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ADVANCES IN NATURAL ORGANIC MATTER AND HUMIC SUBSTANCES RESEARCH 2008-2010

XV Meeting of the
International Humic Substances Society
Puerto de la Cruz, Tenerife, Canary Islands, 27 June - 2 July 2010

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ADVANCES IN NATURAL ORGANIC MATTER AND HUMIC SUBSTANCES RESEARCH 2008-2010

Vol. 1

ORAL PRESENTATIONS

Proceedings Book of the Communications presented to the
15th Meeting of the International Humic Substances Society
Tenerife - Canary Islands. June 27- July 2, 2010

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WELCOME TO IHSS15

THE WORLD CONFERENCE ON HUMIC SUBSTANCES & NATURAL ORGANIC MATTER

During the 14th Meeting of the International Humic Substances Society (IHSS14), held on cruise from Moscow to St. Petersburgh, our research group from IRNAS-CSIC proposed to organize the next IHSS Meeting. Now, two years after, and on behalf of the Organizing Committee I have the great honour to welcome all of you to the 15th Meeting of the International Humic Substances Society (IHSS15) to be held in the Canarian village of Puerto de la Cruz, Tenerife, Spain, during June 27-July 2, 2010. Tenerife is known as the island of eternal spring, one of Spain's most beautiful areas and an ideal place to host this meeting.

This IHSS edition is organized under the auspices of the Spanish Ministry of Science and Innovation (MICINN), Spanish Council for Scientific Research (CSIC), Canarian Government, Canarian Universities of La Laguna (ULL) and Las Palmas de Gran Canaria (ULPGC), and local authorities from Tenerife (Cabildo de Tenerife and Ayuntamiento de Icod de los Vinos).

The International Humic Substances Society (IHSS) was founded in 1981 to bring together scientists in the coal, soil, and water sciences with interests in humic substances. IHSS has a membership of nearly 900 scientists. The Society is recognized as a world leader in fostering scientific education and research, and promoting public understanding of humic substances.

Every other year, this group of scientists organizes this International Meeting that is among the most prestigious conference in the field. In 1988, the 4th IHSS Meeting edition was held in Matalascañas, Huelva, Spain. Now, for the second time and 22 years latter, the IHSS Meeting returns to Spain. We are expecting that 15IHSS in Tenerife will continue the tradition as the premier forum for the presentation and discussion of cutting-edge advances in basic and applied aspects, setting the trend for the development of this discipline.

This edition of the Meeting has exceptional exposure in fields like novel applications of HS and NOM in the environment and industry, latest advances in the knowledge of physical, chemical and biological properties of HS & NOM in terrestrial and aquatic systems, new analytical approaches, and the problems and solutions related to water treatment, carbon stabilization processes (humification & highly refractory forms) and in the structural elucidation of complex organic materials that may help in a better comprehension of the carbon cycle and Global Climatic Change. More than 250 top scientists and industry professionals from 38 countries from all over the world attended the IHSS15. This edition had a very intense programme that, from a grand total of 375 communications received, finally included 13 Invited Key Lectures, 44 Oral communications, a special session for IHSS awarded students with 11 Oral presentations and 212 Posters.

Here I will like to thank IHSS15 members of the Organizing Committee for their constant work in the hard tasks needed for a successful meeting, to the Scientific Committee for valuable advice and the production of our solid and outstanding Scientific Programme and to all donors, sponsoring companies and expositors for their support without which the Tenerife IHSS15 would not be possible.

Finally we wish all participants to IHSS15 fruitful debates and a pleasant stay in Tenerife.

José A. GONZÁLEZ-PÉREZ
IHSS15 Chairman

FOREWORD FROM THE PRESIDENTOF THE INTERNATIONAL HUMIC SUBSTANCES SOCIETY

In 2011 IHSS will reach 30 years since its creation! These have been years of important achievements and activities that fulfilled objectives stated at the beginning. For sure it is time to celebrate and to thank very much those pioneers that started the Society, as well as the many others who have contributed to the development of the Society. We need to thank those who worked to standardize chemical procedures to extract humic substances and produce the highly valued reference and standard collection of humic samples, and those who contributed to our Newsletters, to the organization and operation of National and Regional IHSS Chapters and to the organization of the bi-annual international conferences, which have been held on all continents. This legacy is very important but this effort must continue, to maintain and advance our Society. This is the commitment of IHSS Board Members, and all members are invited to join us in current and future efforts to strengthen IHSS, as well as to celebrate the 30 years of IHSS, at this 15^{th} IHSS Meeting, in Tenerife.

It is very well accepted that natural organic matter (NOM) research is much more relevant than ever, being associated with global climate change, one of the more challenging issues for the future of the Planet. A new emphasis in research has emerged related to carbon compounds in different environments. We are involved in this research field, which offers many opportunities and we must be aware of the best ways to take advantage of the opportunities, and make a positive contribution to IHSS. We face much greater competition from several other groups of researchers, with different scientific backgrounds, and there has been a multiplication of the number of scientific meetings and organizations involved in NOM associated research. For IHSS it is crucial to develop a strategic plan to take advantage of the opportunities in the best way that we can.

In addition to good planning, for our Society to stay healthy we must invest in young researchers and motivate them in their activities. In this IHSS can be proud, because for many years we have sponsored the IHSS Travel Award, a worldwide competition that provides an outstanding opportunity for PhD students to participate in our bi-annual meetings. In 2008, for the meeting in Russia, IHSS Board members decided to support 17 students, the highest number until that meeting. For the 15^{th} IHSS meeting the number is even higher, and 22 students from all continents were given awards to attend the meeting. These 22 were chosen from 47 applications, a record, which demonstrates growing interest in the IHSS Travel Award.

Finally I would like to recognize those who have contributed to the organization of this bi-annual international meeting, and so on behalf of IHSS I would like to thank very much all members of the Organizing and Scientific Committees of the 15th IHSS Meeting in Tenerife, Spain, and particularly Dr Jose A. GONZÁLEZ-PÉREZ and Dr Francisco J. GONZÁLEZ-VILA, Chair and Co-Chair, for their willingness to organize the meeting and their strong dedication and motivation. I am sure that we will have a very high level scientific conference that is productive and enjoyable, on the Island of Eternal Spring!

Thank you very much for your attendance.

Best regards.

Ladislau MARTIN-NETO
President of IHSS

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CONTENT Vol. 1
ORAL PRESENTATIONS

SESSION 1: OPENING

Advances in the Non-invasive Molecular Characterization of Humic Substances by NMR and Fourier Transform Ion Cyclotron Resonance Mass Spectrometry: A Revolution in Molecular Understanding

Patrick G. Hatcher*, Rachel L. Sleighter, Georgina A. McKee, Hongmei Chen, Aron Stubbins, Rajaa Mesfioui, and Junyan Zhong

Old Dominion Univ. Department of Chemistry and Biochemistry, Norfolk, VA 23529, USA
E-mail: phatcher@odu.edu

1. Introduction

Recent developments in both NMR and mass spectrometry have revolutionized our understanding of complex mixtures of biological substances in living organisms from both a molecular standpoint and a reactivity standpoint. This work focused on humans, plants, and other organisms has opened the door for studies of even more complex systems- the humic substances in soils, sediment, and water. Using multinuclear NMR and Fourier transform ion cyclotron resonance mass spectrometry, we can begin to realize the dream of most individuals who have studies humic substances in their lifetime- to molecularly characterize them.

2. Analytical Methods

We employed a variety of Nuclear Magnetic Resonance (NMR) approaches alongside ultrahigh resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) to gain insights into the molecular composition of both solid and soluble humic substances. The complementary methods each provided information for NOM at near ambient concentrations. This capability makes it possible to obtain a great deal of chemical information without the need for special isolation or extraction methods, which inevitably fractionate the humci susbtances sample.

For solid humic substances, use of High Resolution Magic Angle Spinning (HRMAS) NMR allows solid polymers to be swollen by addition of a solvent (e.g. DMSO-d_6) resulting in enhanced molecular mobility and NMR ‘visibility’. We obtained two-dimensional [^{1}H-^{13}C Heteronuclear Single Quantum Coherence (HSQC)] HRMAS spectra of dried sediment samples to “visualize” the structural entities. One can also obtain other traditional solution-state multi-dimensional data. Such high-resolution spectra were not previously possible for solid untreated sediment samples. Pyridine extracts of the sediments were also analyzed by FT-ICR-MS using a Bruker Daltonics 12T Apex Qe spectrometer to obtain highly resolved and accurately calibrated masses from which elemental formulas were calculated. The elemental compositions of the peaks allow us to propose structures for the multitude of N-containing molecules present in the extract and to identify a new abiotic process for the incorporation of nitrogen into humic substances.

For soluble humic substances, we have employed new approaches in which the samples are examined spectroscopically at or near ambient concentration levels. FT-ICR-MS is sensitive enough at 12 Tesla to analyze freshwater riverine and lacustrine samples directly without use of isolation methods. Likewise, solution ^{1}H NMR spectra can be obtained directly. Comparing the results of data obtained directly, with those in which isolation methods are utilized, shows that isolation methods are selective in their representation of humic substances. In humic substances associated with saline or marine waters, salts need to be removed prior to analysis. This is accomplished by use of electrodialysis.

3. Results

Analyzing humic substances by advanced, non-invasive NMR and MS methods minimizes selectivity for compounds that fractionate due to isolation but does not remove selectivity inherent in the techniques. We present a series of case studies of how these combined techniques have enlightened our views of humic substance composition in the last several years. We also provide some new information on the molecular nature of specific humic materials that have huge implications for the humic substances field of study in soil, sediment, and aquatic systems.

Nanoparticles and humic substances in the aquatic and soil environment

Etelka Tombácz
Univ. of Szeged, Department of Physical Chemistry and Material Science, H-6720 Szeged,
Aradi Vt. 1, Hungary
E-mail: tombacz@chem.u-szeged.hu

Tremendous variety of inorganic and organic particles with at least one dimension in the nanoscale (<100 nm) or colloidal range (<1000 nm) can be found in soils and most aquatic environments. Besides the naturally occurring environmental nanoparticles such as clay minerals, metal (hydr)oxides, and humic substances, the products of nanotechnology market are emerging constantly and lead to the release of nanoparticles, e.g., silver, Ti-, Fe- and Mn-oxides mentioning only the most abundant ones, into the environment. Although their mobility, reactivity, ecotoxicity and persistency are hardly understood yet, the interfacial and colloidal interactions probably govern their existence and fate in the environment. In the natural systems, a great selection of dissolved small and macromolecular organic compounds rich in anionic functional groups (e.g., phenolic compounds, humic and fulvic acids) can be found besides the alkali and alkali earth cations situated as hydrated (water-surrounded) ions in bulk solution and as the charge-neutralizing cations in the local electrostatic field of environmental particles. The solution composition influenced surface properties of colloidal clay and metal oxide particles play the governing role in the particle-particle interactions resulting in either dispersing or aggregation of nanoparticles. Particle aggregation is of great importance in environmental point view considering such fields as soil erosion, particle transport, sediment formation, etc. In aquatic systems, the surface of mineral particles is coated by a polyanionic organic layer formed spontaneously in the presence of macromolecular components (humic and fulvic acids) of dissolved organic matter (DOM). This surface modification inherently influences particle interactions. The colloidal stability of an organic rich composite system can improve to such a degree that even a humate-assisted transport of nanoparticles becomes probable. Considering the role of the solution composition in the colloidal stability of composite aqueous dispersions in environment, the presence of humic substances as multifunctional organic complexants is of crucial importance besides the well-known master variables, the pH and ionic strength, as well as the alkali, alkali earth and multivalent inorganic cations dissolved generally in soil solutions, surface, ground and subsurface waters.

In the presence of indifferent cations (typically Na^+), the bound humate polyanions smear out the differences between the surface charge properties of metal oxides and clay mineral particles above an appropriate adsorption loading, and the polyanionic humic coating on the surface of mineral particles hinders the aggregation of particles, therefore enhances their colloidal stability in aqueous systems. This effect of humic substances (HS) promotes the dispersing of nanoparticles and enhances their colloidal transport in most aquatic environments. However, if specific cations (typically Ca^{2+}) in the sense of chemical affinity for carboxylic groups of humic substances and ion exchange sites of clay minerals are also present, the interfacial distribution of both humic molecules and Ca^{2+} essentially changes, so that their accumulation in the solid phase is mutually enhanced. The interfacial Ca^{2+} distribution is shifted to the solid phase, humate polyanions are bound through Ca bridges to the surface, which results in advanced humic acid accumulation in the solid phase and its adsorptive fractionation, leaving small residual fractions of HS in the aqueous phase. The formation of Ca bridges between humate polyanions and clay lamellae goes together with an inherent structural change, the significant solidification in particle network formed in clay and clay-oxide mixed suspensions, especially since both the faces and the edges, and humate coated oxide particles are also involved in Ca bridges.

