cycles. For that matter, the main objective of this study was to quantify LOM and ROM in water samples from the Amazonian rainforest and to correlate them with optical properties of the chromophore dissolved organic matter (CDOM), as absorption and fluorescence.

## Materials and Methods

The sampling of freshwater was done during drought and flooded seasons. Representative samples of black-water (BW) rivers were collected in Carú river (3°1'6.10"S; 58°34'43.64"W) and Anebá river (2°57'46.28"S; 58°29'57.17"W). White-water (WW) samples were collected in the Amazonas river (3°29'10.95"S; 58°53'3.36"W) and Madeira river (3°10'56.13"S; 58°31'10.21"W). Samples were filtered through a 0.45 µm acetate cellulose membrane. The LOM and ROM concentrations were quantified [1]. For that purpose, microcosm experiments were conducted in jacketed glass reactors (19°C) which received $H_2O_2$ until 7.1 µmol $L^{-1}$, $H_2O_2$ disappearance was quantified [2]. Dissolved organic carbon (DOC) was carried out using catalytic combustion (TOC-VCSN analyzer, Shimadzu, Japan). Aliquots were collected and DOC alteration during the experiment was characterized by $SUVA_{254}$ ($A_{254}$/[DOC]), and Spectral Slope ratio $S_R$ ($S_{275-295}/S_{350-400}$) employing UV-Vis Spectrophotometer (2600, Shimadzu, Japan) and Molecular Fluorescence (Cary Elipse, Varian, Australia) [3, 4, 5]. Fluorescence spectra scans were obtained in Emission-Excitation Matrix (EEM) using a Spectrofluorimeter Cary Eclipse (Varian, Australia). The Spectra 3D (EEM) of the samples were treated by mathematical method Parallel Factor Analysis (CP/PARAFAC) to evaluate the contribution of fluorophores present in the CDOM samples during the kinetic experiment.

## Results and Discussion

In the low flow period, BW showed higher DOC content and lower pH as expected (Table 1), commonly observed in riverine systems that receive great input of organic-rich horizons runoff and leaching processes. For all freshwater samples ROM amounts were predominant (Table 1). For BW, LOM concentrations were higher (1.01 and 0.79 mg $L^{-1}$) than WW (0.73 and 0.61 mg $L^{-1}$). In the rainy period, the opposite behaviour was observed to DOM lability. WW (Amazonas 1.49 and Madeira 1.03 mg $L^{-1}$) showed LOM content three times higher than BW (Carú 0.49 and Anebá 0.61 mg $L^{-1}$), despite lowest DOC concentration.

Some optical properties were determined as $SUVA_{254}$, values ranged from 8.8 to 9.8 mg $C^{-1}$ $m^{-1}$ and from 10.7 to 15.3 mg $C^{-1}$ $m^{-1}$ for BW and WW in drought season, respectively. These results indicated that WW showed more dissolved aromatic carbon content. $S_R$ range 0.78-1.08 (drought) and 0.77-0.89 (flooded) as expected by DOM terrestrial origins (< 1), being highest values obtained to WW (drought) and BW (flooded) are related with lower molecular weight [3].

CP/PARAFAC treatment identified 3 components (C1, C2 and C3) with a Concordia of 89.8%. Fluorophores were humic-like formed by two peaks not separated (C1 - principal $\lambda_{em}/\lambda_{exc}$ = 420/250 nm and 420/310 nm and C2 - principal $\lambda_{em}/\lambda_{exc}$= 466/250 nm and

$\lambda_{em}/\lambda_{exc}$= 466/365 nm) as well. A single peak for Tryptophan-like (C3 - $\lambda_{em}/\lambda_{exc}$= 337/275 nm) [6].

**Table 1** – Dissolved organic carbon (DOC), Labile (LOM) and Recalcitrant (ROM) organic matter concentrations in black water and white water samples from the Amazonian region collected during drought and rainy seasons.

| | Samples | pH | DOC mg $L^{-1}$ | LOM mg $L^{-1}$ | ROM[a] mg $L^{-1}$ |
|---|---|---|---|---|---|
| *Drought season* | *Carú* | 5.35 | 3.20±0.23 | 1.01 | 2.20 |
| | *Anebá* | 4.80 | 3.15±0.31 | 0.79 | 2.36 |
| | *Amazonas* | 7.26 | 2.43±0.11 | 0.73 | 1.70 |
| | *Madeira* | 7.30 | 1.60±0.14 | 0.61 | 0.99 |
| *Rainy season* | *Carú* | 6.67 | 6.56±0.06 | 0.49 | 6.07 |
| | *Anebá* | 6.75 | 6.70±0.09 | 0.61 | 6.09 |
| | *Amazonas* | 7.04 | 4.35±0.11 | 1.49 | 2.87 |
| | *Madeira* | 7.33 | 2.11±0.06 | 1.03 | 1.08 |

[a]ROM = DOC - LOM (mg $L^{-1}$)

Decreasing of the components during the kinetic experiment were determined, they were normalized by DOC concentration. Mostly, during drought season, FDOM from BW rivers had higher decrease for all components (C1: 71%, C2: 80% and C3: 96%). Meanwhile, for WW rivers variation of components were much less (Amazonas river: C1: 5.5%; C2: 13.4%; C3: 44% and Madeira river: C1 not decreased; C2: 14.2%; C3: 64.9%). These results indicate the presence of fluorescence dissolved organic matter (FDOM) humic-like and protein-like more labile in BW rivers than WW rivers. For this study, FDOM protein-like (C3) as the more labile component for all rivers, and they had mainly humic-like components more recalcitrant FDOM as showed by $SUVA_{254}$ index also. Decreasing order of lability was similar when compared to decreasing of FDOM components (Carú river> Anebá river > Amazonas river > Madeira river). These results corroborate with the supramolecular hypothesis, where the humic-like content, present in all samples, would be formed by small labile molecules associated [7].

This study contributes to a better understanding of spectroscopic characterization of DOM in Central Amazonian region. Unpublished results for LOM and ROM were obtained for BW and WW. We highlighted the importance of the chemical composition of DOM as a major factor responsible to lability, and fluorescence data could provide the lability approach used in this work.

We can conclude that BW showed high organic matter content and lower pH as expected. LOM was higher for BW, indicating that around 30% of organic matter content was not degraded by chemical or biological processes. WW showed more aromaticity in organic matter content and presented the highest values to $SUVA_{254}$ (drought season). These results will be very important in order to understand organic matter behaviour in the aquatic environment.

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# Production of hydroxylating species from DOM model sensitizers

K. D. COUCH [(a)], G. MCKAY [(a)], F.L. ROSARIO-ORTIZ[(a)]

(a) Department of Civil, Architectural, and Environmental Engineering, University of Colorado, 4001 Discovery Drive, Boulder, CO
* E-mail: Kylie.Couch@colorado.edu

**Keywords:** DOM, photochemistry, hydroxyl radical

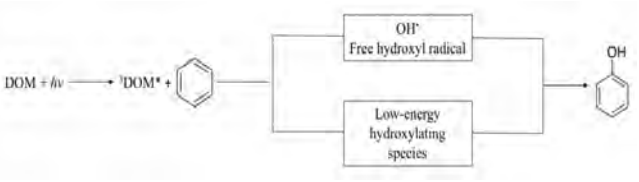
**Abstract.** The photochemical production of hydroxylating species from dissolved organic matter (DOM) has been well documented. Lower energy hydroxylating species, in addition to free hydroxyl radical, are present upon the photolysis of DOM. The ability of DOM model sensitizers to photohydroxylate •OH probe compounds was examined, with the goal of identifying species responsible for low-energy photohydroxylation. The main classes of sensitizers tested were aromatic ketones, quinones, and hydroxybenzoic acids. Preliminary results suggest that quinone moieties are the primary species responsible for low energy hydroxylators observed in DOM samples.

## Introduction

Organic matter is present in natural waters in the form of dissolved molecules, colloids, and particulate matter. Dissolved organic matter (DOM) is involved in a wide range of chemical and physical processes in water, including sorption, precipitation, aggregation, and aquatic photochemistry. Aquatic photochemistry involves interaction of natural waters and sunlight, which results in the transformation of organic compounds present in the system. This has quickly become a field of interest to scientists and engineers alike because aquatic photochemical processes can be utilized to remediate contaminated waters. The photochemistry of DOM in particular has been in question for quite some time, however, due to its inherent structural ambiguity its photochemistry is not well understood.

There are two kinds of photochemical reactions in surface waters, direct and indirect. Direct photochemistry involves the degradation of organic compounds through interaction with light. Indirect photochemistry involves the creation of short lived reactive intermediates (RI) when a system is exposed to light and the subsequent degradation of organic compounds by those RI [1]. In natural waters the presence of DOM heavily influences the overall photochemical behavior of the system. Although it has been shown that the interaction of DOM with sunlight produces a number RI including hydroxyl radicals ($OH^{\bullet}$), singlet oxygen ($^1O_2$), and triplet state dissolved organic matter ($^3$DOM*) [1], the mechanisms by which this occurs are not well understood. Ultimately, the presence of RI in natural waters influences the fate and transport of organic contaminants.

The photochemical production of hydroxylating species from dissolved organic matter (DOM) has been primarily classified as free hydroxyl radicals [1]. It has been shown by removing $H_2O_2$ that these quantum yields are, in part, independent of photo-Fenton chemistry. Initially, it was thought that hydroxyl radicals were responsible for the observed hydroxylating capacity, however, more recently it has been shown that lower energy hydroxylating species, in addition to free hydroxyl radical, are present upon the photolysis of DOM, as seen in Scheme 1 [2].




**Scheme 1.** Different pathways by which hydroxylating species can be produced by photolysis of DOM.

Although the hydroxylating capacity of DOM has been documented, the chromophoric DOM moieties responsible for photohydroxylation have not been identified. In this study, the ability of DOM model sensitzers to photohydroxylate •OH probe compounds was examined, with the overall goal of identifying species responsible for low-energy photohydroxylation reactions.

## Materials and Methods

**Model Sensitizers.** The main classes of sensitizers tested were aromatic ketones, quinones and hydroxybenzoic acids and probe compounds included benzene and caffeine. These model sensitizers were chosen to represent a range of one-electron reduction potentials and DOM structural moieties [3]. All chemicals were purchased from either VWR or Sigma Aldrich. Sensitizer solutions were prepared at a concentration of 100 uM in ultrapure water.

**Photochemistry Experiments.** Photochemistry experiments were performed with an Oriel solar simulator, model number 81171 (Newport Corporation, California). This device was equipped with a 1000 W xenon lamp and an air mass 1.5 global filter. Chemical actinometry was used to determine what the lamp irradiance was with a p-nitroanisole/pyridine system.

## Results and Discussion

To date, photochemical experiments have been performed with the model sensitizers listed in Table 1, using benzene as the probe for detecting the presence of hydroxylating species. The product of hydroxylating benzene is phenol; therefore, the presence of phenol is representative of the hydroxylating capacity of the model

sensitizer in question. As seen in Figure 1, preliminary results suggest that quinone moieties are the primary species responsible for low energy photohydroxylation reactions observed in DOM samples because there was not notable production of phenol upon the photolyzation of ketone sensitizers.

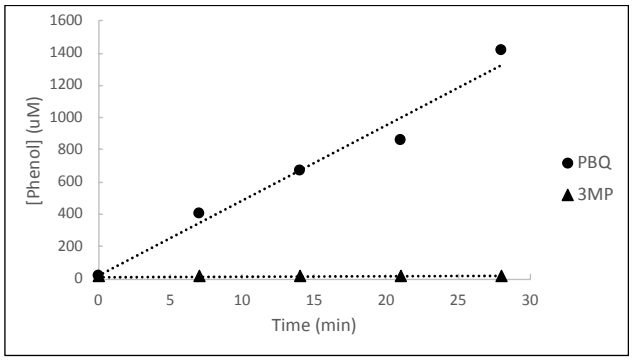
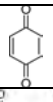
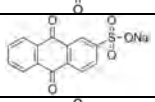
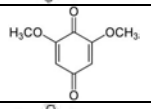
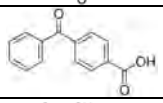
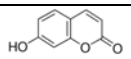
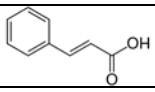



**Figure 1.** Hydroxylation of benzene from the photosensitization of p-benzoquinone (PBQ) and 3-methoxyacetophenone (3MP).

A summary of results to date can be found in Table 1, with the presence of phenol being indicative of the phtotohydroxylating capacity of the model sensitizer. In addition to benzene, caffeine will be used as a probe compound because the hydroxylation of caffeine has different activation energy than the hydroxylation of benzene.

Additionally, competition kinetics experiments will be conducted employing methanol as a hydroxyl radical quencher at concentrations ranging from 200 µM to 0.1 M in order to determine the relative reactivity of these low energy hydroxylators compared to free hydroxyl radical. Also, activation energies of the hydroxylation of benzene by the low energy hydroxylating species produced by quinone model sensitizers will be performed. This will allow for comparison to the activation energy of the hydroxylation of benzene by free hydroxyl radical and in turn will give a better understanding of the nature of the low energy hydroxylating species produced by quinones.

**Table 1.** Summary of preliminary results of irradiation of quinones (red), ketones (blue), and other sensitizer species (green) with benzene as a probe for hydroxylating species.

| Model Sensitizer | Structure | Production of Phenol from Benzene |
|---|---|---|
| p-Benzoquinone |  | Yes |
| Anthraquinone-2-sulfonate |  | Yes |
| 2,6-Dimethoxy-p-benzoquinone |  | Yes |
| 4-Benzoylbenzoic acid |  | No |
| 3-Methoxy acetophenone |  | No |
| Umbelliferone |  | No |
| *trans*-Cinnamic acid |  | No |

Providing the scientific and engineering community with information about the fundamental photochemistry of DOM will contribute to understanding how sunlight driven remediation of natural waters works and will contribute to modeling the photochemistry of natural aquatic systems.

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# Use of iron mining waste and natural organic matter (NOM) in the synthesis of magnetic hybrids for remediation of chromium and nitrophenol

D. CRUZ [(a)], B. SANTOS [(a)], I. SILVA [(a)], G. CUNHA [(a)], L. ROMAO [(a,b)*]

(a) Laboratory of Natural Organic Matter, Department of Chemistry, Federal University of Sergipe, Brazil
(b) Institute of Chemistry, UNESP, National Institute of Alternative Technologies for Detection, Toxicological Evaluation and Removal of Micropollutants and Radioactive Materials (INCT-DATREM), SP, Brazil.
* E-mail: lucianeromao@uol.com.br

**Keywords:** Iron mining waste, NOM, $CoFe_2O_4$, chromium, nitrophenol

**Abstract:** This work proposes the recycling of iron mining residues as precursors in the manufacture of magnetic hybrids using NOM-rich water. XRD and FTIR analysis confirmed the formation of the cobalt ferrite phase and the presence of NOM in the HbSF and HbLM hybrids. These presented removal percentages of chromium from tannery effluent of 96.0% (HbSF) and 84.0% (HbLM) achieved in 30 minutes. It was also possible to reuse them in six consecutive cycles. The recycled matrices and the recovered chromium ions showed excellent catalytic activity in the reduction of nitrophenol.

## Introduction

The mining and extraction of iron are vital in the global economy, but produce large amounts of hazardous wastes that can lead to extensive environmental degradation [1]. The sludge, which is the principal waste generated, has no commercial value, so it is accumulated in ponds that require expensive and complicated maintenance. Hence, there is a need to develop techniques for the permanent safe disposal of waste from iron ore extraction, in order to avoid catastrophic accidents [1]. One promising technology for the use of iron mining waste is to employ recovered iron ions in the synthesis of magnetic hybrids. These materials optimize techniques of adsorption and catalysis, since they can be easily removed from aqueous media by the simple application of an external magnetic field, enabling their reuse and the recovery of the adsorbed metals after desorption [2].

The objective of this study was to synthesize magnetic hybrids using iron ions recovered from iron mining tailings as precursors and NOM-rich water. Evaluation was made of the potential of the products in the removal of total chromium from tannery effluent, as well as the ability to reuse the adsorbents. Studies were performed of the catalytic potential of the adsorbent matrices impregnated with chromium, and also of the recovered chromium ions, in the reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP), a compound considered a valuable input in the pharmaceutical and dye industries. The NOM was used to compose the organic phase of the hybrid materials because it is rich in functional groups, such as carboxyls and phenolic hydroxyls, which can form complexes with metal species, hence contributing to interactions with the ions of the inorganic phase and enhancing the adsorptive properties of the materials [2].

## Materials and methods

The method used to synthesize the hydrids was adapted from previous work [2], substituting the synthetic iron precursor by either iron salts derived from wastes previously treated by the mining company, or material extracted from the Rio Doce river after failure of the Fundão dam in Mariana in 2015. The resulting hybrids were denoted HbSF and HbLM, respectively. These materials were characterized by infrared absorption spectroscopy (FTIR) and X-ray diffraction (XRD). Adsorption assays and reuse cycles were performed according to the methodology described previously [2].

After the cycles of reuse, the samples and solutions resulting from the final desorption were dried, producing the materials denoted HbSF_Rec, HbLM_Rec and Cr_Rec, respectively. The catalytic reduction of 4-NP was performed in the presence of $NaBH_4$ as reducing agent, described previously [2].

## Results and Discussion

It can be seen from Figure 1 that the most intense diffraction peaks obtained for the hybrid materials were in agreement with the diffraction data for the $CoFe_2O_4$ standard available in the Inorganic Crystal Structure Database (ICSD #192031), showing that this phase was formed, and evidencing the efficiency of the proposed route using iron ions recovered from wastes as precursors and NOM-rich water.

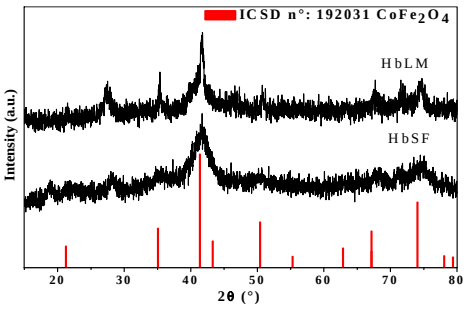



**Figure 1.** XRD patterns of the HbSF and HbLM hybrids and the ICSD #192031 standard.

Figure 2 shows broad and intense bands at around 3430 $cm^{-1}$, attributed to the stretching of O-H groups, indicative of water, alcohols, phenols, and/or carboxylic acids. A band at 1626 $cm^{-1}$ corresponded to stretching of

C=C bonds of aromatic groups, while a band at 1366 $cm^{-1}$ was attributed to symmetric stretching of $COO^-$, characteristic of NOM. A band at 596 $cm^{-1}$ could be attributed to the Fe-O bond characteristic of ferrites [2]. A band at around 1100 $cm^{-1}$ in the HbSF spectrum, attributed to the stretching of S=O bonds, was indicative of the presence of sulfate derived from the precursor used in the synthesis, which was mainly composed of ferric sulfate. In the spectrum of HbLM, a band at around 990 $cm^{-1}$ could be attributed to the Si-O bonds of silicate impurities derived from the tailings sludge from which the iron ions were extracted [3].

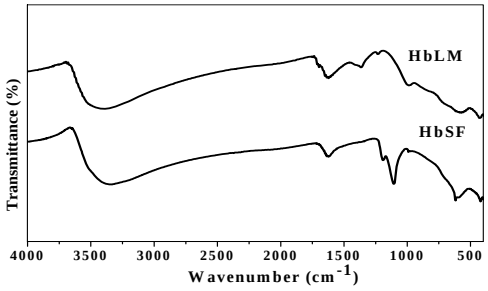



**Figure 2.** FTIR spectra of the HbSF and HbLM hybrids.

The adsorption kinetics for the HbSF (Figure 3a) and HbLM (Figure 3b) hybrids showed excellent adsorption in short periods of time. In only 30 min, the removal percentage and adsorption capacity values achieved were 96.0% and 7.21 mg $g^{-1}$ for HbSF, and 84.0% and 6.31 mg $g^{-1}$ for HbLM. These results showed the suitability of these materials for use in environmental remediation, since time is a very important variable in terms of potential environmental harm.

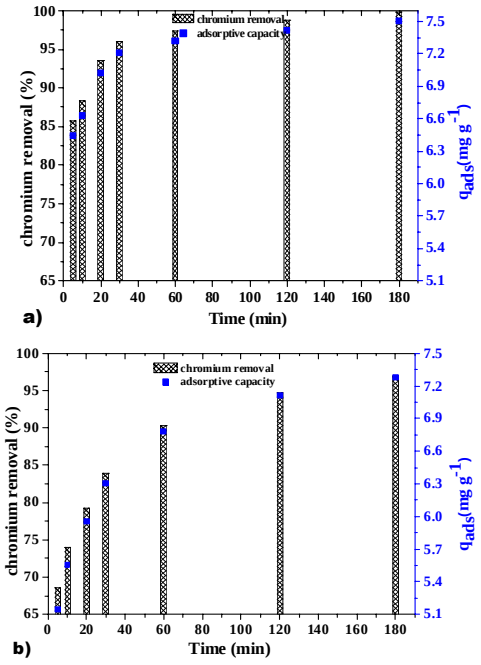



**Figure 3.** Chromium removal percentages (%) and adsorption capacities ($q_{ads}$), as a function of time, for: a) HbSF and b) HbLM. Conditions: $C_i$ = 75.0 mg $L^{-1}$, pH = 4.7, $m_{ads}$ = 0.1 g.

The adsorption sites and metal speciation were among the factors that influenced the adsorptive performance of materials. At the natural pH of the effluent, the main complexation sites of NOM are in deprotonated ($-COO^-$) and protonated (-OH) states, while the chromium species are present in the cationic form ($CrOH^{2+}$). Hence, the effectiveness of the adsorption depends on two types of interactions: electrostatic attraction between the $CrOH^{2+}$ species and the $-COO^-$ groups, and ion exchange between the -OH groups and the $CrOH^{2+}$ species [2].

The reuse assays (data not shown) revealed that the adsorption percentages obtained for the HbSF and HbLM hybrids decreased slightly from 91-98% in the first cycle to 77% in the second cycle, and remained constant at around 56% in the last four cycles. Nonetheless, the results showed that these materials could be satisfactorily reused in the removal of chromium from a real effluent.

Figure 4 shows the UV-Vis spectra for the reduction of 4-NP to 4-AP. Before addition of the catalysts, a band at 400 nm corresponded to 4-nitrophenolate ions formed by the addition of 4-NP to $NaBH_4$ solution. After the addition, there was disappearance of the band at 400 nm and the appearance of a band at around 296 nm, associated with the formation of 4-AP. Hence, the 4-NP was completely reduced.

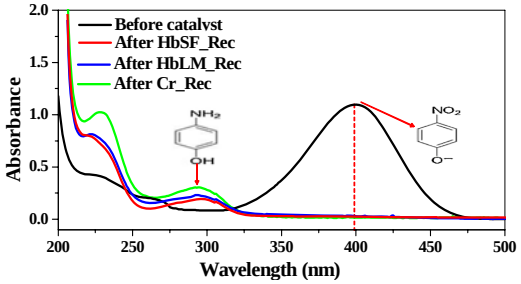



**Fig. 4.** Adsorption/desorption percentages for the hybrids employed in consecutive reuse cycles.

The materials resulting from the adsorption/desorption cycles provided 4-NP conversion rates of 99.9%, requiring times of 37, 42, and 1 s for HbSF_Rec, HbLM_Rec, and Cr_Rec, respectively. High catalytic potential was exhibited in all three reuse cycles, together with short processing times (data not shown). The excellent performance of the hybrids highlighted the effectiveness of the synthesis performed using a simple and inexpensive procedure, at ambient temperature, employing a natural reagent and precursors considered to be wastes.

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# The specific role of endophytic microbiome in the mechanism of action of humic substances as plant growth promoters

D. DE HITA [(a)*], M. FUENTES [(a)], J. M. GARCÍA-MINA [(a)]

(a) Biological agriculture and Chemistry group, Department of Environmental Biology, University of Navarra, 31008 Pamplona, Spain
* E-mail: ddehita@alumni.unav.es

**Keywords:** endophyte, microbiome, humic substances, plant growth promotion.

**Abstract:** Endophytic microbiome composition has been evaluated in order to determine the effect of the humic acid in microorganisms inhabiting the cucumber tissues. 16S and ITS region were sequenced as marker genes to assess the bacterial and fungal communities changes induced by humic acids. Major changes were found in bacterial microbiome; on the other side, fungal microbiome seemed to remain stable after humic acids treatment.

## Introduction

Humic substances (HS) are widely described as plant growth promoters. The HS mediated growth promotion may be due to the existence of plant hormones or hormone-like molecules embedded in HS structure (IAA or CK) [1] or to an action of the HS "per se" [2]. Our group has reported significant increases in cucumber shoot and root growths upon the radicular application of humic acids (HAs) obtained from leonardite [3, 4]. Nevertheless, many aspects of the mechanisms responsible for these beneficial effects of HS on plant growth remain unexplored. One of them is related to the action of HS on plant endophytic microbiome.

The study of microbiomes is increasing the knowledge of microorganism interaction with environments, human beings, animals and plants. These novel studies are clarifying our vision about diseases, our way of life or the effect of some agricultural practices. Endophytes are the microorganisms inhabiting a plant and can be isolated from a sterilised plant tissue. The inner plant is an interesting environment due to its stability and lack of microbial competence in comparison with soils or rhizosphere environments. Some of their beneficial plant growth promotion (PGP) traits are biological nitrogen fixation, plant hormone biosynthesis, siderophore production, P solubilization, etc…[5, 6]. In order to explore this new field, our study focused on the effect of a leonardite humic acid on cucumber endophytic microbiome.

## Material and methods

### Plant growth and DNA extraction

An experiment was carried out to assess the effect of humic substances on the endophyte microorganisms. The cucumber seedlings were grown up in a hydroponic system inside a growth chamber which settings were: photoperiod 15h-day and 9h-night, temperature range used between 25ºC (day) – 21ºC (night) and 250 µmol $m^{-2}$ $s^{-1}$ of PAR (Photosynthetic active radiation). One third of these plants were treated with 100 mg/kg C of humic acid applied to the roots (root application), other third were treated with a foliar application (100 ppm C of HA + Tween 20 as surfactant) and the final third were control plants treated with a foliar application of deionized water and Tween 20. Plants were harvested at two different times: 72 hours after treatment and 192 hours. Plants were sterilized, freeze and ground for the DNA extraction. DNA was extracted separately from leaves, stem and roots in order to evaluate the endophyte inner distribution. Then, DNA was sequenced by a Mi-seq System (Illumina) in Argonne National Laboratory (USA) facilities.