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SESSION 2: CHARACTERIZATION OF NOM AND HS

Heteromolecular Chemistry of Humic Substances

Irina Perminova

Department of Chemistry, Lomonosov Moscow State University,
Leninskie Gory 1-3, Moscow 119991, Russia
E-mail: iperm@org.chem.msu.ru

1. Introduction

Humic Substances (HS) belong to many natural heteromolecular systems composed of many non-identical molecules. Crude oil comprises one of those systems composed of hydrocarbons from different chemical classes and of different molecular weight. The major unifying feature of those hydrocarbons is the reduced form of carbon constituting the backbone of those molecules. This provides for volatility of petroleum hydrocarbons and their high energetic potential. Humic substances, in opposite to oil, are composed of many oxidized organic molecules belonging to different classes of oxygen-containing compounds of predominantly acidic nature, formed due to oxidation and stochastic synthesis from many different compounds which biomass is composed of. They produce a heterogeneous assembly of many oxygen-rich molecules. The properties of such an assembly are consistent with steady – state dynamics of humification process and as such, should bear information on ecosystem functioning [1]. This information might be encoded by different molecular markers or by the structural patterns available within those systems. To reveal this information, many different approaches are being used by the researchers. One of them employs investigation of repetitiveness of the structure that allows for determination of the most abundant building blocks available within the system. We have recently proposed a new algorithm for revealing repetitive units in the system by performing total difference statistics on high resolution data of Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR MS) on humic materials and synthetic polyeletrolytes [2]. It allowed us to identify a new repetitive building block in the humic system with a formula $C_7H_6O_4$ that we have assigned to dihydroxyl-benzoic acid which is consistent with the expected structural blocks of fulvic acids stemming from oxidized lignin structures.

The goal of this research was to extend and deepen this approach upon fractionating humic isolates prior to the FTICR MS analysis. For achieving this goal, we have conducted size fractionation of humic isolates from different sources using size exclusion chromatography (SEC) and performed off-line analysis of the obtained fractions using high resolution FTICR MS.

2. Materials and Methods

Humic materials used for fractionation included humic (HA) and fulvic acid (FA) from peat and from the Suwannee River. The preparative fractionation was performed using size exclusion chromatography facilities located at the Water Chemistry Division of the University of Karlsruhe (Germany) and at the Department of Chemistry of the Lomonosov MSU (Moscow, Russia). Two different buffers were used for fractionation – 0.028 M phosphate and 0.044 M ammonium carbonate buffers. Toyopearl gel HW-55S was used as immobile phase. Electrospray ionization FRICR MS measurements were acquired using a 12 Tesla Apex Qe (Bruker Daltonics, Billerica, MA, USA) FTICR mass spectrometer equipped with an Apollo II electrospray ion source located at the facilities of the HelmholtzZentrum Munich for Environmental Health (Germany). The following conditions were used for electrospray: flow rate 2 ul/min, negative ion mode, spray shield 3 kV, capillary voltage 3.5 kV. Ions were externally accumulated in the hexapole for 1 s. The time-domain data were coadded, base-line zeroed, zero-filled once and fast Fourier transformed using ApexControl 1.2 and Data Analysis (Bruker Daltonics, Billerica, MA, USA).

3. Results and Discussions

To obtain the relationship between molecular weight and structural features of HS, HA and FA from peat and from the Suwannee River were fractionated into five fractions differing in size using SEC, and the obtained fractions were analyzed using FTICR mass spectrometer. The results for the Suwannee River FA are shown in Fig.1.

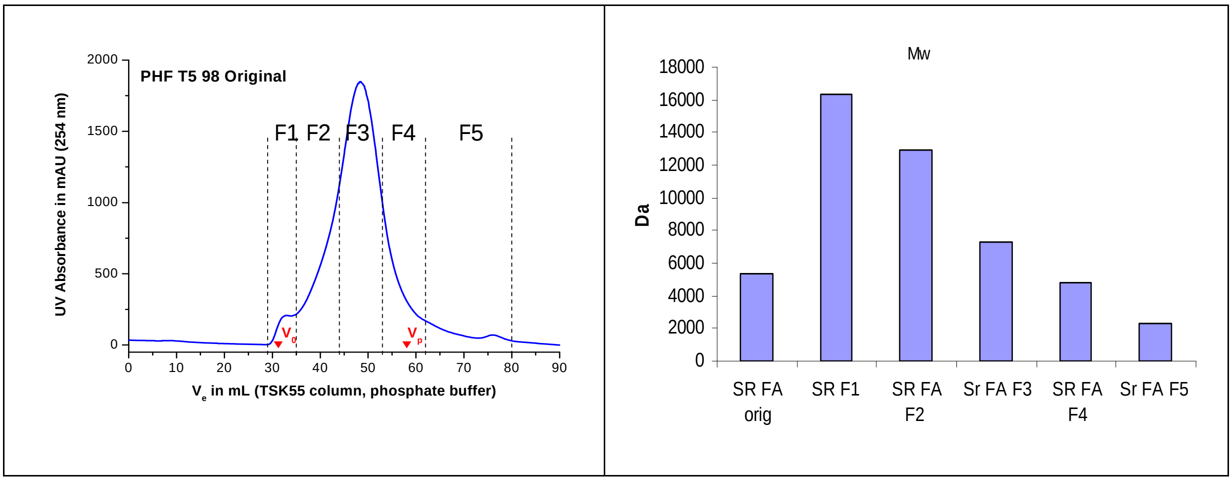

Figure 1: Size exclusion chromatogram of the SRFA sample and the corresponding estimates of molecular weight of the obtained five fractions used further for FTICR MS analysis

As it can be seen, a use of SEC enables separation of the original humic sample into five fractions with decreasing molecular weight ranging from 16000 Da to 1500 Da. The obtained fractions were further analyzed using FTICR MS and the structural trends were estimated. Double bond equivalents (DBE) versus molecular weight relationships are shown in Figure 2.

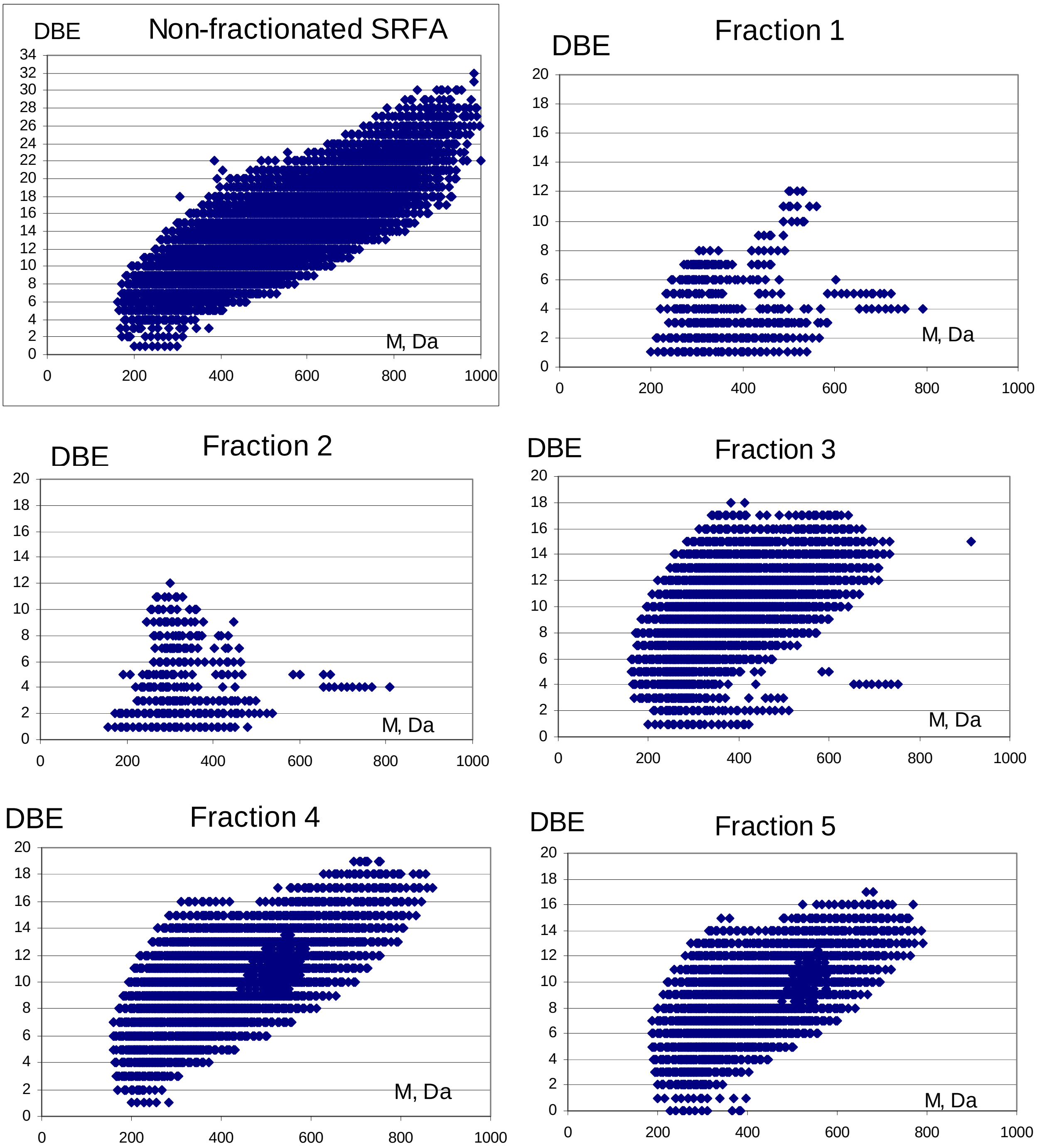

Figure 2: Double bond equivalent (DBE) versus Molecular weight (M, Da) for non-fractionated sample of SRFA (upper left corner) and for five size fractions of SRFA as shown in Figure 1

The DBE vs MW trends obtained for different size fractions show that the high molecular weight fractions of SRFA are much less aromatic as opposed to the low molecular weight fractions of the same sample. This corroborates well the reported findings on much higher content of aliphatic sugar-like units in the high molecular weight fractions of riverine HS [3]. We believe that the structural trends obtained provide sound grounds for classification of chemical compounds constituting HS, that is, for segregating them into the chemical classes. The next straightforward step is linking the obtained trends to the NMR data on the respective samples. This will enable assignment of isomers otherwise impossible within FTICR MS methodology.

4. Conclusions

Molecular complexity of humic isolates from different environments was explored using size fractionation approach accomplished with high resolution FTICR MS analysis. It was convincingly demonstrated that humic materials represent heteromolecular system consisting of different classes of oxygen-rich unsaturated compounds. The obtained structural trends of DBE versus MW can be used for developing classification of humic components into chemical classes. Of particular value will be complementary studies using high resolution 2D NMR spectroscopy. The term “heteromolecular chemistry” is proposed to characterize the chemical processes in the systems of such molecular complexity composed of non-identical molecular entities.

Acknowledgements

The author would like to express her deepest appreciation to all partners and friends from the University of Karlsruhe (group of Professor F. Frimmel), the HelmholtzZentrum Muenchen (group of Drs. Ph. Schmitt-Kopplin and N. Hertkorn) and the Institute of Energy Problem of Chemical Physics of RAS (Moscow) (Prof. E. Nikolaev). This work was supported by the grant of RFBR-CRDF 09-03-92500-ИК, RFBR 10-03-00803 and NATO CLG 983197.

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Two-Dimensional Gel electrophoresis of Humic Acids

Masakazu Aoyama*, Saiful Karim, Yuji Okuyama
Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, 036-8561 Japan
E-mail: aoyamam@cc.hirosaki-u.ac.jp

1. Introduction

Humic acids (HAs) are heterogeneous substances and consist of components with different molecular sizes and chemical properties. To investigate the chemical heterogeneity of HAs, it is necessary to develop a method for separating HAs into their components. Therefore, we applied two-dimensional (2-D) gel electrophoresis to fractionate HAs. In this method, HAs are firstly separated by isoelectric focusing (IEF) and then separated by polyacrylamide gel electrophoresis (PAGE) based on the molecular size.

2. Materials and Methods

Sample preparation: The HA samples used were prepared from Entisol (Fujisaki soil) and Andisol (Chitose soil). The HAs were extracted twice from the soil samples with 0.1 M NaOH and precipitated by acidification with HCl. The dissolution-precipitation cycle was repeated several times for the purification of HAs. The resultant precipitate was dialyzed against ultrapure water and freeze-dried.