### Data processing and analysis

The marker genes selected for sequencing were 16S; single copy gene in bacteria chromosome highly use in microbiome projects and there is a number of available datasets; and on the other side, ITS gene was chosen for similar reasons to assess fungi endophyte microbiomes. The gene sequencing data were trimmed, chimaeras debugged and representatives clustered. That was achieved following the RDP-UPARSE pipeline [7]. Once the data were processed, the sequencing data were analysed using R packages such as phyloseq, DESeq2, MetagenomeSeq, vegan or ggplot2.

## Results and discussion

### Bacterial endophytic microbiome on cucumber plants

In order to assess the effect of HA from leonardite on plant endophytic microbiomes, the following barplot (Fig. 1) shows the Phylum distribution according to the HA treatment applied to cucumber plants.

As shown in Figure 1, the major groups occurring in endophytic microbiome on cucumber plants are *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*. These phyla are commonly found in plant microbiomes [8]. The main differences observed at this taxonomic level are the increase in *Firmicutes* presence in treated plants, remarkably high in those plants treated with foliar HA and the absence (or barely presence) of minor groups such as *Parvachaeota*, *Pacearchaeota*, *Fusobacteria* or *Gemmatimonadetes* in HA-treated cucumber plants.

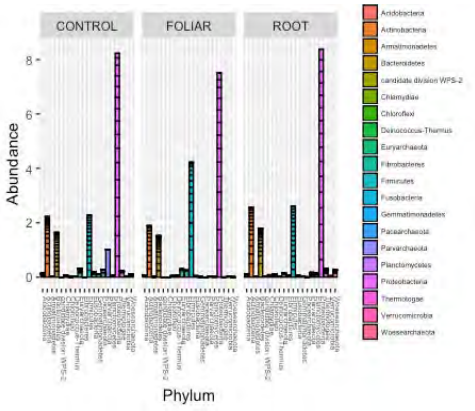



**Figure 1.** The figure shows the distribution of the relative abundances according to phylum taxa in cucumber when humic acids are applied by a foliar aerosol, by direct root application or without humic acids application (control).

The impact of these changes in plants is potentially convenient for agriculture. The analysis at class taxonomic level (Fig. 2) shows major changes in agriculturally important groups as *Bacilli*, *Bacteroidia*, *Clostridia* or *Sphingobacteriia*. These major changes in HA-treated plants (foliar and root application) could be due to a bacterial abundance increase caused by the growth promotion mediated by HA (for instance, an increase in roots surface) or HA itself contributes to this bacterial abundance increase.

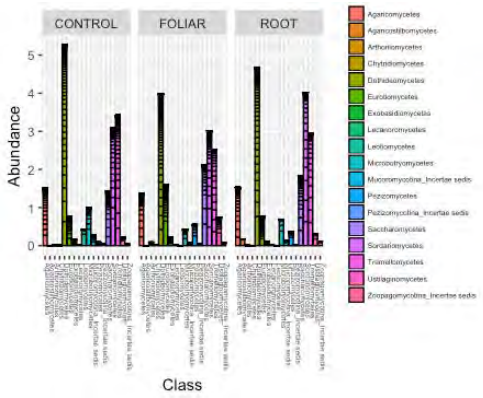



**Figure 2.** The figure shows the distribution of the relative abundances according to class taxa in cucumber plants when humic acids are applied by a foliar aerosol, by direct root application or without humic acids application (control). Only groups with 1% relative abundance unless in one sample appear in this figure.

### Fungal endophytic microbiome on cucumber plants

The effect of HA from leonardite on plant endophytic microbiomes was assessed by a class taxa barplot (Fig. 3) that shows the most abundant taxa in cucumber plants.

Fungal endophytic microbiome on cucumber is diverse but similar between different treatments. Only minor changes appear among HA-treated plants and control plants, such as the increase of *Saccharomycetes* relative abundance or the *Microbotryomycetes* abundance decreasing. It is not clear if these changes are beneficial, neutral or harmful to the plant. New experiments should be addressed to clarify the HA application effect on fungal endophytic plant microbiome.

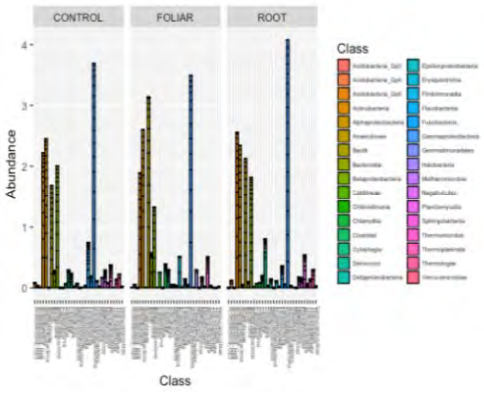



**Figure 3.** Relative abundance distribution according to class taxa in cucumber tissues when humic acids are applied by a foliar aerosol, by direct root application or without humic acids application (control). Only groups with 1% relative abundance unless in one sample appear in this figure.

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# Influence of feed additives of humic nature on coagulation disorders in laboratory rats after combined stress

L. M. DIACHENKO [(a)]*, L. M. STEPCHENKO [(a)]

(a) Dnipro state agrarian and economic university, Serhii Efremov Str., 25, Dnipro, 49600, Ukraine
* E-mail: linadyach@ukr.net

**Keywords:** hemostasis, feed additives of humic nature, stress, fibrinogen

**Abstract** The results of the study of all stages of hemostasis in rats using the feed additives of humic nature "Eco-Impulse Animal" and "Gumilid" are presented in the work on the effects of combined stress. The effect of combined stress on the studied parameters of blood coagulation in rats was determined. The comparative estimation of influence of the studied fodder additives on the indices of hemostasis of rats was conducted.

## Introduction

The effect of stress on a living organism may not cause significant violations, due to the presence of natural mechanisms to eliminate its negative effects. The nature of violations against the background of stress necessarily depends on its type, strength and duration. Combined stress makes it difficult to work for the natural mechanisms of body protection and as a result we can follow the manifestations of oxidative stress, hypoxia and changes in homeostasis, including indicators that characterize coagulation hemostasis [1]. It is known that coagulation hemostasis is a three-stage step-by-step process for the activation of serine proteases, which ends with the formation of a fibrinous thrombus and depends on a large number of plasma and cellular factors of blood coagulation. This creates a large number of targets for the effects of combined stress. A current promising area is the search for substances that can reduce the negative impact of hypoxia and oxidative stress, and thus increase the nonspecific adaptive capacity of the organism. It is important to find the very natural substances that could exhibit protective properties against the background of stress. It is established that feed additives of humic nature can quickly absorb and incorporate into metabolic processes of an organism and can increase the natural resistance of an organism, however, their influence on indicators of coagulation hemostasis has not been investigated to date [1]. Therefore, the purpose of this study is to evaluate the effect of feed additives of humic nature on all levels of coagulation hemostasis in rats against the background of combined stress.

## Materials and methods

The studies were conducted on white, sexually-mature, young male rats with a body weight of 180-200 g based on vivarium. Throughout the experiment, rats were kept under standard conditions, with free access to water and feed. Animals were divided into four groups of 5 animals each: 1 (control) intact animals; 2 - rats, which were kept under standard conditions; 3 - rats received supplements to the diet of the feed additive "Gumilid" (TU U 15.7-00493675004 2009) in the amount of 5 mg/kg of body weight, and 4 - animals received supplementary dietary additive "Eco Impulse Animal" (TU U 10.9-00493675-008 2016) at a rate of 2.5 mg/kg body weight. Water and feed additives were administered orally to animals of all experimental groups, individually for 18 days. After that, animals from 2, 3 and 4 groups were subjected to simulated stress. Based on the model of water-immobilization stress [2] in combination with the model of emotional stress, due to which the effect of combined stress was achieved.

Blood for research was taken from the heart (right ventricle) under thiopental anesthesia (60 μg/kg) the day after the stress modeling. In rats' citrate plasma was determined: activated partial thromboplastin time (APTT), thrombin time (TT), prothrombin time (PTT), prothrombin by Quik (PQ), number of fibrinogen by Rutberg method, and prothrombin ratio (PR), and prothrombin index (PI) were calculated. All manipulations with animals were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (Strasbourg, 1986). The probability of differences was estimated by t-criterion of Student. The results were considered probable at $P \leq 0.05$.

## Results and discussion

For the evaluation of the coagulation level of rat's hemostasis against the background of the use of feed additives of humic nature and the effects of combined stress, standard tests were selected: APTT, TT, PTT, and others.

In the first group of animals, activated partial thromboplastin time (APTT) is within the control values, and after simulating combined stress in animals of group 2 it is reliably prolonged by almost 3 times ($p < 0.001$), as compared to the results in rats 1 group. APTT characterizes the internal pathway for activating coagulation hemostasis in rats, and its value depends on the large number of components that participate in the process of formation of the prothrombin complex. The result may indicate the presence of a large number of fibrin degradation products that could have been formed as a result of the activation of blood coagulation processes in rats, with the subsequent formation of fibrin fibers against the background of combined stress. At that time, the animals in the 3 group who received the feed additive "Gumilide", after the simulation of combined stress, APTT was significantly shorter compared with values in

animals of group 2 by 60% ($p < 0.01$), but significantly increased by 17% ($p < 0.01$), respectively, in comparison with values in animals of the control group. In animals, 4 group who received the feed additive "Eco-Impulse Animal" APTT were significantly shorter by 55% ($p < 0.01$), respectively, compared with values in animals in group 2 and prolonged by 32% ($p < 0.001$), compared with group 1 values. Combined stress causes prolongation of APTT, but due to the use of feed additives humic nature, there is a significant decrease in this indicator.

The prothrombin time (PTT) and prothrombin ratio (PR) in animals of group 2 significantly increased by 23% ($p < 0.001$), compared to values in rats in group 1. In animals of the 4 group receiving the feed additive "Eco-Impulse Animal", the PTT and PR values increased by 66% ($p < 0.01$), respectively, compared with values in animals in group 1. In animals of 3 group, the values of PTT and TR were within the reference values and did not differ significantly from the control ones. Thus, changes in the PTT indicate a violation of the course of external pathway activation of the process of coagulation of blood of rats for the effects of combined stress. Use of the feed additive "Eco Impulse Animal" causes more pronounced growth of PTT and PR in the background of combined stress, which completely differs from its effect on the internal mechanism of blood coagulation in rats.

The prothrombin index (PI) in the rats of group 2 significantly decreased by 19% ($p < 0.001$), respectively, in comparison with values in rats in group 1. In animals, 4 group of PI significantly decreased by 39% ($p < 0.001$), compared with values in animals in the control group. Such PI values indicate increased blood coagulation and the potential risk of thrombosis. This indicator in animals of the 3 group, which in addition to the diet supplemented with dietary additive "Gumilide" before the stressful period, did not differ significantly from the values of the control group.

In rats of group 2, after the simulation of combined stress, the value of prothrombin for Quik (PQ) significantly decreased 3 times ($p < 0.001$), compared with values in animals in group 1. PQ in animals of the 3rd group remained within the reference values. Therefore, the use in the stressful period of the feed additive "Gumilod" supports the survey values within the normal range or brings them closer to it.

Thrombin time (TT) in animals of group 2 was prolonged by 54% ($p < 0.001$) compared with the value in animals of group 1. In rats, 4 group, which additionally added the feed additive "Eco-Impulse Animal" in before the stressful period, observed a significant prolongation of PM in 2.6 times ($p < 0.001$) compared with values in animals in group 1 and 72% ($p < 0.001$) in comparison with animals of the 2 group that were modeled by combined stress. In animals, 3rd group receiving the feed additive "Gumilide" in a stressful period (18 days), the TT value was within the reference values. The results of the TT show the process of thrombin formation (the 2nd phase of blood coagulation of rats) and the dependence on the external and internal pathway of the formation of prothrombinase, the elongation of which may indicate the presence of indirect anticoagulants or the deficiency of plasma coagulation factors at each stage of hemostasis.

The content of fibrinogen in rats of group 2, which was modeled by combined stress, increased by 5.5 times ($p < 0.001$) in comparison with control animals. Such a sharp increase in the content of fibrinogen may be due to the activation of all stages of blood clotting against the background of prolonged combined stress, which led to a shortage of intermediate reaction components and the accumulation of end-product products. In animals, 3rd group that received a feed additive "Gumilide" before the stressful period, there was a significant decrease of 2.5 times ($p < 0.001$), compared with the value in the rats of group 2, but compared with the value in animals of group 1, is marked increase in the content of fibrinogen in 2 times ($p < 0.001$). In the plasma of rats, the 4 group that received the feed additive "Eco Impulse Animal" before the stressful period, the fibrinogen content was 3 times higher ($p < 0.001$) higher than that of animals in group 1 and 1.5 times smaller ($p < 0.001$), in comparison with values of group 2.

Modeling of combined stress in rats causes a violation of the flow of external and internal mechanisms of the formation of the prothrombinase complex, the transformation of prothrombin into thrombin and fibrinogen into fibrin.

In rats receiving the "Eco Impulse Animal" feed additive, the effect on the test species was not unambiguous, which may depend on the method of obtaining the feed additive and the source of the source material. Therefore, the use of this fodder additive requires further research.

The use of the supplements "Gumilide" promotes the maintenance of the main indicators of coagulation hemostasis within the reference values, which indicate the value of APTT, PTT, PR, PI and PQ, which can be considered as preventive protection against the influence of stress factors.

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# Hydrochar and process water from hydrothermal carbonization of sugarcane industry by-products: germination studies and humic substances characterization

L. G. FREGOLENTE [(a)*], F. S. MAZATTI [(a)], J.V. DOS SANTOS [(a)], A.B. MOREIRA [(a)], O.P. FERREIRA [(b)], M. C. BISINOTI [(a)]

(a) Instituto de Biociências, Letras e Ciências Exatas, UNESP, Universidade Estadual Paulista, Campus São José do Rio Preto, Departamento de Química e Ciências Ambientais, Laboratório de Estudos em Ciências Ambientais. Cristóvão Colombo, 2265, São José do Rio Preto, São Paulo State, Brazil, 15054-000.
(b) Laboratório de Materiais Funcionais Avançados (LaMFA), Departamento de Física, Universidade Federal do Ceará (UFC), Fortaleza, Ceará State, Brazil, P.O. Box 6030, Zip Code 60455-900.
* E-mail: laisfregolente@hotmail.com

**Keywords:** germination, plant growth, hydrochar, vinasse.

**Abstract** Brazil is one of the largest agricultural producer countries, but it imports around 90% of its fertilizers. Hydrothermal carbonization of sugarcane by-products could generate a hydrochar, capable of acting as the soil conditioner or as fertilizer and process water, rich in carbon and nutrients. Therefore, the goal of this study was to evaluate process water and hydrochar carbon released solutions in the maize germination process. The results showed that diluted process water stimulated shoot and root development, and the hydrochar carbon released from water solution stimulated root growth and the one from acid solution stimulated shoot development.

## Introduction

The soil organic matter controls the fertility in the soil, which is formed by two fractions, a no humificate one and humic substances (fulvic acids, humic acids, and humin) [1]. Brazil is one of the largest agricultural producers and it imports large amounts of fertilizers. There is a lot of research focusing on producing biochar by means of pyrolysis, or hydrochar, produced by hydrothermal carbonization (HTC), to be used as the soil conditioner and a source of nutrients. Several biomass sources have been applied in HTC, such as starch, wood, etc. [2]. The HTC process generates a solid material (hydrochar) and also a liquid phase (process water). Both of them are rich in carbon and nutrients, showing great potential for agricultural purposes. Studies have been evaluating the effect of the use of hydrochar and process water in experiments with plants, evaluating the germination processes and plant growth.

Studies conducted by this group showed the viability of the use of vinasse and sugarcane bagasse in HTC process, which led to a hydrochar that showed potential for application as a soil conditioner [3,4].

Therefore, the goals of this study were to evaluate the maize germination process in different process water concentrations, and in hydrochar carbon released, both in water and in diluted acid solution extracts. The hydrochar and process water were obtained from hydrothermal carbonization of vinasse and sugarcane bagasse mixture. The humic and fulvic acids of hydrochar used were also characterized for identifying the characteristics of humic substances of the material that maybe support changes in the soil when hydrochar was applied as soil conditioner.

## Material and Methods

For process water, the germination studies were conducted at concentrations of C 50, 100, 250 and 500 mg $L^{-1}$ of TOC (TOC-VCSN, Shimadzu, Japan) present in process water. For hydrochar, the extractions of soluble fraction were conducted following the proportion of 5:1, extract solution (mL) and hydrochar (g) respectively. The extract solutions used were ultrapure water (AQ) and 0.1 mol $L^{-1}$ HCl solution (AC). The supernatant was separated from hydrochar by centrifugation and filtered. The TOC of AQ and AC solution was determined, and dilutions of 10, 50 and 100 mg C $L^{-1}$ of TOC were used in germination studies. Germination experiments were made using 5 replicates for each treatment, with seeds of maize (*Zea mays*) using agarose as a growth medium for 7 days. Germination index, the percentage of germination, and shoot and root length were evaluated, and the comparison of data was made by statistical analysis.

The humic and fulvic acids of hydrochar were extracted following the IHSS recommendations. The characterization by UV/Vis (UV-2600, Shimadzu, Kyoto, Japan) and infrared spectroscopy (Spectrum Two – UART Two, PerkinElmer, Llanstrisanti, UK) of samples were obtained.

## Results and Discussion

Comparing the shoot and root growth was observed that the studies conducted at concentration of 50 mg C $L^{-1}$ presented germination behavior similar to the control showing no delay in germination, unlike the other concentrations, although there is no statistical difference for the final number of germinated seeds between the control and all dilutions evaluated [5]. Process water at a concentration of 250 mg C $L^{-1}$ stimulated root and shoot growth better than the other concentrations of carbon evaluated, showing positive statistical significance in relation to the control [5].

The germination of the carbon solution released from the hydrochar showed similar behaviour, once it was not observed statistical difference among all concentrations for both extracts for seed germination.

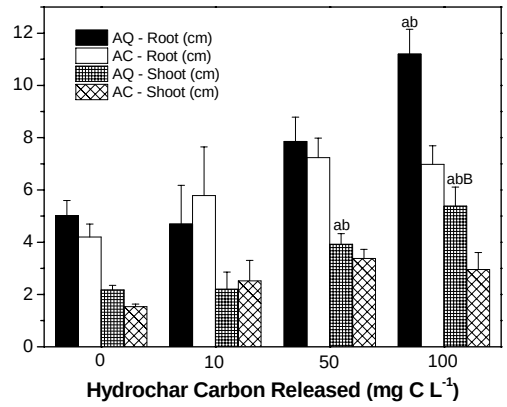



**Figure1.** Shoot and root growth for AQ and AC hydrochar carbon released solutions.

[a] Indicates positive difference in relation to the AQ solution control group; [b] Indicates positive difference in relation to the AC solution control group; [B] Indicates positive difference in relation to all concentrations from AQ and AC solutions.

The AQ extractor with a concentration of 100 mg C $L^{-1}$ showed positive statistical significance for root growth compared to all concentrations and extractive solutions (Figure 1). Shoot growth exhibited positive statistical significance in concentrations of 50 and 100 mg C $L^{-1}$ for AC extract.

The FA $E_4/E_6$ ratio (12.0) showed that its aromatic groups are found less condensed, presenting lower molecular weight than HA (3.1). Similarly, it was observed $E_3/E_5$ ratio that indicated FA (28.4) with smaller molecular size than HA (1.4). The $A_{254}/A_{203}$ indicated more substituted aromatic rings by hydroxyls, carbonyls, esters and carboxyl groups in HA (0.7) than in FA (0.3), as it was confirmed by FTIR analysis.

## Conclusion

Diluted process water with 250 mg C $L^{-1}$ stimulated the development of roots and shoot. Hydrochar carbon released by HCl solution with 100 mg C $L^{-1}$ better stimulated shoot growth, and hydrochar carbon released by water at 100 mg C $L^{-1}$ better stimulated root development. HA and FA extracted from hydrochar exhibited characteristic capable of improving soil organic matter.

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# The molecular composition of humic acids, as reflected by FTICR-MS, varies systematically in terms of the total C storage of the corresponding soils

M. A. JIMÉNEZ-GONZÁLEZ [a,c]*, D. WAGGONER [b], A. M. ÁLVAREZ [c], F. J. GONZÁLEZ-VILA [d], P. HATCHER [b], G. ALMENDROS [a]

(a) Department of Biogeochemistry and Microbial Ecology, National Museum of Natural Sciences, 28006 Madrid, Spain.
(b) Department of Chemistry and Biochemistry, Old Dominion University, 23529 Norfolk (VA), USA.
(c) Department of Geology and Geochemistry, Autonomous University of Madrid, 28049 Madrid, Spain.
(d) Department of Biogeochemistry, Plant and Microbial Ecology, Institute of Natural Resources and Agrobiology of Seville, 41012 Sevilla, Spain.
* E-mail: majimenez@mncn.csic.es

**Keywords:** carbon sequestration, FTICR-MS, humic acid

**Abstract.** Long-term stabilization of soil organic matter (SOM) as recalcitrant forms plays an important role in the Earth's carbon cycle. Hence, understanding biogeochemical mechanisms of soil C sequestration is crucial to control its emission to atmosphere. In this research the composition of humic acid (HA) is analyzed by Electrospray Ionization (ESI) Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS). Using Partial Least Squares (PLS) regression, a forecasting model for total soil C is obtained from the common compounds and its importance in the prediction is plotted in the space defined by their atomic ratios.

## Introduction

Global change is a well-publicised concern regarding the need of implementing appropriate soil management practices. In fact, soil is the greatest reservoir of carbon at the Earth's level. For this reason, recent research have highlighted the interest of describing the molecular composition of SOM as a source of information to be related with the humification mechanisms involved in soil carbon storage [3]. In consequence, the origin of the SOM from plant or microbial biomass, as well as the extent of its biogeochemical transformation can be scrutinized from the analysis of a series of molecular proxies such as signature compounds that are part of the macromolecular structure of the HAs, which represent one of the most recalcitrant and structurally complex pools of the SOM.

Nevertheless, the classical problem to study the molecular composition of the HAs without the unavoidable structural alteration produced by e.g., wet chemical degradation methods, cannot be definitively resolved from non-destructive approaches e.g., infrared spectroscopy (IR) or nuclear magnetic resonance spectroscopy (NMR), that do not provide accurate structural information at the level of the units of the macromolecules. In particular FTICR-MS is a useful technique for direct and fast analysis of structural compounds of SOM with minimal chemical alteration, which has been recently used to study the composition of humic substances [2, 4].

## Materials and methods

Topsoil samples (0–10 cm) were collected from 35 different forest or agricultural ecosystems from Spain with a large variability in climatic conditions, vegetation, geological substrate, use and management.

The free organic matter (FOM) was removed by flotation in 2M $H_3PO_4$. The extractable humic substances were isolated with standard method using 0.1M $Na_4P_2O_7$ and 0.1M NaOH in inert atmosphere. The HAs and fulvic acids (FAs) were fractionated by precipitating the total humic extract with 6M HCl. The HAs were purified by high-speed centrifugation (46.000 × g) followed by dialysis to remove the minerals. The main HA structural groups were quantified using solid-state $^{13}C$ NMR.

The molecular-level analysis of the HAs was carried out using a Bruker 12 Tesla Apex-Qe FTICR-MS instrument equipped with an Apollo II ESI source. For this analysis: *c.* 0.5 mg of HA was dissolved in 1% $NH_4OH$, then diluted with methanol:water 1:1. The empirical molecular formulas were assigned in the range from 200 to 800 m/z. The mass calibration was carried out using naturally present fatty acids. In order to use appropriate data matrices for chemometric approaches, only common compounds identified in the HAs of the different soils were selected. These compounds were represented in a Van Krevelen (VK) diagram (Fig. 1) using the H/C and O/C ratios calculated from each empirical formula. Prior to statistical analyses, the total abundances of the compounds were managed as compositional data and subjected to centered log-ratio (CLR) transformation [1]. The PLS was used to explore the utility of the common compounds as predictors of SOC concentration.

## Results and Discussion

More than 1000 compounds from the HAs were identified by FTICR-MS, but only up to 131 compounds, with a mass from 309 to 555 Da, were systematically present in the HAs from all the soils. These compounds consisted of C, H and O atoms and in some few cases N. Graphical representation of the abundances of the compounds in the H/C and O/C space is helpful to obtain an insight on their origin and composition (Fig. 1).

This diagram shows that the common compounds

can be classified into two main groups either corresponding to lipid- or lignin-derived molecules, with comparatively low number of compounds in regions characteristic for polycyclic aromatics and protein.

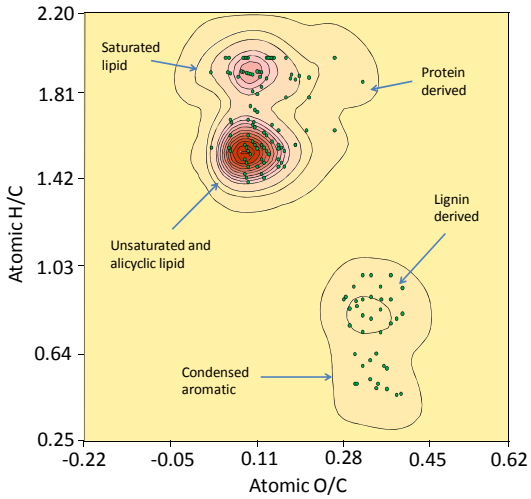



**Figure 1.** Van Krevelen diagram of common compounds in all the HAs. The different regions (lipid, protein, lignin and condensed aromatics) are represented: Colour intensity in the contour map indicates relative abundance of the compounds.

In the PLS prediction models, a very significant model was obtained for SOC content ($R^2$ = 0.68). The Akaike's information criterion (AIC) and comparisons with models using randomized data of SOC were used to check for overfitting in the model. The PLS showed that a correlation exists between the molecular composition of the HAs as reflected by the common compounds analyzed by FTICR-MS and the SOC levels. Therefore, the variable importance in the projection (VIP) for the 131 individual compounds was also represented in the Van Krevelen diagram to compare the extent to which the different compounds contribute to explain the soil carbon levels (Fig. 2a).