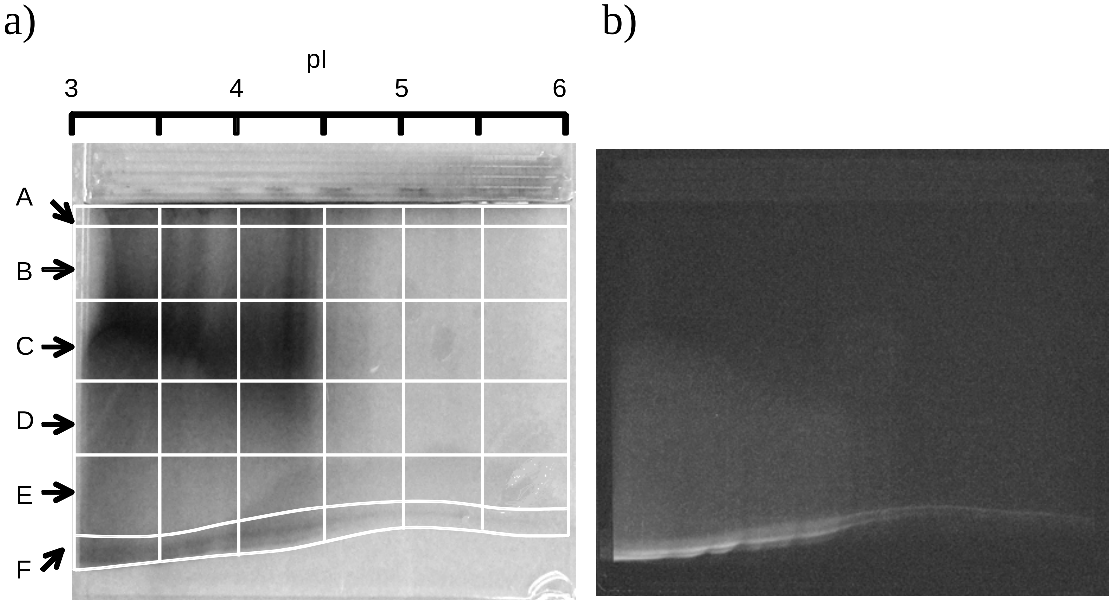

Figure 1: Electrophoretogram of the Chitose soil HA (a) and fluorescence under blue-light (b)

2-D gel electrophoresis: IEF was performed using commercially available immobilized pH gradient (IPG) strips (Bio-Rad, ReadyStrip IPG Strips, 17 cm, pH 3–6). HA samples were solubilized in 2% IPG buffer containing 7M urea and 2% CHAPS (0.5% w/v), then 0.3 mL of the solution was applied on the IPG strip. Electrophoresis was carried out at 3500 V for 5 h. After the completion of IEF, the IPG strips were transferred on 10% polyacrilamide slab gels containing 7 M urea (0.1 × 18 × 18 cm). Electrophoresis was carried out at a current intensity of 25 mA for 70 min using TBA buffer (pH 8.3). After electrophoresis, the gels were divided into 36 sections (Fig. 1a). HAs were extracted with 0.1 M NaOH from the individual sections and precipitated by acidification with HCl. The precipitates were dissolved in small amount of 0.1 M NaOH and precipitated again by acidification with HCl. The dissolution-precipitation cycle was repeated twice. The resultant precipitates were washed with ultrapure water and freeze-dried. The analysis of total carbon content, high performance size exclusion chromatography (HPSEC), and the measurement of diffuse reflection infrared Fourier transform (DRIFT) spectra were carried out for the individual electrophoretic fractions.

3. Results and Discussion

Figure 1a shows the gel after 2-D gel electrophoresis for the Chitose HA. Dark-colored substances were mainly observed in the low isoelectric point (pI) region (< 4.5). Furthermore, green fluorescence was observed in the lower left of the gel, when blue-light was illuminated (Fig. 1b). HPSEC of the electrophoretic fractions confirmed that the HAs were separated based on their molecular sizes upon PAGE. The fact that the fluorescence components had low molecular sizes is in agreement with our previous finding that most of the fluorescence was contributed by the relatively small molecular size fractions of soil HAs [2, 3].

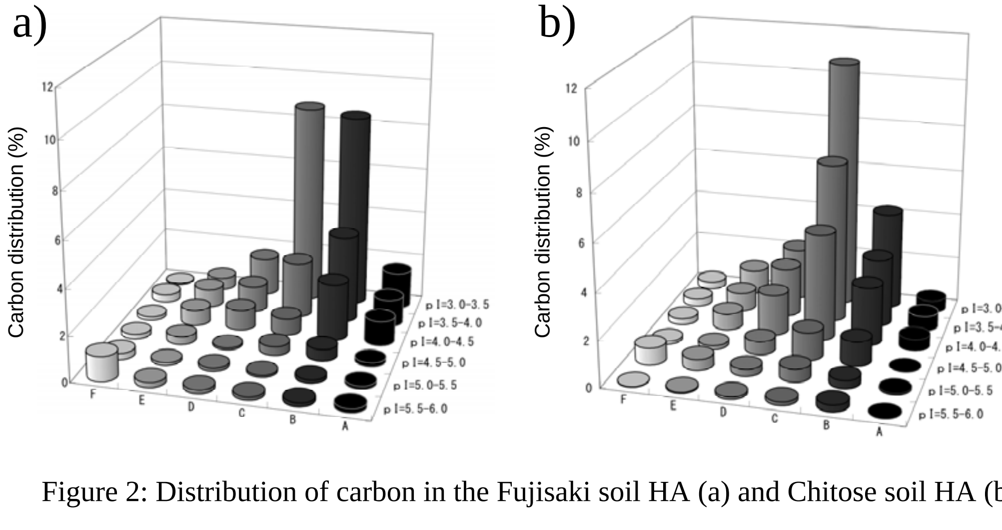

Figure 2: Distribution of carbon in the Fujisaki soil HA (a) and Chitose soil HA (b)

The analysis of organic carbon showed that HAs consisted mainly of components with pI less than 4.5 and middle and higher molecular sizes (Fig. 2). The Fujisaki soil HA contained larger proportion of high molecular size component than the Chitose soil HA. The total carbon recoveries were 48% for the Fujisaki soil HA and 54% for the Chitose soil HA. The low recoveries suggested that the considerable amount of HAs was lost during the extraction and purification steps.

The DRIFT spectra of the selected electrophoretic fractions were shown in Figs. 3 and 4. All the fractions exhibited the distinct COOH bands at 1720 and 1240 cm^{-1}. The fractions with relatively high molecular size (B) showed the bands at 1660 and 1540 cm^{-1}. These signals are associated with the amide I and amide II bands of peptidic or proteinaceous materials. The shoulder at 1040 cm^{-1} attributable to carbohydrates was observed in the relatively high molecular size fraction (B). For the Fujisaki soil HA, prominent peaks at 2850 and 2940 cm^{-1} were observed in the C and D regions at the pI range of 4.0–4.5. These peaks were assigned to the symmetric and asymmetric CH_2 stretching bands in aliphatic chains. In contrast, for the Chitose soil HA, the band at 1600 cm^{-1} due to C=C stretching in aromatic rings was remarkable in relatively low molecular size fractions (C and D). Thus, the chemical properties were different from each other among the electrophoretic fractions of HAs.

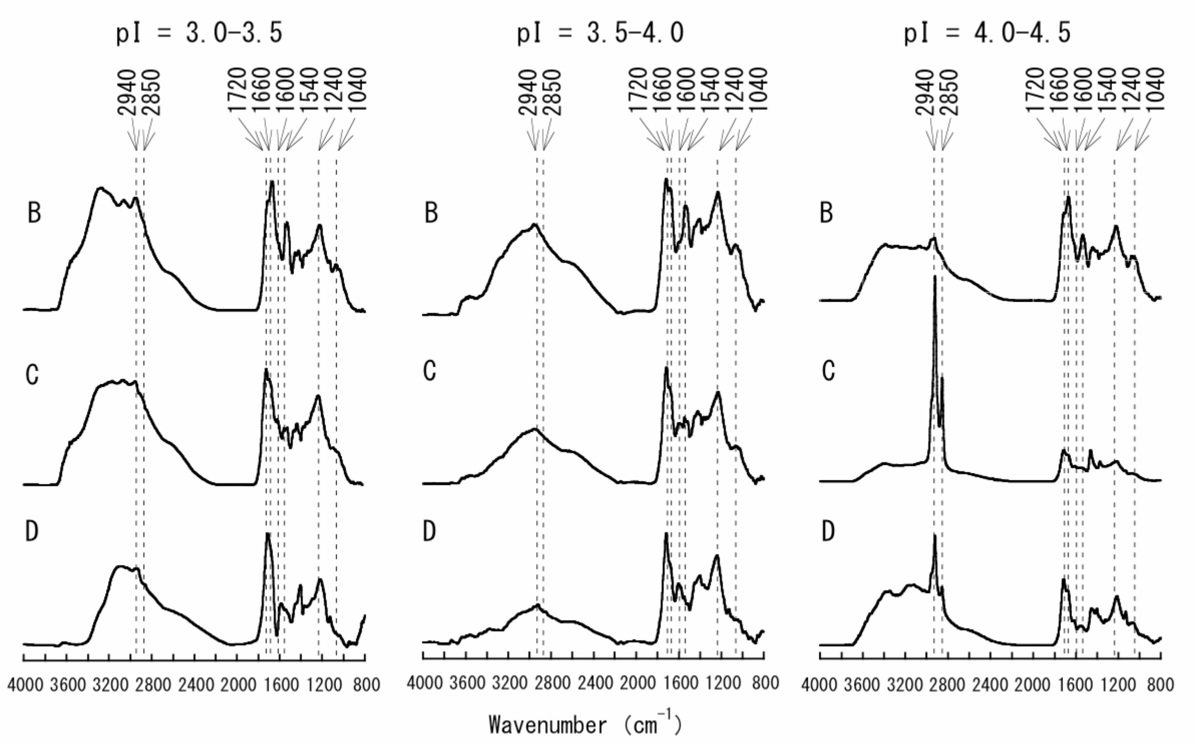

Figure 3: DRIFT spectra of electrophoretic fractions of the Fujisaki soil HA

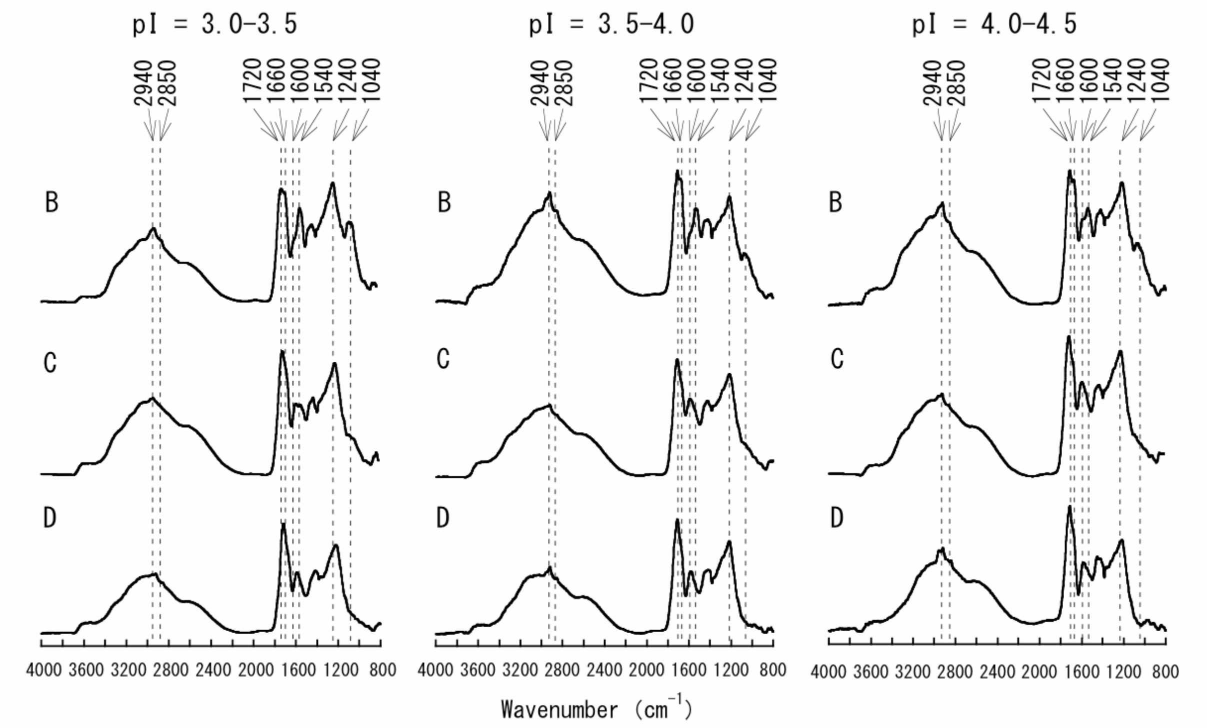

Figure 4: DRIFT spectra of electrophoretic fractions of the Chitose soil HA

4. Conclusions

We succeeded in separating soil HAs on a single polyacrylamide gel by using 2-D gel electrophoresis. The electrophoretic fractions had different molecular sizes and chemical properties. This novel technique offers an effective method for fractionating and isolating the components that constitute HAs.

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Separation and Characterization of Soil HA Fluorescent and Non-fluorescent Fractions by Ultrafiltration in 7M Urea Followed by PAGE Coupled with Transilluminator Technique

Olga Trubetskaya[a], Alexander Shashkov[b], Lubov Shaloiko[a], Dmitrii Demin[c], Bernardo Hermosin[d], Cesareo Saiz-Jimenez[d], Oleg Trubetskoj[c*]

[a]Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 142290 Pushchino, Moscow region, Russia; [b]Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia; [c]Institute of Basic Biological Problems, Russian Academy of Sciences, 142290 Pushchino, Moscow region, Russia; [d]Instituto de Recursos Naturales y Agrobiologia, Consejo Superior de Investigaciones Cientificas, Apartado 1052, 41080 Sevilla, Spain
E-mail: olegi03@yahoo.com

1. Introduction

It is well known that soil HAs are fluorescent compounds with fluorescence arising from many different fluorophores present in humic compounds. However, due to the chemical complexity and molecular size (MS) polydispersity of soil HA, the separation of soil HA in different fluorescent and non-fluorescent fractions have not yet been carried out. In this work we describe the results of soil HA separation on non-fluorescent and different fluorescence units using ultrafiltration (UF) in the presence of 7 M urea as a denaturating agent. All the UF fractions have been assayed by polyacrylamide gel electrophoresis (PAGE) coupled with transilluminator technique for rapid fluorescence detection. Using PAGE with detection in UV and white lights on transilluminator is a quite routine methodology for study of proteins and DNA. However, we are no aware of any literature data using this set-up in HS research.

Further the ^{1}H-NMR spectroscopy and thermochemolysis with subsequent identification of products by gas chromatography-mass spectrometry technique were used for the UF fluorescence and non-fluorescent fractions characterization.

2. Materials and Methods

The soil sample used in this study was taken from the A horizon (10-20cm) of typical chernozem soil (Kursk region, Russia). Isolation of HA from soil was done according to IHSS extraction procedure. The HA was dialyzed through a dialysis tubing (10-12 kDa) during seven days against distilled water. Some characteristics of the soil and soil HA, as well as the method of PAGE used for analysis of HA samples were previously reported [1]. For electrophoresis we have applied on the gel 0.1 mg of HA sample completely dissolved in 0.1 ml of sample buffer containing 89 mM Tris-borate, pH 8.3, 7M urea, 1 % SDS and 1 mM EDTA.

Immediately after electrophoresis the results of HA fractionation in polyacrylamide gel were observed in a dark room using a TFP UV/WL – mixed transilluminator both in white and UV (312 nm) light.

Ultrafiltration of HA was performed using Millipore Amicon regenerated cellulose membrane with nominal retention size 5 kDa in nitrogen gas atmosphere. Fifty mg of HA were dissolved in 50 ml 7M urea and placed into the ultrafiltration cell. After finishing the ultrafiltration procedure, 30 ml of the ultrafiltrate (MS < 5kDa) and 20 ml of the retentate (MS > 5kDa) were obtained. Further, the retentate was diluted with 7M urea up to 50 ml and again passed through the same 5 kDa UF-membrane. This procedure was repeated 30 times until a clear ultrafiltrate solution was obtained. At the end of the experiment we had 30 ultrafiltrates (the volume of each was 30 ml) and a final retentate R (20 ml). All ultrafiltrates were combined into a fraction named UF (MS<5kDa). Then R and half volume of UF were dialyzed using 1kDa dialysis tubing during seven days against distilled water, lyophilized and assayed by PAGE. A second half volume of UF was further passed through a series of ultrafiltration membranes starting with Millipore Amicon regenerated cellulose membrane with nominal retention size 3kDa and then through 1kDa. Two subfractions $UF_{3\text{-}5kDa}$ and $UF_{1\text{-}3kDa}$ were obtained, dialyzed through the 1kDa dialysis tubing during seven days against distilled water, lyophilized and used for further physical-chemical analysis.

The original HA, retentate R and subfraction $UF_{3\text{-}5kDa}$, were dissolved in alkaline deuterated water (D_2O + 0.1N NaOH) at a concentration 5-10 mg/ml. The subfraction $UF_{3\text{-}5kDa}$ was treated by $CDCl_3$ for extraction of lipid components. ^1H-NMR spectra was acquired with a Bruker Avance 600 spectrometer.

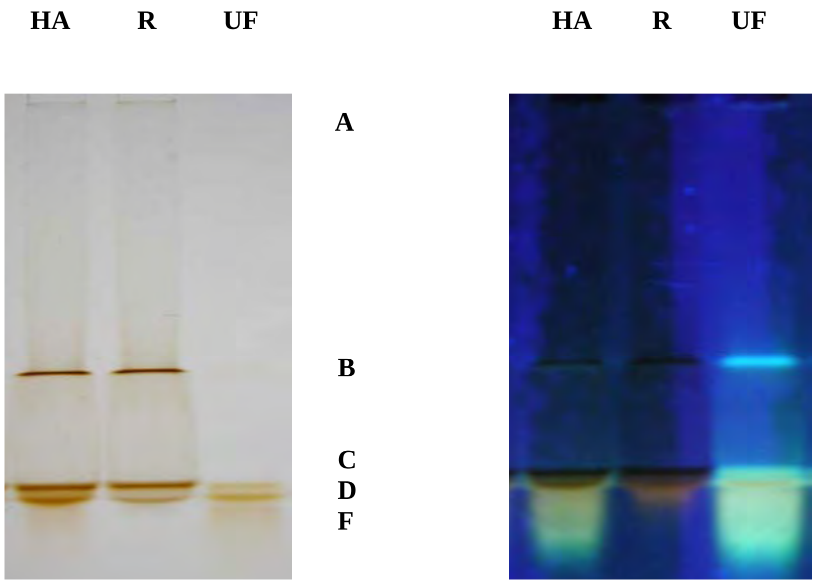
Thermochemolysis, or thermally assisted hydrolysis and methylation of the original HA, retentate R and UF, was performed in a Fisons gas chromatograph GC 8000/mass detector MD 800 coupled to a Fischer 0316 Curie-point pyrolyzer, using a 30 m x 0.25 mm SPB-5 column (film thickness 0.25 μm).

3. Results and Discussions

The bulk HA, retentate R (MS>5kDa) and ultrafiltrate UF (MS<5kDa) were assayed by PAGE. In white light (Figure 1, left) HA originated four discrete fractions: A or the start zone, which did not move into the gel and B, C, and D, which were three narrow, intensively naturally colored zones. The R and UF samples showed some differences. In retentate R zone D was pale and in the ultrafiltrate UF zones A and B absent and zone C was paler than zone

D. To visualize the distribution of emitting constituents among retentate R and ultrafiltrate UF, the gel was detected in UV light (312nm) as well (Figure 1, right).

Under UV light, HA showed the new zone F, which possessed the highest EM and showed intensive overlapped yellow and blue fluorescence, demonstrating that this zone is still not homogeneous, weak blue fluorescence was detected jointly with non fluorecent fraction B. Fractions A, C and D were seen as brown non-fluorescent traces. Retentate R demonstrated a narrow weak red-light band associated with the fraction D and non fluorescent fractions A, B and C. The UF showed bright yellow-blue fluorescence associated with fractions C, D and F and a small part of blue fluorescence formed a narrow zone in the mid part of the gel.



White light **UV light (312 nm)**

Figure 1: Electrophoresis of 0.1 mg HA, R and UF. A, B, C and D – naturally colored zones, detected in white and UV light. F – fluorescent fraction, detected in UV light

Taking into account that ultrafitration was made in 7M urea, which destroy hydrogen bonds, it should be assumed, that humic material which passed through the membrane (nearly 20% in weight of the whole HA) and shown yellow-blue fluorescence, was connected with HA non-fluorecent matrix through intermolecular hydrogen bonds.

Further we have performed subfractionation of UF sample in 7M urea using 3 and 1 kDa ultrafitration membranes. The subfractions UF_{3-5kDa} and UF_{1-3kDa} showed in the transilluminator on UV light (312 nm) yellow and blue fluorescence, respectively.

The deuterated aqueous solutions of original HA, retentate R, and subfraction UF_{3-5kDa} were analyzed by ^{1}H-NMR. These spectra show only non-exchangeable unhindered protons. An essential differences in the region 6.0-9.0 ppm have been observed between R and $UF_{3\text{-}5kDa}$. Most of the aromatic components belong to $UF_{3\text{-}5kDa}$ (hump with max at 7.8 ppm), while R contained minor quantity of other type of aromatics or olefinic protons (hump with max at 7,3 ppm). The spectrum of $UF_{3\text{-}5kDa}$ have been considerably more resolved in aliphatic part (0-4,5 ppm) in comparison with HA and R. For further purification $UF_{3\text{-}5kDa}$ subfraction the extraction of lipids has been done by $CDCl_3$. After lipid extraction the polymethylenic peak (1,3 ppm) essentially decreased and the peaks at 2,2 and 2,4 ppm practically disappeared. On the other hand the mixture of long chain lipids were detected in $CDCl_3$ extract.

HA, retentate R and UF fraction were subjected to pyrolysis-gas chromatography-mass spectrometry analysis. The HA sample yielded a typical soil HA chromatograms, while in fraction R was found alkanes, alkenes and fatty acids with C>22, compounds that were less apparent in fraction UF. On the other hand, fraction UF was enriched in unsaturated C16 and C18 fatty acids and contained considerably less pyrolysis products than HA and fraction R.

4. Conclusions

Using a repeated ultrafiltration in 7M urea followed by PAGE coupled with transilluminator technique with detection in UV-light, we successfully separated soil HA on fluorescent and non-fluorescent fractions. Further the fluorescent material has been separated into rather homogeneous subfractions $UF_{3\text{-}5\ kDa}$ and $UF_{1\text{-}3\ kDa}$, which contained yellow and blue fluorophores, respectively. These subfractions contained considerably less aliphatic components in comparison with the bulk HA. The data obtained could help in deciphering the components of different fluorescent and non fluoresecent humic compounds.

Acknowledgements

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Role of the Aliphatic-Rich Biopolymers Cutin and Cutan and their Transformation Products as Natural Sorbents in Soils

Benny Chefetz[a]*, Baoshan Xing[b] and Michal Shechter[a]

[a] The Hebrew University of Jerusalem, Dept. Soil and Water Sciences, P.O. Box 12 Rehovot 76100, Israel; [b] Department of Plant, Soil and Insect Sciences, University of Massachusetts, Amherst, MA 01003, USA.
E-mail: chefetz@agri.huji.ac.il

1. Introduction

Plant cuticular materials are important precursors for soil organic matter (SOM). The plant cuticle is a thin, predominantly lipid layer that covers all primary aerial surfaces of vascular plants. Plant cuticle has been found in considerable amounts in both natural and agricultural soils. In most plant species, the major structural component of the plant cuticle is the cutin — a high-molecular-weight, insoluble, polyester-like biopolymer. In some plant species, this biopolymer is associated with a base and acid hydrolysis-resistant polymethylene-like biopolymer, known as cutan.

Recently, it has been documented that plant cuticular matter exhibits high sorption capabilities for polar and nonpolar organic compounds [1–4]. The aliphatic-rich structure of the cuticle has been suggested to provide nonspecific and specific adsorption domains [2,4]. The effects of decomposition and transformation in soils of the individual biopolymers (cutin and cutan) on their sorptive capabilities are not well understood. Therefore, the objective of this study was to evaluate the role of humification of the cutin and cutan biopolymers on their ability to act as natural sorbents for organic compounds in soils.