The overall pattern shown by the VIP plots is comparable to that obtained by subtracting average compound abundances in HAs from soils with high SOC content (1st quartile, Q1) from those in soils with low SOC content (4th quartile, Q4). This plot (Fig. 2b) illustrates the common compounds which are more abundant in soils with high SOM levels (red color) and in soils poor in SOM (yellow). It was found that lignin-derived structures and complex, unsaturated and alicyclic lipid were more abundant in HAs from soils with high SOC content. On the other hand, aliphatic compounds mainly saturated alkyl molecules predominant in microbial biomass were in characteristically high proportions in the HAs from soils of low C content.

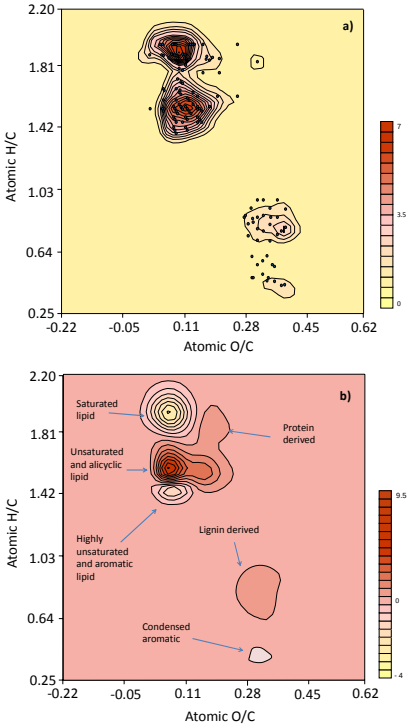



**Figure 2.** Van Krevelen diagram with VIP values is represented in a). Subtraction Q1-Q4 is presented in b) with positive values in red (Q1, high SOC content) and negative values in yellow (Q4, low SOC content).

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# Dissolved organic matter diversity in Antarctic lakes and streams (Soya Coast, East Dronning Maud Land)

M. KIDA [(a)*], T. KOJIMA [(a)], Y. TANABE [(b,c)], K. HAYASHI [(d)], S. KUDOH [(b,c)], N. MAIE [(e)], N. FUJITAKE [(a)*]

(a) Graduate School of Agricultural Science, Kobe University, Japan
(b) National Institute of Polar Research, Japan
(c) SOKENDAI (The Graduate University for Advanced Studies), Japan
(d) Institute for Agro-Environmental Sciences, NARO, Japan
(e) School of Veterinary Medicine, Kitasato University, Japan
* Research Fellow of Japan Society for the Promotion of Science
* E-mail: fujitake@kobe-u.ac.jp

**Keywords**: EEM, humic substances, optical index, PARAFAC, polar region, UV-vis

**Abstract.** This study examined the properties of DOM obtained from various lakes and streams at Soya Coast, East Dronning Maud Land, East Antarctica ($n$ = 81). The samples were analyzed for basic water chemistry, humic substances concentration, UV-vis spectroscopy, and excitation emission matrix spectroscopy (EEM). The EEM results were further analyzed by parallel factor analysis, and five components were validated. The compositions and optical properties of DOM differed considerably between water types, such as streams vs lakes, fresh vs saline lakes, and pore water vs lakes directly fed by a glacier.

## Introduction

Dissolved organic matter (DOM) exists ubiquitously in water and is involved in various biogeochemical processes. In Antarctica, where no higher plants are present (except in the Antarctic Peninsula), most DOM is considered to originate from lower plants. This unique feature of Antarctica is ideal to unveil the structural properties and functionality of DOM, which depend on the source materials and environmental processing. The differences in the properties of DOM between Antarctica and other continents are well known, but differences within Antarctic DOM are less understood. In this study, we present the diversity in DOM properties obtained from various lakes and streams at Soya Coast, East Dronning Maud Land, East Antarctica.

## Materials and Methods

Water samples were collected from two streams ($n$ = 16), and the center ($n$ = 36) or shore ($n$ = 29) of lakes in ice-free areas of Soya Coast, where Japan has its base Syowa Station and >100 lakes exist with various sizes and water chemistry. They were filtered with a pre-combusted glass fiber filter (0.3 µm), and the UV-vis spectroscopy and excitation emission matrix spectroscopy (EEM) were measured immediately. The EEMs were corrected for all necessary steps to remove biases and normalized in Raman units [1]. A portion of water samples was stored in glass bottles, spiked with 0.02% (final concentration) $NaN_3$, and transferred to Japan, where DOM in the samples was fractionated into humic substances (HS) and non-HS based on adsorption onto Supelite™ DAX-8 resin [2]. Optical indices such as $A_{254}$, $SUVA_{254}$, $S_{275–295}$, FI, BIX, and HIX were calculated from the absorbance and EEM data to elucidate the structural properties and origin of DOM [1, 3]. We further analyzed the EEMs by parallel factor analysis (PARAFAC) to deconvolute them into distinct fluorescent components [4].

## Results and Discussion

Electrical conductivity (EC) values of the samples ranged from fresh (1.3 mS $m^{-1}$) to hypersaline (22.6 S $m^{-1}$). DOM concentration ranges were 0.27−146 mg $C^{-1}$ and increased as a function of EC (Fig. 1). A similar trend was also observed for total dissolved nitrogen and $A_{254}$, indicating general evaporative concentration of solutes. The proportion of HS in DOM (%HS) ranged from 11.4% to 55.7% with a mean ± standard deviation of 35.0 ± 9.3%. The %HS tended to be lower in lakes directly fed by a glacier (11.4−36.6%, $n$ = 9) and higher in the bottom and pore water of lakes (29.1−55.7%, $n$ = 8).

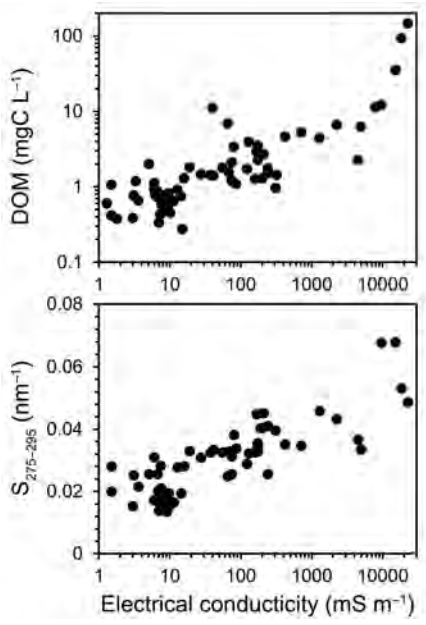



**Figure 1.** DOM concentrations and $S_{275–295}$ versus electrical conductivity.

Values of the optical indices also indicate the variability in the DOM properties depending on water type. The $S_{275-295}$ values increased as a function of EC (Fig. 1), suggesting removal of high molecular weight DOM by aggregation and selective preservation of low molecular weight DOM. BIX (1.02−1.61) and FI (1.58−2.05) also showed high values in saline lakes, indicating autochthonous DOM production. Interestingly, DOM from bottom and pore water of lakes and the two streams had similar optical properties; high $SUVA_{254}$ and HIX values and low $S_{275-295}$ values. These similarities suggest the less photo-degraded states of DOM and preservation of photo-sensitive HS in these environments, which were probably because of low sunlight penetration in pore water and short water residence time in streams.

Five PARAFAC components were validated (Fig. 2), indicating two protein-like (C1, C5), two microbial humic-like (C3, C4), and one ubiquitous (C2) component [5]. The protein-like components had an average summed proportion of 54% and were most dominant. Clear negative relationships were found between %(C1 + C5) and %C3, and %(C1 + C5) and %C4, suggesting microbial consumption of C1 and C5, and producing of C3 and C4 [5]. The ubiquitous component C2 had a positive relationship with %(C3 + C4), and a negative relationship with %(C1 + C5), suggesting its generation by the same process as that of C3 and C4. The relative contribution of each PARAFAC component to the EEMs differed considerably depending on water type. For example, stream DOM was characterized by high contribution of C2 and C4 and low contribution of C5 (~14%), whereas some saline lakes had very high (~60%) contribution of C5.

Overall, a considerably high variability was observed both in the composition (%HS) and in the optical properties within Antarctic DOM. We are currently analyzing dissolved metals and nutrients and structural composition of DOM by nuclear magnetic resonance spectroscopy to better understand the relationships between environmental parameters and DOM properties and functions.

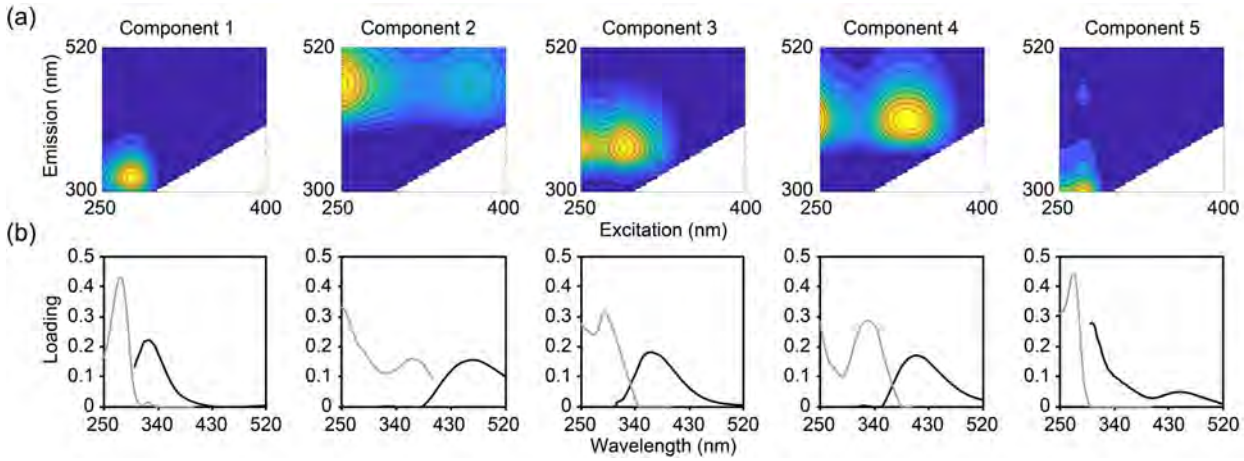



**Figure 2.** Contour plots of the five PARAFAC-derived fluorescent components (a) and excitation (grey) and emission (black) loadings produced from the PARAFAC model (b).

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# Low molecular weight organic acids of peat permafrost-affected soils

O. S KUBIK [(a)*], E. V. SHAMRIKOVA[(a)], D. A. KAVERIN [(a)], A. V. PASTUKHOV [(a)] , V. V. PUNEGOV [(a)]

(a) Federal State Institution of Science, Institute of Biology, Komi Science Center, Ural Branch, Russian Academy of Sciences, 167982, Syktyvkar, Russia
* E-mail: kubik-olesia@yandex.ru

**Keywords**: water-soluble organic compounds, permafrost-affected peat soils

**Abstract.** The composition of the water extracts (pH water, the weight concentration of the total carbon in the water-soluble organic compounds and low-molecular-weight organic acids) in active layer and upper permafrost in peat soils of the South-East of Bolshezemelskya tundra has been studied. It has been shown that differences in the genesis of peat have a significant impact on soil properties both at soil profile and landscape levels.

## Introduction

Large areas (about 10 %) of weakly drained watershed terraces and lacustrine-alluvial valleys of South Bolshezemelskya tundra are occupied with peatlands cobered by permafrost-affected soils. That determines the need for their consistent and detailed study. Terms of soil formation determine the complexity of the soil cover of permafrost peatlands where organogenic soils of fens, mounds and peat circles are developed. The influence of permafrost affects the processes of transformation and movement of substances in the soils [1, 2]. Information about the component composition of water-soluble organic compounds in permafrost peat soils is still missing. The study of the peat soils is also important in connection with increasing anthropogenic impact and climate change.

## Materials and methods

Soil survey was carried out within the permafrost-affected peatland (67º03' N, 62º55' E, 100 m a.s.l.) located in an ancient lake bed. The trench 10 meters length was filled on a peat mound. We selected three oligotrophic peat soils to be studied (Soil Classification of Russia, 2004) [3]. The peat soil of the peat circle belongs to subtype of oligotrophic peat destructive soils (I). Further along the trench – oligotrophic soil of the edge zone of peat circle (II) and oligotrophic peat soil of the peat mound slope (III). All the soils in accordance with World Refereed Base for soil resources WRB (2006) [4] are characterized as Cryic Histosols. Soil profiles were studied according to the structure of two-component system “active layer (AL) - permafrost layer (PL). The samples were taken at different depths: 0-10, 10-30 cm (respectively, AL-1, AL-2) and 50-80, 220 to 250 cm (PL-1 and PL-2). All the samples represent the organic substrate.

Mass concentration of low-molecular-weight organic acids (LMWOAs) was estimated by method of gas-chromatography and chromato-mass-spectrometry (GC/MS) [5, 6].

The aim of this work is to study the content of water-soluble organic acids in the complex of peat permafrost-affected soils of southern tundra in the European North-East.