2. Materials and Methods

Cutin and cutan biopolymers were isolated from the fruits of tomato (*Lycopersicon esculentum* Mill.) and leaves of the succulent plant *Agave americana* L., respectively. Isolation was performed using previously reported methods [3,4]. Cutin and cutan (4–6 g) were placed in nylon bags with a mesh size of 45 μm. The bags were placed in plastic containers containing 2500 g of air-dried and sieved (<2 mm) sandy loam soil. N, P, and K were added to the soil at a level of 60 mg/kg. The containers were incubated at 25 ± 1°C for 20 mo. During the incubation period, the moisture level was maintained at a constant level (80% of the soil field water capacity) by wetting the soil on a daily basis according to weight loss. Sampling was performed after 0, 1, 3, 6, 12 and 20 mo of incubation. Characterization of cutin and cutan biopolymers was conducted by: elemental analysis, formation of humic substances [5], CPMAS ^{13}C-NMR spectroscopy, and differential scanning calorimeter (DSC) measurements.

Sorption-desorption experiments were performed with 1-naphthol and phenanthrene using a batch equilibrium technique. Solutions with initial concentrations ranging from 0.01 to 0.8 mg/L for phenanthrene and from 0.1 to 600 mg/L for 1-naphthol were added to the samples (i.e, cutin and cutan) previously weighed into 40-mL Teflon or 100-mL Pyrex bottles. The tubes were agitated in the dark for 5 d based on kinetics measurements. After the sorption experiments, three sequential desorption steps were performed.

3. Results and Discussion

During the first 3 mo of incubation, the level of cutin and cutan decreased rapidly by 15 and 30%, respectively. From that point on, the level of cutan remained stable, while the level of cutin was stable up to 12 mo and then continued to decrease. After 20 mo of incubation, about 35% of the cutin and 30% of the cutan were decomposed. The intact cutin and cutan samples exhibited a high level of alkyl-C (obtained by ^{13}C NMR spectroscopy), making up 60 and 74% of the total C in the samples, respectively. This moiety was decomposed by 45% and 38% after 20 mo of incubation, respectively.

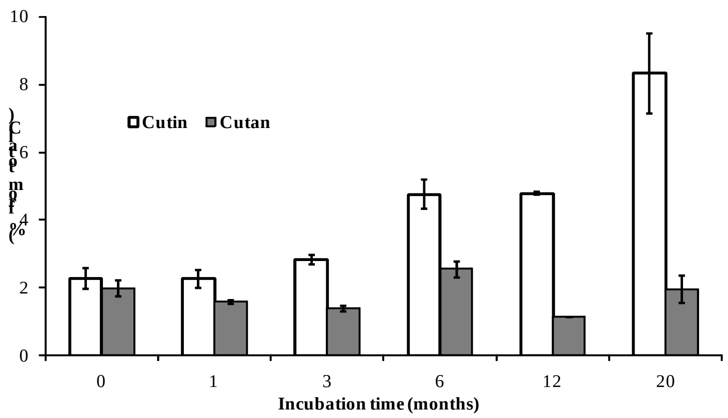

Figure 1: Level of humic substances formed during incubation of cutin and cutan in soil

With the cutin microcosm, the level of humic-like substances was stable during the first 3 mo (2.8% of the total C), then it increased to 8.3% after 20 mo of incubation (Fig. 1). The level of humic-like substances in the cutan microcosm was lower (~2% of the total C) and did not show significantly change over time. The cutan sample exhibited a distinct T_g at 43 °C, corresponding to melting of the rigid (crystalline) polymethylene domain [6,7]. Moreover, a reduction of the enthalpy associated with this phase transition was observed during incubation. The enthalpy decreased from 38.7 J/g C for the fresh cutan to 15.1 J/g C for the 20-mo decomposed cutan. This suggests a decrease (i.e., degradation) in the amount of the rigid (crystalline) polymethylene moieties in the cutan with time.

Phenanthrene exhibited reversible sorption behavior (desorption hysteresis was not obtained) with both cutin and cutan. With the cutin, the K_d values decreased by 80% during 20 mo of incubation whereas for the cutan samples the K_d value decreased only by 40%. 1-Naphthol exhibited nonlinear sorption isotherms. The isotherms linearity decreased with incubation. In addition, 1-naphthol exhibited a pronounced desorption hysteresis with all cutan samples and only with the 20-mo-decomposed cutin sample. As cutan decomposition proceeded, desorption hysteresis became more pronounced (Fig. 2). The maximum sorption capacity (q_{max}) of 1-naphthol decreased by 90% due to cutin decomposition. With cutan, these values did not show any changes correlated with incubation time. After 20 mo of incubation, the q_{max} values obtained for cutin and cutan were similar. This emphasizes the remarkable affect of decomposition and transformation on the sorption domain of the cutin residue.

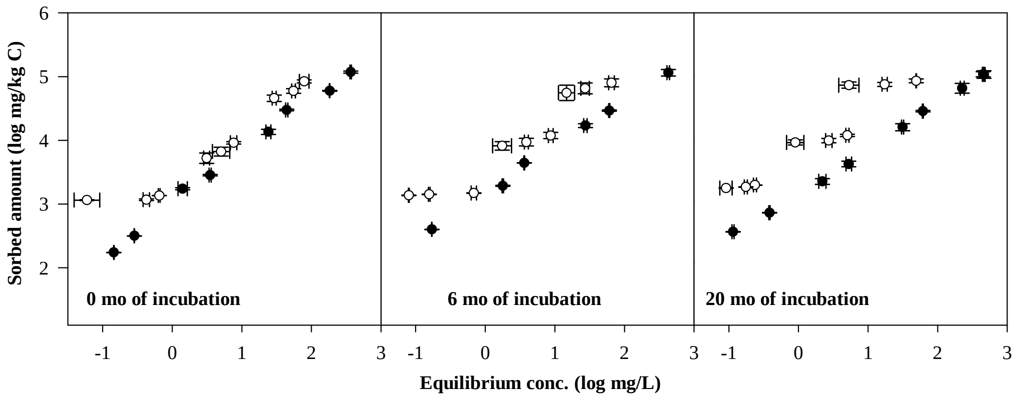

Figure 2: Sorption-desorption of 1-naphthol by cutan biopolymer during incubation. Filled symbols are for sorption data, open symbols are for the desorption points

The ^{13}C NMR and DCS analyses suggest a decrease in the amount of rigid (crystalline) polymethylene moiety due to cutan decomposition. However, the sorption linearity and sorption reversibility of 1-naphthol with the cutan increased as the rigid (crystalline) C content decreased. We therefore suggest that the "rigid" alkyl-C content, calculated as the area in the 30.5–33 ppm regions of ^{13}C NMR spectra [8] is composed of a heterogeneous mixture of rigid (crystalline) and mobile (amorphous) polymethylene C rather than "pure" rigid (crystalline) C.

4. Conclusions

This study demonstrates the important role of the aliphatic biopolymers cutin and cutan as natural sorbents in soil. Although they were subjected to decomposition, they still exhibited a high sorption capacity. However, with humification and degradation, cutan is mostly like to act as highly efficient aliphatic-rich sorbent in soil. The cutan biopolymer is more likely to accumulate in soils via selective preservation, whereas the decomposed products of the cutin are probably transformed into humic-like substances during humification processes.

Our data show that the "rigid" (crystalline) alkyl-C moiety was biodegraded, opposite to previous reports. We assume that the "rigid" (crystalline) alkyl-C moiety (33 ppm) is a heterogeneous mixture of rigid (crystalline) and relatively dense amorphous polymethylene C rather than "pure" crystalline C as previously defined by Hu et al. [8].

Acknowledgements

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Assessment of Hydropyrolysis As a Method for the Quantification of Black Carbon Via the Testing of Standard Reference Materials

Will Meredith[a*], Philippa L. Ascough[b], Emma L. Tilston[b], Colin E. Snape[a], David J. Large[a], Michael I. Bird[c]

[a]Department of Chemical and Environmental Engineering, University of Nottingham, UK;
[b]SUERC, Scottish Enterprise Technology Park, Rankine Avenue, East Kilbride, UK;
[c]School of Earth and Environmental Sciences, James Cook University, Cairns, Australia
E-mail: william.meredith@nottingham.ac.uk

1. Introduction

Black Carbon (BC) is produced from the thermal degradation of biomass under conditions of restricted oxygen (pyrolysis), a process which transforms the starting material into a range of products, including soot, char and charcoal, with BC defined chemically as having both high aromaticity and high resistance to oxidative degradation [1]. BC plays an important role in atmospheric, terrestrial and marine systems, and a number of different chemical, thermal and optical methodologies have been developed for BC quantification in each case, although the results they give are known to vary widely [2]. Hydropyrolysis (hypy), which is pyrolysis assisted by high hydrogen pressure has recently been proposed as a new method for the quantification of BC [3]. This study seeks to assess the efficacy of this method on a wide range of samples that have previously been well characterised as part of a major inter-comparison study of methods for determining BC [2].

2. Materials and Methods

The samples tested were the 12 reference materials used in the BC inter-comparison study, for which detailed sample descriptions have been previously published [2]. Briefly they comprised 3 laboratory-produced BC-rich materials (two chars and *n*-hexane soot); 5 BC-containing environmental matrices (two soils, harbour marine sediment, dissolved organic matter and urban aerosol); 4 non-BC containing potentially interfering materials (shale, lignite, bituminous coal and melanoidin).

Fixed bed hypy tests were performed using the apparatus described in detail previously [3]. Briefly, the samples were first loaded with the Mo catalyst (10% by weight) using an aqueous/methanol solution of ammonium dioxydithiomolybdate [$(NH_4)_2MoO_2S_2$], and pyrolysed with resistive heating from 50 °C to 250 °C at 300 °C min^{-1}, and then from 250 °C to the final temperature (between 450 and 600 °C), where it was held for 2 min at 8 °C min^{-1}, under a hydrogen pressure of 15 MPa. A hydrogen sweep gas flow of 5 L min^{-1}, measured at ambient temperature and pressure, ensured that the products were quickly removed from the reactor for subsequent trapping on dry ice cooled silica. Three replicate tests with a final temperature of 550 °C were performed on all samples to assess the reproducibility of the hypy technique. The BC content of each sample was derived from comparing the TOC of the fresh samples with that of its hypy residue, with elemental analyses being performed in duplicate on a Thermo Scientific 1112 Flash EA.

3. Results and Discussion

The change in residual BC content as a function of temperature for the two reference soils are shown in Fig. 1, with both soils showing the same trend of decreasing carbon content until 550 °C, corresponding to the loss of carbon from the labile (i.e. non-BC) organic matter in the soil. Following this phase a plateau in BC content is reached. This is consistent with other hypy studies for lignocellulosic material which has indicated that up to 100% conversion to volatile products is achieved by approximately 500°C [4]. At 600°C, following the plateau phase, there is the beginning of a further loss of carbon which marks the onset of BC hydrogasification to yield methane. The residual TOC content over the plateau region is taken as the BC content of the sample material, and represents the conditions under which the total BC portion of a sample may be recovered. The results, 20.7% for Vertisol, and 17.1% for Chernozem (at 550°C) are consistent with values obtained for this sample in the BC inter-comparison study [2].

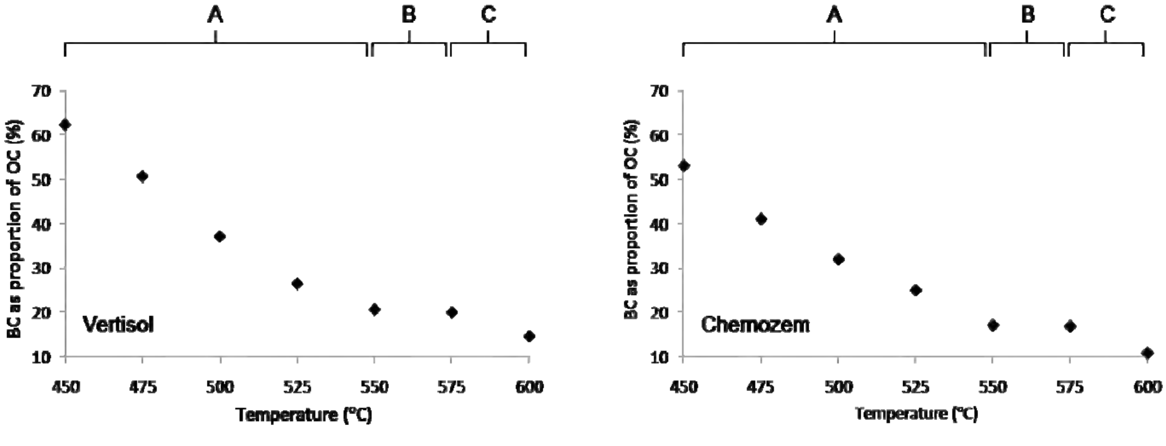

Figure 1: Variation in BC content (as proportion of organic carbon) for reference soil sample hypy residues; showing the zone of labile C loss (A), plateau of BC content (B) and onset of sample hydrogasification (C). Figure adapted from [3]

A similar profile with increasing temperature is observed for the marine sediment, with the plateau at 550 °C suggesting a BC content of 27.9%. In contrast the carbon content of the urban dust aerosol is stable across the whole temperature range with a BC content of 27.9% at

550 °C. These results are again comparable with those from the inter-comparison BC study, suggesting that the ability of hypy to isolate a chemically resistant BC fraction is independent of the environmental matrices in which the BC is found.

For the laboratory produced BC-rich materials hypy is able to differentiate clearly between the very high BC soot and the chars, as shown in Fig. 2, with the soot containing 83% BC and the grass and wood chars 23 and 46% respectively. The lower BC content of the grass char relative to the wood is consistent with the data from dichromate oxidation, although the other methods reported less variation between the chars [2]. It should be noted that these chars were both formed at 450°C and as suggested for chemo-thermal oxidation methods for quantifying BC [2], chars produced at higher temperatures (e.g. 1000 °C) would likely be more resistant to degradation (so give higher BC contents) under hypy conditions.

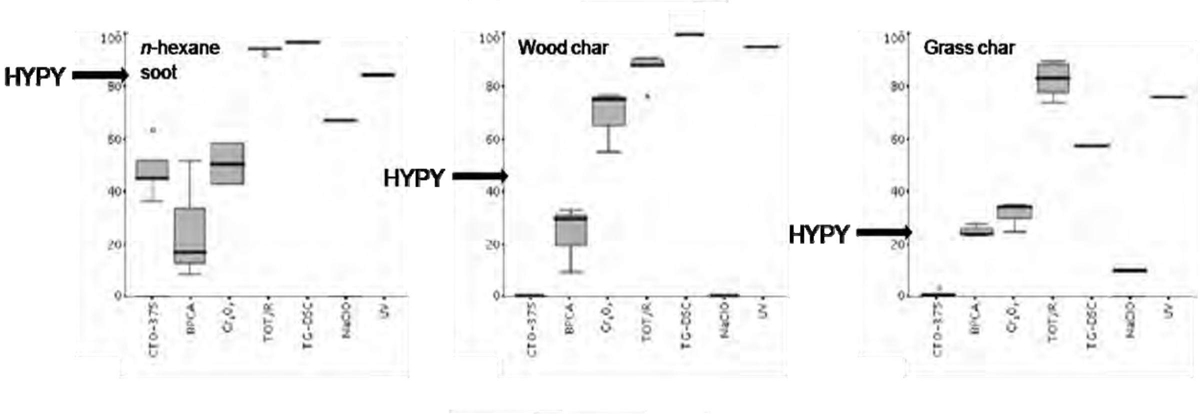

Figure 2: Comparison between hypy and the methods used in the BC inter-comparison study for the quantification of BC in laboratory produced chars. Figure adapted from [2]

The results for the non-BC containing potentially interfering materials show that hypy did not report false positives for the melanoidin, lignite or Green River Shale. It did however, in common with all but one of the other methods in the inter-comparison study [2] report a significant BC content for the bituminous coal (45%), due in part to its significant inertinite content derived from ancient fire occurrences [5].

One potential benefit of hypy is therefore the ability to provide a methodology by which uniform and standardized operating conditions can be used for isolation of BC from a wide range of materials. Additionally, the results are very reproducible, with BC determinations from replicate analysis typically within ±1% across all samples. This indicates that provided the sample is well homogenized prior to treatment, a high degree of measurement precision can be achieved using the hypy method in order to consistently remove the same (non-BC) components over different runs using the same methodological protocol for a wide range of sample types.

4. Conclusions

The results of this study suggest that hypy is reproducibly and rapidly able to isolate the most resistant BC fraction from carbonaceous samples, independent of the environmental matrices in which it is found. The fact that hypy can reduce labile organic matter to volatile products in a controlled manner makes it an attractive new approach for BC quantification across a wide range of sample materials.

The BC contents as determined by hypy for all of the 12 samples fall within the range reported in the BC inter-comparison study. Hypy is also shown to distinguish successfully BC from all of the potentially interfering materials, with the exception of the high rank bituminous coal, which, due to its significant inertinite content that is chemically indistinguishable from BC.

Acknowledgements

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Mid-infrared Spectroscopic Properties of Humic Acid and Fulvic Acid-soil Mixtures

Francisco J. Calderón[a*], James Reeves III[b], and Merle F. Vigil[a]

[a]USDA-ARS, Central Great Plains Research Station, Akron, Colorado, USA; [b]USDA-ARS Environmental Management and ByProducts Utilization Lab., Beltsville, Maryland, USA
E-mail: francisco.calderon@ars.usda.gov

1. Introduction

The detection of humic materials in soils is essential in order to determine organic matter (SOM) stability and C sequestration on agricultural land. Mid-Infrared (MidIR) spectroscopy has been used to characterize SOM quality [1], study extracted soil humic acids [2], develop calibrations for quantifying SOM [3], and to study decomposition of organic matter in soil [4]. However, infrared spectra from soils are the result of a multitude of combined absorbances from organic and mineral bands, and some of the spectral signatures of humic acids can be lost or confounded in soil matrices.

In this study, we added different amounts of authentic humic and fulvic acid standards to ashed soil in order to identify reliable spectral MidIR bands for marking the presence and amount of stable organic matter in soil.

2. Materials and Methods

In order to obtain soils free of organic matter background, we ashed Elliott Silt Loam Soil (International Humic Substances Society (IHSS), St Paul, MN) at 550 °C for 3 h. Besides the Elliott soil standard, we also ashed two soils from Akron Colorado, one was an eroded (high erosion) soil of low SOM, and the second was a prairie top soil of moderately high SOM (low erosion). We obtained the following standards from the IHSS to be mixed with the ashed soils: Suwannee River Humic Acid, Suwannee River Fulvic Acid, Elliott Soil Humic Acid, Pahokee Peat Humic Acid, and Pahokee Peat Fulvic Acid. The standards were mixed with the soils using a dilution series of 1/10, 1/100 and 1/1000 standard/soil ratios before scanning. The mixtures, as well as the neat soils and standards were scanned on a Digilab FTS7000 Fourier transform spectrometer (Varian, Inc., Palo Alto, CA) in line with a Pike AutoDIFF diffuse reflectance sampler (Pike Technologies, Madison, WI) in the MidIR range (4000 to 400 cm^{-1}).

3. Results and Discussion

Mid IR observations of the spectra form the pure humic acids, fulvic acids, soils, and ashed soils. The humic acids absorbed prominently at the broad band around 3400 cm^{-1}, commonly assigned to O–H (or N–H) stretching in plant-derived materials, although it will be largely to O–H in the humic acids [4]. This prominent wide band can be observed in the Suwannee River Humic Acid spectrum (Fig. 1). Whole soils and fulvic acids absorb moderately in this region. Ashed soils lost absorbance at 3400 cm^{-1} because of the combustion and loss of organics. From 2870–2950 cm^{-1} lies the aliphatic C–H stretching band that marks the presence of methyl and methylene groups [1, 3, 4]. Humic acids absorb in this band (Fig. 1), with whole soils and fulvic acids having intermediate absorbance, and ashed soils having the least absorbance. At 2600 cm^{-1} is the carboxylic acid O–H stretching band, which forms a shoulder on humic acid spectra (Fig. 1). At 1730 cm^{-1} is the C=O bond stretching band of carboxylic acid [3,4], and esters [1], which is one of the most marked spectral features in humic and fulvic acids. Cox et al., [5] state that this band is characteristic to soil humic compounds. Our results support this, as seen in the Suwannee River Humic Acid spectrum (Fig. 1). Bands at 1640 and 1560 cm^{-1} mark the presence of amides in organic materials, but others have shown them to be present in humic acids, which can contain N due to free radical coupling of nitrogenous substances [2]. The Pahokee Peat and Elliott Soil humic acids were strong absorbers, and ashing soils diminished the absorbance in this spectral region (data not shown). The same pattern was found for absorbance at 1610–1620 and 1517 cm^{-1}, both aromatic bands present in the stable fraction of soil organic matter. The Suwannee River fulvic acid, Pahokee Peat humic acid, and Elliott Soil humic acid absorb strongly in the region between 1400 and 1350 cm^{-1} dominated by phenolic, or COO stretching vibrations of oxidized carbon and CH_3 bending [3, 4]. At 1335 cm^{-1} is a peak of possible mineral influence given the high absorbance of the ashed soil (Fig. 1). The fulvic and humic acids absorb in the region around 1230 cm^{-1} for aromatic –CH (Fig. 1), and at 1030–1160 cm^{-1}, a region assigned to –COH stretching. Note that bands at 2200–2000, 1230, 1030-1160 cm^{-1} have been found by others to be important for soil organic C calibrations [3]. The 1030–1160 cm^{-1} region lies in the quartz inversion band, which explains the low absorbance in the neat soil samples (data not shown). Note that absorbance between 1030 and 400 cm^{-1} varies widely among the humic and fulvic acids. This region is difficult to interpret, with many bands of uncertain mineral or organic origin.

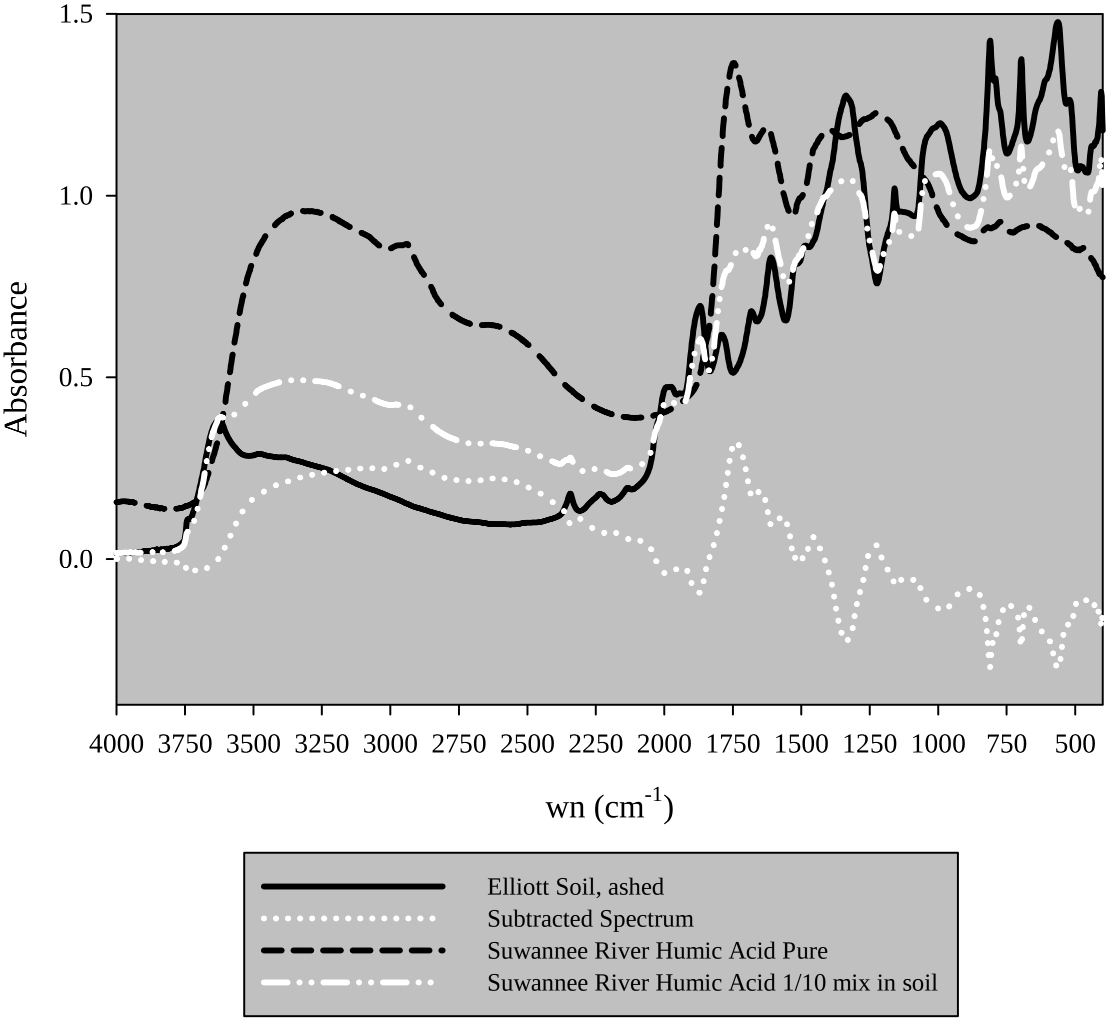

Figure 1: Mid Infrared spectra of the Elliott ashed soil, Suwannee River Humic Acid, the soil and humic acid mix. The subtracted spectrum is: (ashed soil and humic acid mixture) – (ashed soil).