## Results and Discussion

Formation and development of peat soils under conditions of cryogenesisis inseparably linked with the microtopography which defines the temperature regime, the redistribution of solid precipitation, the presence and structure of vegetation cover.

Complex mechanism of formation and functioning of peat soils is reflected in the variability of the composition and properties of water-soluble organic compounds at the level of the soil profile as well as at the landscape level (in layers).

In all the soils the common patterns are observed. First, expressed increase in $pH_{water}$ values down the profile and pH of active layer is 0.7-1.0 more in comparison to underlying permafrost. This difference is less marked in the soil of the peat circle because of higher decomposition degree of the peat on the circle surface. On the other hand, at all four depths from the first profile to the third one the acidity is increasing by 0.4-0.6 pH units. Thus the most acidic water-soluble components occur at the early stages of decomposition of organic substrate in the surface horizon, and in a higher degree in the soil occupying the lowest position.

The total carbon content of water-soluble organic compounds is 70-190 mg/dm$^3$ in all the studied objects. A single regularity is marked in each profile: a decrease in water-soluble organic compounds content from top soil up to a depth of 100-130 cm. According to pH the soil of peat circle is the least changeable. A general pattern of gradual decrease in $C_{WSOC}$ is disturbed deeper than 2 m and a slight increase of this indicator is revealed. Still, the total carbon content of water-soluble organic compounds in PL - 2 horizon is invariable within the all studied complex (68-72 mg/dm$^3$). This layer was preserved earlier perhaps in the period of its formation the territory had similar conditions of peat formation such as homogeneous microrelief and similar composition of the biota. In other layers (AL - 1, AL - 2, PL - 1) on the contrary, at the landscape level, significant variability of this indicator is revealed: from the first profile to the second one and then to the third one the consistent growth of $C_{WSOC}$ on average 1.5-2 times is noted. In active layer this is a consequence of the obvious differences in microtopography, hydrothermal regime and composition of the biota.

Variations of this indicator in PL - 1 (100-130 cm), in addition, can be defined by a variety of paleogeographic conditions against periodic thawing of the horizon during the subatlantic period.

The proposed method identified 0.1-20.3 mg/dm$^3$ low molecular weight organic acids that corresponds to 0.4-14.5 % of the total carbon water-soluble organic substances. In all the profiles maximum diversity and acid content as well as total carbon content of organic compounds, which are for the surface layer decreases down the profile. In the soil of peat circle this trend is less seen in contrast to the second and more in the third section, in which the total content of certain acids within the active layer is 7 times less, then in permafrost 30 times less. Such changes are connected with features of hydrothermal regime and composition of the vegetation.

Most of spatial variability in the content of acids is typical for seasonally-thawed horizons where the content of these compounds along the trench from the circle to the slope increases as much as 15. Thus, the acid content defined by GC/MS is more variable characteristic compared to the total carbon content of organic compounds - both in time (organic substrate of different age) and in space (microtopography).

Figure 2 shows a dendrogram of the similarity of soil and permafrost layers. For all the profiles the uniformity of all studied characteristics have been obtained at the landscape level both for permafrost at depths of 2 m and 100-130 cm. Being homogeneous in space, these two strata differ significantly. The “lifting” the surface of the underlying peat layers of soil of the circle and the “removal” of its surface horizon due cryogenic processes have been evidenced. First, the presence in the group horizons PL - 1 of three soils of lower active layer of the soil. Secondly, the similarity of the AL - 1 circle and the AL - 2 of the edge zone of peat circle. Features of genesis of soil under shrub vegetation contribute to the formation of the surface layer, distinct from all other objects. Thus, by the dendrogram on experimental data it is shown that the active three horizons of soils find are maximally variability among themselves. For these depths is typical frequency of cycles of freezing-thawing, the magnitude of the temperature and average annual temperature in organogenic profile of peat soils that confirms the previously obtained results.

Using the dendrogram of the similarity of horizons the scheme of the soil profile structure has been constructed where the objects, similar to each other are uniformly painted out. The diagram clearly shows the manifestation of cryogenic processes - frost heave and abrasion (Fig. 3).

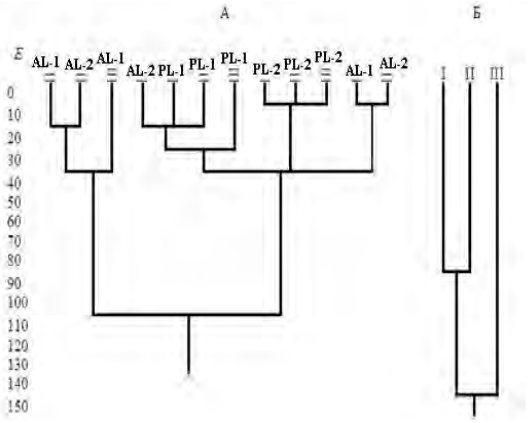



**Figure 1.** Dendrogram of the similarity of horizons (A), soil sections (B), E - Euclidean distance: I - Peat soil of the circle, II - Peat soil of the edge zone of the circle, III - Peat soil of peat mound; AL – active layer, PL – permafrost layer.

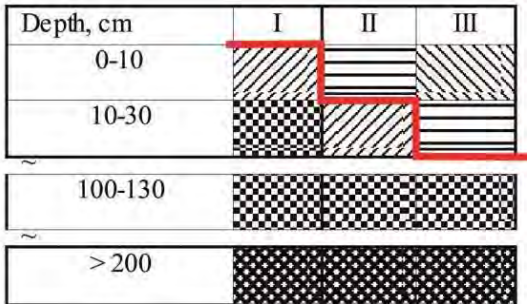



**Figure 2.** Scheme of the structure of soil profile (according to the dendrogram of the similarity of aquifers).

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# Upgrading from the lab to a pilot plant and an industrial bath process in the production of biochar from three types of Brazilian biomasses residues.

T. T. S. MATOS [(a)], J. SHULTZ [(a)], E. M. C CARDOSO [(a)], M. R. FORNARI [(a)], L. P. C ROMÃO [(b)], R. O. C. RIBEIRO [(a)], A.S. MANGRICH [(a)*]

(a) Department of Chemistry, Federal University of Paraná, 81531-980 Parana, Brazil
(b) Department of Chemistry, Federal University of Sergipe, 49100-000 São Cristóvão, Brazil
* E-mail: asmangrich@gmail.com

**Keywords:** biomass, Agro-industrial waste, pyrolysis.

**Abstract.** Agro-industrial wastes for the making biochars, as sugar cane bagasse (SB), exhausted husk (EH), and elephant grass (EG), were used in this work: (1) tubular furnace, (2) pilot plant; and (3) industrial muffle. The order of the processes that favored the formation structures with condensed aromatic rings was (3) ≥ (2)> (1). The pyrolysis processes caused increase of the organic free radicals (OFR) (spin concentration) in the order (3) ≥ (2) > (1). Moreover, the OFR that previously remained centered on oxygen atoms tended to become more near carbon atoms in the biochars structures.

## Introduction

In Brazil, the vast agricultural production and processing of various products from agroindustry generate significant amounts of crop biomass wastes. Some biomass residues are abundant and could serve as feedstock of others industrial processes [1]. Examples of such abundant residues include the sugar cane bagasse, husk of the black wattle (Acacia mearnsii de Wild) from the production of tannin, and elephant grass, that is a grass produced in practically all over the country.

Raw materials coming mainly from agriculture are used, through pyrolysis, to produce biochar. This material is a valuable soil amendment for agriculture and, together with the other two products, bio-oil and bio-gas are also excellent sources of energy [2].

The aim of this work was using three different type of biomasses from agroindustry in Brazil to study the upgrade of biochar production from laboratory furnaces, to a pilot plant and to an industrial furnace. From the characterization of the biochars produced in this study, we can understand their characteristics and interactions, for a future application in soils.

## Materials and methods

Biomass waste from sugar cane bagasse (SB), exhausted husk (EH), and elephant grass (EG) were dried in an oven at 105°C for 24 hours. The exhausted husk was provided by the company Tanac S.A. (Rio Grande do Sul, Brazil). After, they were pyrolysed until it reached the temperature of 350°C in three different modes: (1) batch process in tubular furnace in the laboratory, (2) flow process in a pilot plant; and (3) batch industrial process in muffle.

FTIR analyses were performed using a BIORAD spectrophotometer. CHN analyses were performed at the Chemistry Institute of University of São Paulo. The EPR analyses were carried out on a Bruker EMX spectrometer operating in X-band (≈ 9.5 GHz).

## Results

### FTIR Spectroscopy

All the prepared biochar samples showed broad bands in the region of 3429 $cm^{-1}$ attributed to O-H stretching vibrations of phenols or alcohols. In addition, bands of the aliphatic CH stretches occurring in the region of 2920 $cm^{-1}$ and 2850 $cm^{-1}$ were observed and confirmed with bands appearing in the region of 1458 and 1317 $cm^{-1}$ attributed to the CH-bending folds of aliphatic [3]. The batch process in muffle (3) provided materials with the disappearance of these absorptions, in relation to the in nature biomasses, that is, demonstrated a greater degree of loss of the functional groups through the pyrolysis process. The shoulder near 1740-1700 $cm^{-1}$ corresponding to C=O, stretching of carboxyl, aldehyde, ketone and ester groups. A band at 1616 $cm^{-1}$ was attributed to the stretching of aromatic C=C. An absorption band at 1513 $cm^{-1}$, attributed to the bending of the amine N-H bond, was present in all biomasses but disappeared in all biochars spectra.

Intensive absorptions were observed in the region close to 1000 $cm^{-1}$ and 1050 $cm^{-1}$ in the EH and EG biomasses, attributed to the carbohydrate C-O stretching vibration, confirmed structures, by the low and wide absorptions, in the region slightly above 2000 $cm^{-1}$, assigned to combining bands of these carbohydrate group C-O.

### Elemental Analyses

Pyrolysis increases C content. It is explained by the increase of carbonization degree and consequently reducing the O and H content, due to the formation of cycling aromatic groups. There was increase of the N contents, it is suggested that temperature can conserve the N in coals by incorporating it into complex structures resistant to heating and not easily volatilized [4]. The ashes content for all biochars is higher comparing to respective used biomasses.

The Van Krevelen diagram (Fig. 1) shows O/C vs. H/C ratios decreased with pyrolysis. The biochars produced by the processes (2) and (3) obtained the highest

ash values and seem to have formed more aromatic structures.

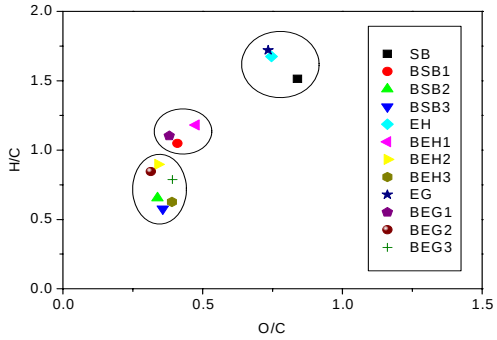



**Figure 1.** Van-Krevelen diagram of the biomasses and their respective biochars of pyrolysis.

The low O/C ratio indicates the arrangement of aromatic rings, forming crystal graphite-like structure with high stability. The biochars produced by procedures (2) and (3) showed the lowest O/C ratio favoring this structure profile [5] The biochar produced by process (1) had the highest H/C ratio, indicate they are more similar to the original biomasses. With respect to the formation of condensed aromatic structures, the processes could be placed in the following order (3) ≥ (2)> (1).

### EPR Spectroscopy

The biochars spectra showed a narrow line, accentuated and centered at 350 mT with $g \approx 2.0$. Biochars produced in the flow process oven presented EPR signal of $Fe^{3+}$ ions in concentrated domain ($g \approx 2.2$; $\Delta H_{pp} \approx 130$ mT). The biochars produced from EG presented ions $Fe^{3+}$ in diluted domain ($g \approx 4.3$). In Table 1, as can be observed through the *g*-factors, organic free radicals that previously remained centered on oxygen atoms ($g \approx 2.0040$) tended to become a mixture with radicals centering on carbon and oxygen atoms ($g \approx 2.0030$-$2.0040$) after pyrolysis [6]. It was observed that the spins concentrations (spin $g^{-1}$) of all biochars ware bigger than for raw materials a hint that pyrolysis caused increase in the spins concentrations and the biggest values occurred in the biochars prepared by the processes (2) and (3).

**Table 1**. EPR parameters of samples

| Sample | g- factor | Spin $g^{-1}$ |
|---|---|---|
| SB | 2.0040 | 1.58E+16 |
| BSB 1 | 2.0034 | 1.88E+18 |
| BSB 2 | 2.0033 | 1.01E+19 |
| BSB 3 | 2.0033 | 1.12E+19 |
| EH | 2.0041 | 2.40E+16 |
| BEH 1 | 2.0036 | 6.95E+17 |
| BEH 2 | 2.0033 | 9.34E+18 |
| BEH 3 | 2.0034 | 7.78E+18 |
| EG | 2.0044 | 1.61E+16 |
| BEG 1 | 2.0035 | 7.30E+17 |
| BEG 2 | 2.0034 | 4.65E+18 |
| BEG 3 | 2.0033 | 5.91E+18 |

Organic free radicals, occurred an increase of the spins concentration with the pyrolysis processes of the biomasses. The EPR g-factor indicated that the free radicals previously centered near oxygen atoms tend to be blend with that centered near carbon atoms. This result agrees with the elemental analyzes as the carbon content increased and the oxygen content decreased with the pyrolysis processes.