The MidIR spectra also provide useful information about the mineral composition of the soils. All the intact and ashed soils have the clay OH stretching peak at 3600 cm^{-1} (Fig. 1) [6]. The high erosion soils (ashed and intact) have a peak at and 2517 cm^{-1} indicative of the presence of calcium carbonate (data not shown) [6]. This is due to the fact that erosion has removed the original soil surface to a level where the caliche layer is exposed on these soils. All the soil spectra show the peaks at 1970–2000, 1870, and 1790 cm^{-1}, which are quartz overtone combination bands [6] indicative of sand content, and can be seen in the ashed Elliott soil (Fig. 1).

Spectral subtraction of standard-soil mixture spectra from ashed soil spectra. The spectral subtraction approach allows us to mathematically isolate spectral bands of humic and fulvic acid standards when they are in the presence of a mineral soil matrix. The subtracted spectrum in Figure 1 indicates that the addition of humic acid to soil increases absorbance in the region between 3400-2800 cm^{-1}, due to the increased concentration of O–H and C–H groups. Perhaps the most marked effect on the spectra was an increase in the absorbance near 1730–1630 cm^{-1}, possibly due to C=O bonds, amides, and/or aromatics. These effects were observed across most humic and fulvic acids, and in some cases could be found at up to 1/1000 dilution of standard in ashed soil (data not shown).

4. Conclusions

Several regions of the MidIR spectrum decrease in soils upon ashing, and at the same time are highly absorbed by humic and/or fulvic acids. These regions include 3500–2000, 1830–1520, and 1260–990 cm^{-1} and should thus be considered organic matter bands in soils. Other MidIR regions outside these bands are predominantly of mineral absorption or from a mixture of organic-mineral absorption. The 2870–2950 cm^{-1} is one of the few regions of the MidIR soil spectrum where absorbance is almost exclusively from organics, with little absorbance due to mineral sources. Our spectral subtraction approach suggests that 3400–2800 and 1730–1630 cm^{-1} are regions that can be attributed to stable organic compounds in soil spectra.

Acknowledgements

Thanks to Brandon Peterson for all the laboratory work and handling of the spectral data.

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Chemical and Spectroscopic Characterization of Marine Dissolved Organic Matter Isolated Using the Coupled Reverse Osmosis-Electrodialysis (RO/ED) Method

E. Michael Perdue[a*], Nelson W. Green[a], Hongmei Chen[b], Aron Stubbins[b], Kenneth Mopper[b], Jingdong Mao[b], John R. Helms[b], Patrick G. Hatcher[b]

[a]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, Georgia, 30332, USA; [b] Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, VA 23529, USA
E-mail: mperdue@eas.gatech.edu

1. Introduction

Dissolved organic carbon (DOC) in the oceans is one of the largest dynamic reservoirs of carbon on earth, comparable in size to the reservoir of carbon (as CO_2) in the atmosphere, or to the amount of carbon in all terrestrial and aquatic biota (Hedges et al., 1997). The concerted efforts of earth scientists, atmospheric scientists, and biologists who study global biogeochemical cycles and the earth's climate have yielded a rather detailed understanding of carbon in the atmosphere and in biota. Marine dissolved organic matter (DOM) is far less well characterized, principally because it exists as a highly diluted mixture of perhaps millions of organic compounds in a highly saline aqueous solution. This research builds upon many prior efforts to isolate DOM from seawater using XAD resins, C_{18} adsorbents, and cross-flow ultrafiltration, sharing with those studies the goal of obtaining relatively large quantities of representative, low-ash samples that can be studied to learn much more about the origin, reactions, and fate of DOM in seawater. To obtain representative samples of isolated marine DOM that are suitable for the widest array of methods of chemical and spectroscopic characterization, virtually all of the salts in seawater must be removed, the bulk of the water must be removed, and a large, representative fraction of the DOM must be retained.

When DOM is isolated from seawater by ultrafiltration using a 1kDa membrane or by solid-phase extractions, typical yields are 25% or less [1, 2]. We have recently coupled reverse osmosis (RO) for removal of water with electrodialysis (ED) for removal of sea salts to isolate DOM from seawater, and we have recovered an average of 75 ± 12% of DOM from 16 seawater samples [3–5]. Five of the samples have been characterized spectroscopically and chemically [5]. Those published results and new unpublished results from recent cruises to the Mauritanian Upwelling Zone in 2008 and to Station ALOHA in 2009 will be presented.

2. Materials and Methods

Our RO/ED system can currently process 200 L of seawater *at sea* in around 8 hours. In the initial ED phase, the salinity of a sample is reduced from around 50,000 µS cm^{-1} to 15,000 µS cm^{-1}, at which point the osmotic pressure of the sample has been reduced that water can be removed by RO. During the RO/ED phase, water is removed by RO until the volume of the sample is reduced to approximately 5 L, and sea salts are removed concomitantly by ED to maintain a constant conductivity of 15,000 µS cm^{-1}. In the final ED phase, the conductivity of the now-concentrated DOM sample is reduced to its final target value (currently 50 µS cm^{-1}).

3. Results and Discussion

For sixteen seawater samples processed via RO/ED on two cruises in 2006, the yields of DOM averaged 75 ± 12%, and a procedural blank gained only about 3 µmol L^{-1} DOC during processing [5]. Such yields are far greater than the yields of DOM that are most often achieved using older methods of isolation, so the RO/ED method potentially provides a less biased sample of marine DOM.

RO/ED samples of marine DOM have approximately the same DOC/DON ratios as bulk DOM in seawater (16–19, systematically decreasing with increasing distance from the coast), indicating that DOC and DON are isolated in the same yield by the RO/ED method. The normalized UV absorption coefficients (m^{-1}) of initial and final samples are compared in Figure 1. The results are in excellent agreement, indicating an unbiased sampling of chromophores by the RO/ED method.

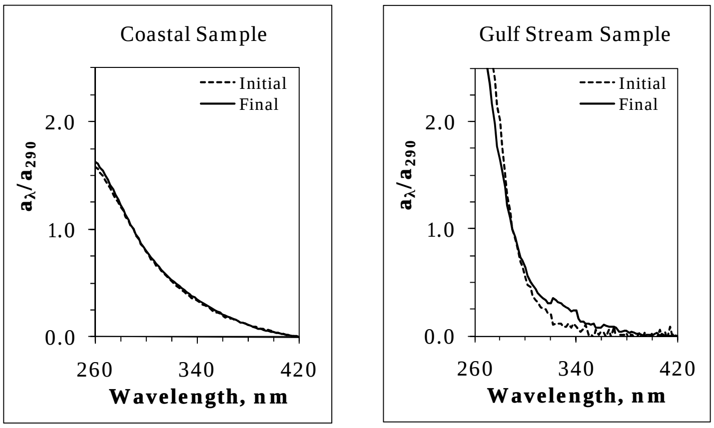

Figure 1: Normalized absorption coefficients (a_λ/a_{290}) for RO/ED samples of marine DOM from the mouth of the Ogeechee River, Georgia, USA (Coastal Sample) and from the open Atlantic Ocean (Gulf Stream Sample)

Both ^{1}H and ^{13}C-NMR spectra for an average RO/ED sample are compared in Figure 2 with corresponding average spectra for samples that were isolated using ultrafiltration and solid-phase extractions. The average spectrum of DOM that was isolated using ultrafiltration exhibits very strong peak intensity in the two ranges of chemical shift that are usually attributed to carbohydrates, and the average spectrum of DOM that was isolated using solid-phase extractions exhibits the strongest signal in the ranges of chemical shift that are usually attributed to non-functionalized alkyl carbon and to aromatic carbon. The peak intensity for the average RO/ED sample is generally intermediate between the peak intensities for the UF and SPE samples across the entire range of chemical shift, in effect averaging out the dramatic differences between the spectra of samples isolated by the UF and SPE methods.

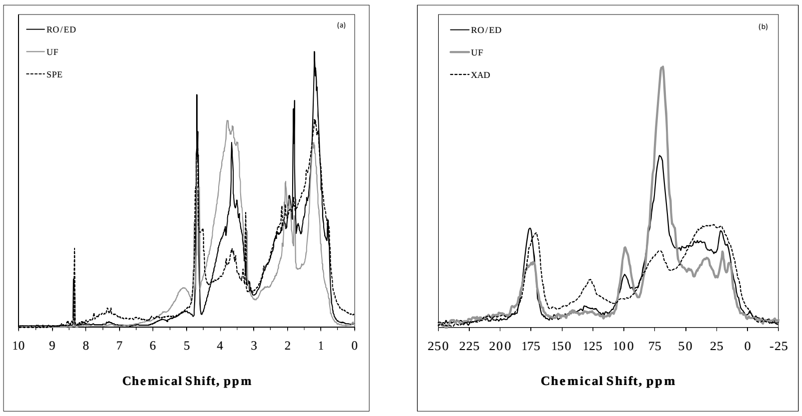

Figure 2: ^{1}H and ^{13}C NMR spectra (a and b, respectively) for the average RO/ED sample and for average spectra of samples of marine DOM that were isolated using ultrafiltration or solid-phase extractions

Finally, Fourier transform ion cyclotron resonance (FTICR) mass spectra show that RO/ED samples of marine DOM from coastal waters contains thousands of compounds having molar H/C < 1, which contrasts sharply with the near absence of these compounds in RO/ED samples of marine DOM from the open ocean. The results are given in the form of van Krevelen plots in Figure 3. The presence of so many unsaturated compounds in coastal waters is consistent with the fact that such DOM is rich in compounds that are derived from lignins and tannins. Their absence in RO/ED samples of marine DOM from the open ocean is undoubtedly due to the combined effects of dilution, photodegradation, and biodegradation.

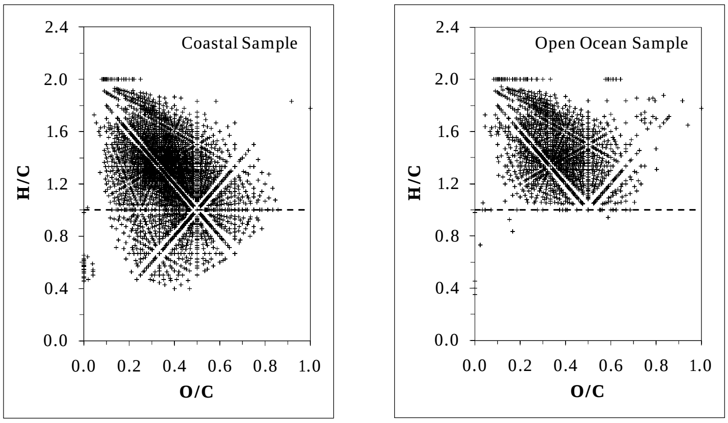

Figure 3: Van Krevelen plots of molecular formulae that were derived from FTICR mass spectroscopic data for RO/ED samples of marine DOM from coastal and open ocean water.

4. Conclusions

The RO/ED process can be used to remove more than 95% of the water and more than 99.995% of the sea salts from a 200 L sample of seawater in 6–8 hours of processing *at sea*, while recovering an average of 75% of DOC in the sample. All results (C/N, UV, ^{13}C NMR, ^{1}H NMR, and FTICR-MS) reveal clear differences between non-coastal and coastal marine DOM, and the observed differences confirm, not surprisingly, that the coastal sample contains a significant amount of terrestrially derived DOM. It is thus reasonable to conclude that the coupled RO/ED process enables the isolation (at sea) of significant quantities of chemically representative DOM in high yield from both coastal and non-coastal surface seawater.

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SESSION 3: NOM AND HS IN SOILS AND SEDIMENTS

Mitigation of GHGs Emission from Soils by a Catalyzed *in situ* Photo-Oxidative Polymerization of Soil Humic Molecules

Alessandro Piccolo*, Riccardo Spaccini, Antonio Nebbioso

Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali,
Università di Napoli Federico II, Via Università 100, 80055 Portici, Italy
E-mail: alessandro.piccolo@unina.it

1. Introduction

In 2005, agriculture accounted for an estimated emission of 5.1 to 6.1 GtCO$_2$-eq/yr (10– 12% of total global anthropogenic emissions of greenhouse gases (GHGs)). However, measures to mitigate GHGs emission from agricultural soils are limited to improved cropland practices such as crop rotation, nutrient management, tillage/residue management, agroforestry, and return to natural vegetation. These practices are not only far from substantially reducing GHGs emissions from soils or permanently stabilizing soil organic matter, but are also predicted to hardly match more than a maximum of 25% of the GHGs reductions required by the Kyoto Protocol within 2050. Despite the knowledge that GHGs release from soil largely derives from biochemical transformations of plant litter and soil humus (SH), no new and much wished biotechnological measures are adopted so far to augment mitigation. Here we propose an innovative approach to mitigate GHGs emissions from soils based on the *in situ* photo-polymerization of SH under biomimetic catalysis. Three Mediterranean soils of different physical and chemical properties were added with a synthetic watersoluble iron-porphyrin, irradiated by solar light, and subjected to 15, and 30 wetting and drying cycles.