Therefore, it is expected that these and other techniques shall enable to understand the interaction behavior of the biochars with the soil not only in their fertilizations improvements, as well as, in the behavior for the emission of greenhouse gases. Ongoing studies in our research group shall produce important data on those topics.

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# Thermally treated sewage sludge: a humic substance precursor with the potential to serve as phosphate fertilizer?

M. PANEQUE [(a)*], J. M. DE LA ROSA [(a)], J. KERN [(b)], H. KNICKER [(a)]

(a) Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), 41012 Seville, Spain
(b) Leibniz-Institut for Agricultural Engineering and Bioeconomy, 14469 Potsdam, Germany
*E-mail: mpaneque@irnas.csic.es

**Keywords:** pyrolysis, hydrothermal carbonization, phosphorus, organic matter, slow-release, RMN

**Abstract.** Sewage sludges (SS) have been suggested to act as soil phosphorus (P) source at the same time that contribute to increase the soil organic carbon fraction. This work studies the evolution of total ($P_T$) and extractable (ext-P) P of SS after pyrolysis and hydrothermal carbonization and the impact of the pyrochars and hydrochars produced in a soil after a 80-days of incubation. Pyrochars showed higher $P_T$ and ext-P contents than hydrochars. Pyrochars and 200ºC-produced hydrochars increased soil ext-P. A pH-dependent slow release P potential was suggested for some treatments.

## Introduction

Sewage sludge (SS) is a nutrient-rich organic waste. The use of this material in agriculture can not only add to the C sequestration potential of soils by contributing to its humic fraction, but also allows recycling of nitrogen (N) and phosphorus (P) for soil fertilization. However, before SS can be used as soil amendment it has to be hygienized. This can be achieved by hydrothermal carbonization (HTC) or pyrolysis. Such treatments not only turn the organic carbon fraction into a pool with assumingly slow turn-over rate in soils but also decreases the biochemically availability of N and P forms.

Previous works suggested the potential of SS-derived pyrochars and hydrochars as slow releasing P and N reservoirs [1, 2]. Pyrolysis and HTC seem to decrease P mobility. However not all this P is immobilized since SS-pyrochars were observed to act as P-source for plant growth [3]. Based on our former study demonstrating the huge influence of the char production conditions on the chemical composition of its organic C and N fractions [2], we suspect a comparable impact on the ratio between plant-available and immobilized P. In order to test this hypothesis we investigated how total and extractable P contents of two SS changed under different thermal treatments and how this was related to the organic matter composition. In addition, the impact of these treated-SS on soil P availability contents was studied in a 80-days greenhouse incubation study.

## Materials and methods

**Sewage sludge collection and treatment.** Two different sewage sludges, named A_SS and T_SS, were collected from the Experimental Wastewater Treatment plant (CENTA), located in Carrion de los Céspedes, Spain. Both sludges were hydrothermally carbonized (HTC) at 200°C and 260˚C for 30 min and 3 h and pyrolyzed (Py) at 600ºC for 1h. The W_Py pyrochar was produced from green waste at 600°C for 20 min and derived from the company Swiss Biochar.

**Greenhouse incubation experiment.** Pots with 250 g of a Calcic cambisol with amounts equivalent to 5 and 25 t $ha^{-1}$ of each char and topped with 25 certified grass seeds of *Lolium perenne* were prepared (n = 4). Controls without any char amendment but with seeds were also prepared (n=6). The pots stayed in a greenhouse for 80 days and were regularly irrigated.

**Analyses.** The following analyses were done to the non-treated and treated SS as well as to the amended soils at the end of the incubation experiment: total phosphorus ($P_T$) by ICP-OES spectrophotometer; extractable P (Extr-P) by using the Olsen-P method; pH by using a 1:2.5 w/v soil:water ratio and a 1:10 w/v chars:water ratio. The chemical composition of the organic fraction of the chars by solid-state $^{13}C$ NMR spectroscopy.

## Results and discussion

According to the $^{13}C$ NMR spectroscopic analysis, temperature rather than residence time affected the chemical alteration during the thermal treatment. Considerably higher aromaticity was achieved for the pyrochars than for hydrochars suggesting a more efficient carbonization by pyrolysis than by HTC. Concomitantly, a higher biochemical stability in soil systems is expected for the pyrochar than for the hydrochars.

Hydrochars produced from both A_SS and T_SS were slightly acidic whereas the pyrochars showed pH around 10. Increasing the temperature during HTC resulted in a relative increase of $P_T$. In contrast applying the harsher pyrolysis at elevated temperature clearly augmented the $P_T$ relative levels. The increasing $P_T$ contents in treated sewage sludges with temperature is best explained by a relative enrichment of this element due to C and O losses during carbonization [3]. In relation to P availability, HTC decreased the amount of extr-P. Pyrolysis, however, did not show a consistent effect for A_Py and T_Py compared to their respective non-treated SS. This points out to different P compounds in A_SS and T_SS and hence a different speciation/complexation during pyrolysis.

The application of hydrochars resulted in decreased pH values of the soils at the end of the experiment (Table 1).

**Table 1.** pH of the control, hydrochars and pyrochars amended soils at the end of incubation

| | **pH** | |
|---|---|---|
| Control | 8.4 ± 0.02 | |
| *Amended soils* | *5 t ha$^{-1}$* | *25 t ha$^{-1}$* |
| A_HTC_200_0.5 | 8.0 ± 0.03 | 7.4 ± 0.07 |
| A_ HTC_200_3 | 8.2 ± 0.03 | 7.0 ± 0.02 |
| A_ HTC_260_0.5 | 8.2 ± 0.05 | 7.5 ± 0.09 |
| A_ HTC_260_3 | 8.0 ± 0.12 | 7.3 ± 0.09 |
| T_ HTC_200_0.5 | 8.0 ± 0.11 | 7.3 ± 0.06 |
| T_ HTC_200_3 | 8.2 ± 0.05 | 7.0 ± 0.04 |
| T_ HTC_260_0.5 | 8.2 ± 0.03 | 7.5 ± 0.04 |
| T_ HTC_260_3 | 8.1 ± 0.07 | 7.6 ± 0.05 |
| A_Py | 8.2 ± 0.04 | 7.3 ± 0.09 |
| T_Py | 8.2 ± 0.04 | 7.8 ± 0.03 |
| W_Py | 8.4 ± 0.04 | 8.5 ± 0.07 |

The pots amended with A_ and T_HTC produced at 200°C for 0.5 h applied at 5 t ha$^{-1}$ showed two to three times more exr-P than the control (Table 2).

**Table 2.** Extractable P (mg kg$^{-1}$) of the control, hydrochars and pyrochars amended soils at the end of incubation.

| | **Extr- P** (mg kg$^{-1}$) | |
|---|---|---|
| Control | 13.1 ± 0.5 | |
| *Amended soils* | *5 t ha$^{-1}$* | *25 t ha$^{-1}$* |
| A_HTC_200_0.5 | 25.8 ± 0.8 | 57.2 ± 1.1 |
| A_ HTC_200_3 | 17.1 ± 0.4 | 32.0 ± 1.1 |
| A_ HTC_260_0.5 | 12.7 ± 1.0 | 20.9 ± 0.7 |
| A_ HTC_260_3 | 11.6 ± 0.7 | 11.5 ± 0.1 |
| T_ HTC_200_0.5 | 35.4 ± 0.7 | 80.0 ± 3.5 |
| T_ HTC_200_3 | 26.9 ± 0.5 | 57.3 ± 2.0 |
| T_ HTC_260_0.5 | 17.6 ± 1.5 | 24.9 ± 1.0 |
| T_ HTC_260_3 | 12.7 ± 0.7 | 15.8 ± 0.3 |
| A_Py | 58.9 ± 1.3 | 200.3 ± 8.2 |
| T_Py | 48.5 ± 1.6 | 148.3 ± 3.4 |
| W_Py | 15.0 ± 1.1 | 14.1 ± 1.1 |

Higher application rates intensified the effect, suggesting that such a treatment may help to reduce alkalinity of basic soils. In spite of the high pH of the pyrochars, its addition did not increase the pH of the soil but it slightly decreased it. Increasing the application rate increased the soil extr-P. Intensifying the thermal treatment during HTC decreased the amount of extr-P, possibly due to lower input with the hydrochar application. The pyrochars A_Py and T_Py increased soil extr-P in a higher extent than their hydrochars. However, W_Py addition had no impact on this parameter at any dose. The latter is in accordance with the low total and extr-P content of this pyrochar.

The difference ($P_{diff}$) between the amount of extr-P in the soil at the end of the experiment ($P_{end}$) and the respective amount at the beginning ($P_{added}$) was calculated. All $P_{added}$ of the 260ºC-produced hydrochars and the W_Py pyrochar seemed to have been used by the plants during the incubation. However, the pots amended with the 200ºC-produced hydrochars and the A_Py and T_Py pyrochars showed positive $P_{diff}$. This indicates that part of the originally no extractable P turned into extractable during the incubation. The extent of this effect increased with increasing the application rate. This may be related to the lower soil pH observed in the 25 t ha$^{-1}$ amended pots. This work confirms that thermal treated SS cannot only serve for increasing the organic matter content in soils but also as a slow P release fertilizer. Further research regarding the pH-dependency of the P-release behavior should be done.

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# A cost effective and slow release humified organo-mineral fertilisers for increasing nitrogen use efficiency and crop yield

BIPLOB K. SAHA [(a)*], MICHAEL T. ROSE [(b)], VANESSA WONG [(c)], TIMOTHY R. CAVAGNARO [(d)], ANTONIO F. PATTI [(a)]

(a) School of Chemistry, Monash University, Clayton, Victoria, 3800, Australia.
(b) NSW Department of Primary Industries, Wollongbar Primary Industries Institute, Wollongbar, NSW 2477, Australia.
(c) School of Earth, Atmosphere & Environment, Monash University, Clayton, Victoria, 3800, Australia.
(d) School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, South Australia, 5064, Australia.
*E-mail: bksah1@student.monash.edu

**Keywords**: humic-mineral interaction, brown coal-urea, crop yield, nitrogen use efficiency

**Abstract.** A novel slow release fertiliser was formed by blending humified brown coal (BC) with urea. A field trial experiment was conducted to study the efficacy of the brown coal-urea (BCU) granules on the N losses and crop yield of sweet corn. Blending of urea with humified BC substantially increased fertiliser N retention by decreasing its losses via $N_2O$ and $NH_3$ emissions and significantly increased plant available N and crop yield. The increased yield and reduced losses of N makes the BCU fertiliser more profitable and environmentally friendly over the use of conventional urea.

## Introduction

Urea is one of the most widely used N fertilisers to enhance growth and yield of crop plants. The very poor use efficiency and low recovery of urea-N in soil-plant systems is associated with its losses by leaching, denitrification and volatilisation [1]. The N lost through this process represents a serious economic loss and environmental burden [2]. Considerable research efforts in recent years have been aimed at developing mitigation strategies to reduce gaseous and leaching losses of N [3]. There is an increasing interest in the application of blended organ-mineral fertilisers, as they can contribute to climate change mitigation through better retention of key nutrients such as N, C sequestration, supply organic matter to soil at the same time [4]. Hence, granulation of inorganic fertilisers with humified organic matter has great potential for enhancing fertiliser use efficiency and concurrently returning organic matter back into soil, restoring soil health and improving crop yield in a more sustainable manner [5].

This study evaluates the efficacy of brown coal-urea (BCU) fertilisers prepared using a pan granulation technology. A field trial was conducted with sweet corn and this paper presents the results of this trial where the growth, yield, N use efficiency (NUE) and uptake of N are reported.

## Materials and methods

The field trial was carried out at the research station of Department of Primary Industries (DPI), Wollongbar Research Institute (28°90'95"S and 153°41'54"E), NSW, Australia. The soil is acidic with pH 4.7, with high concentrations of iron and is classified as a Ferrosol (Isbell, 2002). The individual plot size was 34 $m^2$ (10 m×3.4 m). Seeds were uniformly sown with a seed driller. Two BCU granules (BCU 1 having 20% N and BCU 2 having 10% N) and two rates of N (180 and 90 kg $ha^{-1}$) were evaluated in this field trial. The experiment was laid out following a completely randomised block design with four replicates. The two levels of N added to the soil, required applying varying amounts of BCU granules depending on their N concentration. The fertilisers were mixed with the top (0-15 cm) soil in granular form and uniformly covering the whole surface area. Twelve greenhouse gas (GHG) and volatilised $NH_3$ samples were collected at one week intervals over the plant growth period. From each plot, 10 soil samples were collected at 7, 14, 21, 28, 42, 56, 70 and 98 days after fertilisation to measure the urease activity in the soil. A destructive final harvest was undertaken at 98 days after seed sowing to measure biomass and cob yield. The urease activity was determined colorimetrically using a microplate fluorimeter. The statistical analyses were performed using the statistical software package IBM SPSS, version 20 (SPSS IBM, 2010). A one-way ANOVA was run and the multiple comparisons among the different treatments were done using a Tukey test.

## Results and Discussion

Soil incorporation of BCU exhibited considerable effects on the growth, biomass and cob yield, N uptake, nitrogen use efficiency (NUE) and economic returns for sweet corn, grown in field conditions.

### Urease activity

Addition of BCU significantly reduced the hydrolysis of urea-N at the early growth stage of sweet corn by inhibiting the urease enzyme activity (Figure 1). A sharp increase in urease enzyme activity was observed immediately after N fertiliser addition in all the treatments (Figure 1). The urease enzyme activity peaked at 7 days

after sowing (DAS), after which it decreased rapidly and remained almost same until the harvesting of sweet corn. The urease activity was substantially higher in urea amended soil up to 21 DAS compared to BCU and other treatments including the control.

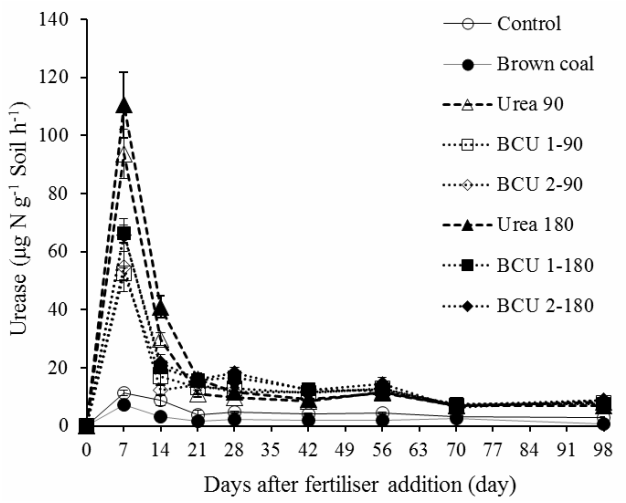



**Figure 1.** Effect of BCU granules and urea on the urease enzyme activity in soil.

### $N_2O$ and $NH_3$ emissions

Due to reduced hydrolysis and mineralisation of N as well as its strong retention by the BC matrix resulted in a significant decrease in gaseous loss of N as $N_2O$ and $NH_3$. Compared to urea, BCU granules considerably decreased N losses via $N_2O$ and $NH_3$ emissions and significantly increased plant available N in the soil (Figure 2).

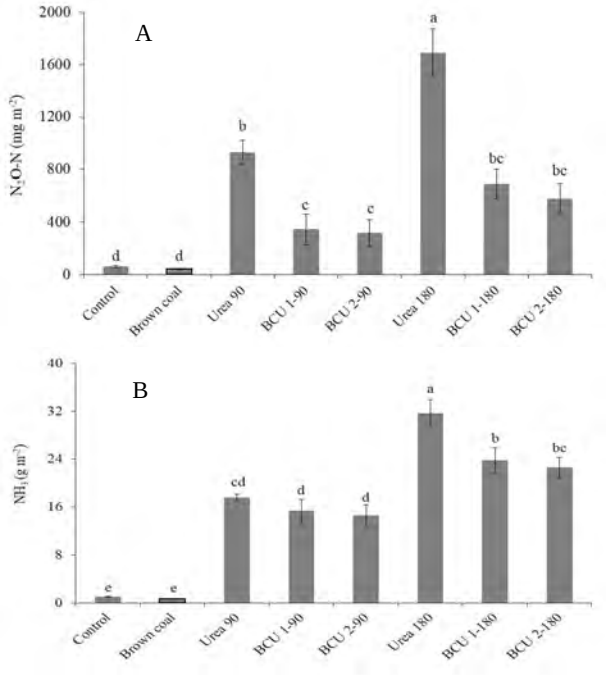



**Figure 2.** Effect of BCU granules and urea on the cumulative $N_2O$ (A) and $NH_3$ (B) emissions from soil.

### N uptake and cob yield of sweet corn

A significantly higher amount cob N uptake was measured from the plants fertilised with 180 kg N $ha^{-1}$ as BCU 1 compared to plants receiving an equivalent amount of N as urea (Figure 3A). The slow and steady release of fertiliser-N increased the availability of mineral N throughout the whole growing season, enhancing N content and its uptake by sweet corn. The increased N uptake resulted in 17% more cob yield from the plots amended with BCU granules compared to commercial urea fertilised plots (Figure 3B). Based on the overall results, the BCU 1 (20% N) can be considered as a more efficient and economically profitable alternative to conventional urea fertiliser (46% N). The use efficiency of commercial urea fertiliser can be improved substantially by granulating it with humified organic matter like BC.

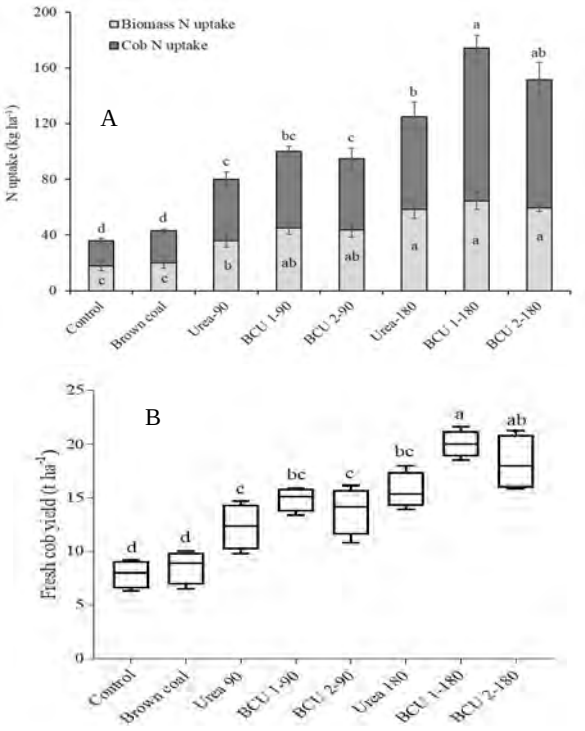



**Figure 3.** Effect of BCU granules and urea on the N uptake (A) and cob yield (B) of sweet corn.

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# Supramolecular organic matter assembly in light of multidetector data analyses

U. J. WÜNSCH[(a,b)*], E. ACAR[(c)], K. R. MURPHY[(b)], C. A. STEDMON[(a)]

(a) National Institute of Aquatic Resources, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark
(b) Water Environment Technology, Chalmers University of Technology, 41296 Gothenburg, Sweden
(c) Simula Research Laboratory, Lysaker, Norway
* E-mail: wuensch@chalmers.se

**Keywords:** FDOM, HPSEC, one-sample PARAFAC, supramolecular assembly, mass spectrometry

**Abstract.** Dissolved organic matter (DOM) plays an integral part in the global carbon cycle. It's capacity as long-term carbon storage is an important factor in climate change mitigation. However, the connection between chemical composition and recalcitrance remains poorly understood. Here, we present novel analytical approaches to elucidate the composition of DOM. These approaches indicate the existence of supramolecules and allow insights into their chemical composition. Our results contribute to an improved understanding of humic substance chemistry, providing novel opportunities to assess the role of humic acids in carbon sequestration.

## Introduction

Dissolved organic matter (DOM) is a major pool of reduced organic carbon in aquatic environments. In the marine environment, it plays an important role in carbon sequestration to great depths through the microbial carbon pump.The measurement of spectroscopic properties of DOM, in particular fluorescence spectroscopy in conjunction with emission excitation matrices (EEMs) and parallel factor analysis (PARAFAC) have provided valuable insights into the humification of DOM as a function of continued processing and production of recalcitrant organic matter [1]. However, much of the chemical origin of the optical properties of DOM remains a mystery. Recent studies suggest fluorophores to be part of supramolecules [2]. Current methodology [3], based on correlation of molecular formula abundances with fluorescence components is not suitable to address the multivariate nature of molecular formulas in supramolecular assemblies and the possible mismatch of analytical windows involved in such comparisons.

Here, we present results from two studies focusing on new analytical frameworks. The first, called one-sample PARAFAC, is designed to evaluate the commonality of fluorescence spectra across diverse, disconnected aquatic systems along with their molecular size distributions. The second approach, using Advanced Coupled Matrix and Tensor Factorization (ACMTF) was designed to jointly decompose fluorescence EEMs and molecular formula tables to investigate the molecular formulas associated with a given fluorescence spectrum.

## Material and Methods.

Full details of the one-sample PARAFAC approach in conjunction with high-performance size-exclusion chromatography (HPSEC) are reported elsewhere[4]. In short, the fluorescence composition of samples from various locations (Rio Negro, Rio Tapajos, Svartan River, Pacific Ocean, Pony Lake) were analyzed using HPSEC in conjunction with online EEM and CDOM absorbance spectra. PARAFAC models were derived for every sample independently.

For ACMTF, 175 FDOM samples from three Artic fjords where characterized using fluorescence EEMs and ultrahigh resolution mass spectrometry. ACMTF jointly models both datasets by fitting a PARAFAC model to EEMs and factorizing molecular formulas in such a way that component scores are identical [5]. The methodology used to obtain valid models was similar to PARAFAC (e.g. split-half validation). A six-component model was able to explain 99.84% of variability in both datasets.

## Results and Discussion.

HPSEC-EEM-PARAFAC was able to reliably identify the underlying fluorescence spectra of FDOM in individual samples [4]. Across allochthonous samples, spectral properties of FDOM were highly congruent (e.g. $C_{510}$ in Fig. 1), while autochthonous samples contained more unique fluorescence spectra. For congruent spectra, the HPSEC-based approach allowed the comparison of molecular size distributions. These distributions were broad and chromatographically unresolved in all cases. Our results indicate that fluorophores are moieties in supramolecular assemblies that consistently have higher apparent sizes when compared to mass spectrometry averages. Importantly, a fluorophore may be incorporated in different assemblies, but also may occur a number of times in a given assembly. Since a given fluorophore occurs across a wide range of apparent sizes, our results show that fluorescence spectroscopy may be limited in its ability to indicate separate biogeochemical fractions. Since a number of fluorophores were found across a wide array of aquatic systems (Fig. 1), our results furthermore indicate that fluorescence fingerprints are not exclusive to certain environments.

Data fusion of fluorescence EEMs with molecular formula tables, measured on the same DOM samples (solid-phase extracts from three Arctic fjords) was used to further investigate the supramolecular assembly hypothesis. If fluorophores are fully independent moieties, this approach should allow the linkage of few molecular formulas with a given fluorescence spectrum, while the remaining formulas can be modeled as unshared factors. However, the first application of this joint

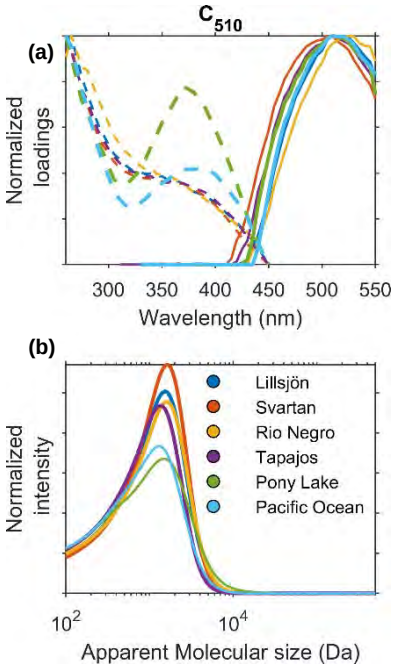



**Figure 1.** Characteristics of a common FDOM fluorescence component. (a): Highly congruent spectral properties (dashed: excitation, solid: emission) between DOM from diverse environments. (b): Apparent molecular size distributions of the component shown in (a).

factorization indicated the opposite. A given fluorescence component was directly associated with numerous molecular formulas across a wide range of molecular masses (Fig. 2 a, b). Conversely, the compositional characteristics associated with a given fluorescence spectrum spanned wide regions of the compositional space (Fig. 2 c), but also showed clear differentiation.

Multidetector approaches, and in particular data fusion combines analytical techniques that were previously isolated, thus mitigating limitations while combining advantages of included techniques to allow a more holistic understanding of DOM composition, its biogeochemical fate, as well as the capacity to function as a long-term carbon storage.

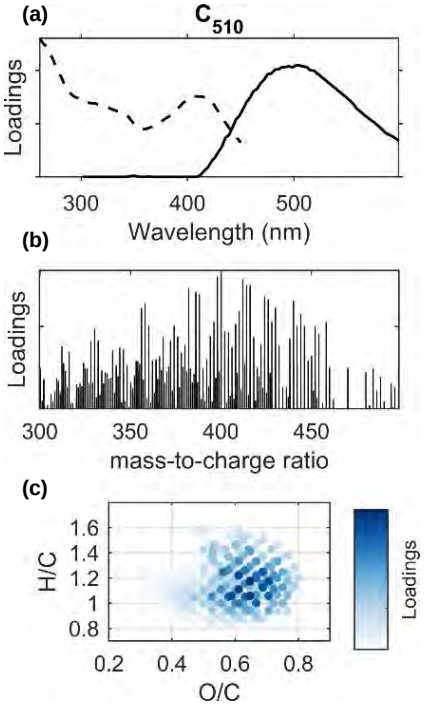



**Figure 2.** Properties of a component identified with ACMTF across three Arctic fjords. (a): Fluorescence properties (dashed: excitation, solid: emission).
(b): Molecular weight distribution. (c): Elemental composition (Oxygen-to-carbon and hydrogen-to-carbon ratios) of compound molecular masses shown in (b).

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