2. Materials and Methods

Soil Samples and Characterization. Soil samples were collected from the surface layers (0-30 cm) of three agricultural plots from south-central Italy: 1. Porrara (Avellino), 2, Colombaia (Caserta), 3. Itri (Latina). Samples were air dried, sieved through a 4.75 mm sieve, and used for characterization and incubation experiments.

Photo-polymerization experiments. For each replicate (n = 3), 60 g of air dried soil sample was placed on a Petri dish (12 cm diameter) and soil moisture was kept at 40% of water holding capacity (WHC) by adding X mL of water (X = 20, 17, 10 mL, for Porrara, Colombaia and Itri soil, respectively) in order to obtain a control series. The polymerized series were similarly prepared (n = 3) and added with 0.24 μmol of synthetic water-soluble iron-porphyrin (*meso*-tetra(2,6-dichloro-3-sulfonatophenyl)porphyrinate of iron(III) chloride, Fe-(TDCPPS)Cl) dissolved in the X mL of water pertaining to WHC of each soil. After preparation, both control and polymerized series were left under natural solar radiation throughout the following treatments: (i) covered with a Petri dish and incubated for 5 d; (ii) submitted to 15 wetting/drying cycles; (iii) submitted to 30 wetting/drying cycles. During wetting/drying cycles, samples were uncovered after 5 d incubation and distilled water was added, whenever samples became dry (approximately once a week), to reestablish WHC.

Aggregate stability. An air–dried sub–sample (30 g) was placed on the top sieve of a set of three nested sieves (1.0, 0.50, and 0.25 mm) and submerged into 2 cm of distilled water for 30 min. After this time, the sieves were manually oscillated (up and down 4 cm) for 30 times during 1 min. Recovered aggregate fractions were oven–dried at 60°C, weighed, and stored at room conditions. The mean weight diameter index in water (MWDw) used for the determination of aggregate stability was calculated according to the equation:

$$\text{MWDw} = \sum_{i=1}^{n} X_i W_i$$

where *Xi* is the mean diameter of each aggregate fraction and *Wi* is the proportion of the total sample weight occurring in the *i–th* fraction. The amount of OC (%) in each aggregate fraction was normalized to the weight of each fraction: OC content in fraction (g kg^{-1}) × mass of recovered fraction (g kg–1) / total OC recovered (g kg^{-1}).

Soil respiration. Soil respiration was evaluated by a dynamic absorption method. Briefly, 9 g of air dried and rewetted soil sample (< 2 mm) were placed on a air-tight soil respiration flask in which a CO_2-free air was continuously fluxed by a peristaltic pump. The CO_2 emitted from soil was then captured in a trap containing a 0.01 M NaOH solution. The amount of CO_2 absorbed in this solution was determined after 27 days by back titration with 0.01 M HCl after addition of 7 mL of 0.5 M $BaCl_2$. The ambient CO_2 concentration was determined by inserting blank samples (i.e.: no soil) into the respiration system.

Statistical analysis. A Student’s *t*-test was used to compare values obtained for control and treatments, and difference was considered to be significant at the level of $P \leq 0.05$.

3. Results and Discussion

The humified organic matter in soil (70–80% of SOM) represents the principal potential C sink in the biosphere, whose advanced comprehension may help to mitigate CO_2 emissions from soil. Soil humus is composed by the hydrophobic and heterogeneous aliphatic and aromatic molecules progressively surviving the microbial transformation of dead biological tissues [1]. Recent scientific evidence shed new light on the chemical nature of humus by describing humic molecules as heterogeneous but relatively small in mass (≤ 1000 Da) [2], rather than the previously assumed macropolymers [3]. Humic molecules were shown to be tightly associated in supramolecular structures, which are prevalently stabilized by noncovalent hydrophobic bonds [1]. Based on this, it is reasonable that small aromatic humic molecules could be covalently linked to each other by oxidative coupling reactions under appropriate catalysis, thereby enhancing their molecular size and complexity. It is already shown that larger and more chemically-stable humic molecules were obtained by treating humic solutions in oxidative (H_2O_2) conditions, with a phenoloxidase enzyme, such as peroxidase [1], or a biomimetic catalyst, such as an iron-porphyrin [4]. Both the enzymatic and biomimetic catalysts accelerate the oxidative coupling of phenols via a free-radical mechanism. Moreover, the biomimetic catalysis increased the molecular dimension of humic matter in solution simply by photo-oxidation under solar radiation, without the need of an additional oxidant [5]. By applying photo-oxidative catalysed conditions on humic phenolic monomers, a rapid formation of new intermolecular C-C and C-O-C bonds led to several identified (up to tetramers) and unidentified oligomers [6–8].

The photo-polimerization technology can be applied *in situ* on soils. The catalyzed photo-oxidative formation of covalent bonds among soil phenolic molecules would chemically stabilize SH by increasing the content of chemical energy in humic structures and consequently reducing the extent of SH biomineralization. Moreover, increasing the mass of humus molecules would result in linking together soil particles to larger soil aggregates and thus improving soil physical quality.

The occurrence of SOM photo-polymerization was suggested by the mean-weight diameter of soil aggregates in water (MWDw), an index of aggregate stability, that significantly increased over control for all the three soils after the 5 d incubation (Fig. 1), though to a different extent depending on the intrinsic composition of each soil. This indicates that the photo-polymerization treatment, by increasing the molecular mass and cross-linking of humic molecules, promoted a tighter association among soil particles and formation of larger water-stable aggregates. An enhanced soil aggregate stability in the photo-polymerized samples was still kept after 15 w/d cycles, but it was lost for all soils after 30 w/d cycles.

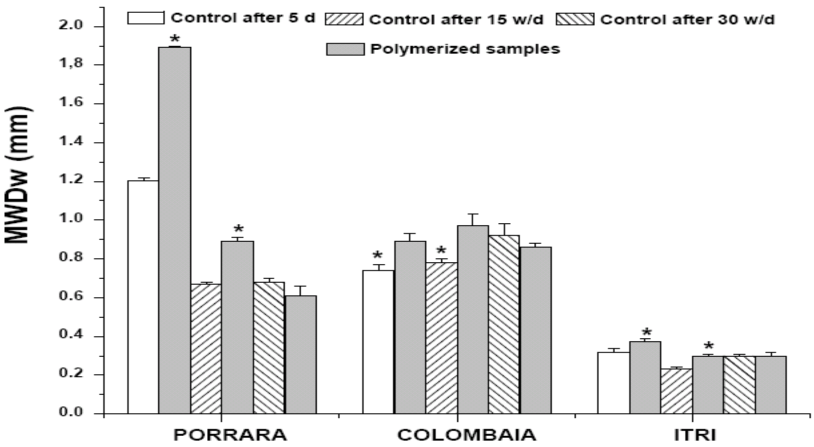

Figure 1: Mean Weight Diameter in water (MWDw) for the three soils, Porrara, Colombaia, Itri, before and after photo-polymerization treatment for 5 d incubation, and 15 and 30 wetting and drying cycles. Error bars indicate standard error (n = 3). The asterisks denote a significant difference between control and treatment at the level of *P* ≤ 0.05

A stronger chemical and physical stabilization of OC after the catalytic photo-polymerization of soils can be inferred by the amount of respired CO_2 (Fig. 2). Microbial mineralization of SH was significantly inhibited in the photo-polymerized samples, as compared to control, for all soils after 5 d incubation and even after 15 w/d cycles. While the reduction of CO_2 emission was significant for Porrara and Itri soils also after 30 w/d cycles, this was no longer true for the Colombaia soil. The CO_2 respiration behaviour of Porrara and

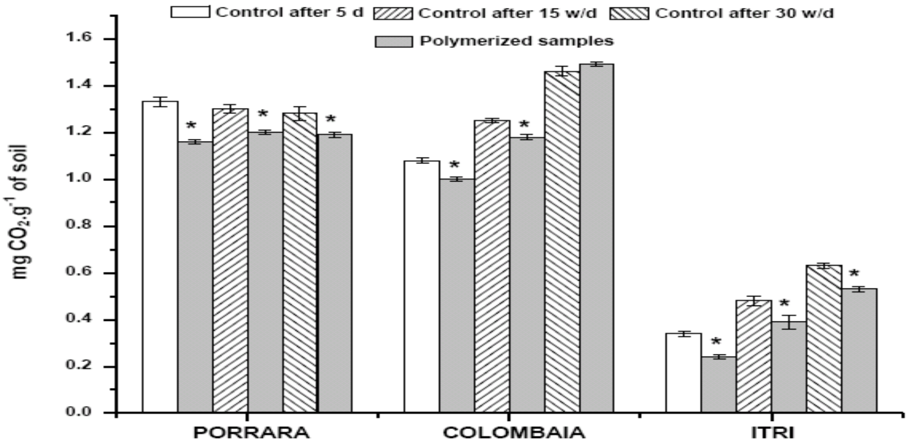

Figure 2: Soil respiration (mg $CO_2.g^{-1}$ of soil) from the three soils, Porrara, Colombaia, Itri, before and after photo-polymerization treatment for 5 d incubation, and 15 and 30 wetting and drying cycles. Error bars indicate standard error (*n* = 3). The asterisks denote a significant difference between control and treatment at the level of *P* ≤ 0.05

Itri soils confirmed that the catalytic photo-polymerization had strongly stabilized their SH. The different result for the Colombaia soil after 30 w/d cycles may be attributed to its larger amount of OC, thus limiting the treatment effect. The OC stabilization obtained in Porrara, Colombaia, and Itri soils mitigated the CO_2 emission by, respectively, 12.8, 7.4, and 29.4 % after 5 d incubation, 8.3, 5.6, 18.7 % after 15 w/d cycles, and 7.0, -2.0, 15.9 % after 30 w/d cycles. Such mitigation corresponded to 0.34, 0.16 and 0.20 Mg of $CO_2.ha^{-1}$ for Porrara, Colombaia, and Itri soils, respectively, after 5 d of incubation, and still to 0.18 and 0.20 Mg of $CO_2.ha^{-1}$ for Porrara and Itri soils, respectively, even after the severe disaggregation of 30 w/d cycles. These results confirm the effectiveness of the proposed technology.

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Application of the DFRC Method to Humic Substances of an Ombrotrophic Peatland. Study of Monolignols and Lipids

Céline Estournel, Laurent Grasset*, André Amblès
Université de Poitiers, SRSN, UMR-CNRS 6514, 40 Av du Recteur Pineau, Poitiers, France
E-mail: laurent.grasset@univ-poitiers.fr

1. Introduction

The DFRC method (Derivatization Followed by Reductive Cleavage) is commonly used in wood chemistry for lignin characterization. It efficiently cleaves aryl ethers present in lignin and leads to monomers soluble in organic solvents which could be characterized by gas chromatography. It has been applied to an ombrotrophic peat bog (Frasne, Jura, France). Aromatics such as monolignols and several series of lipidic compounds such as *n*-alkanes, fatty acids, fatty alcohols, ketones, pentacyclic alcohols and sterols were identified. Comparison has been made with the results obtained for the corresponding humic acids and humin.

2. Materials and Methods

Frasne peatland (46°49'N, 6°10'E) is undisturbed *Sphagnum*-dominated mire situated in the Jura Mountains (France). The site is protected by the UE Directive "Natura 2000". Sample was taken in the wooded part of the peatland (covered since two centuries by *Picea Abies* or *Pinus Uncinata*).

Peat sample was first submitted to a Soxhlet extraction with chloroform to remove free lipids. Afterward, humic acids and humin were extracted according to the IHSS procedure. The DFRC procedure was almost identical to the one reported in the literature [1] (Fig. 1). DFRC is a three simple steps pathway: bromination of the benzylic position and simultaneous acetylation of free hydroxyl groups by acetyl bromide (AcBr); reductive cleavage of the *β*-aryl ether bonds via zinc metal coordination and acetylation of the newly generated phenolic hydroxyl groups.

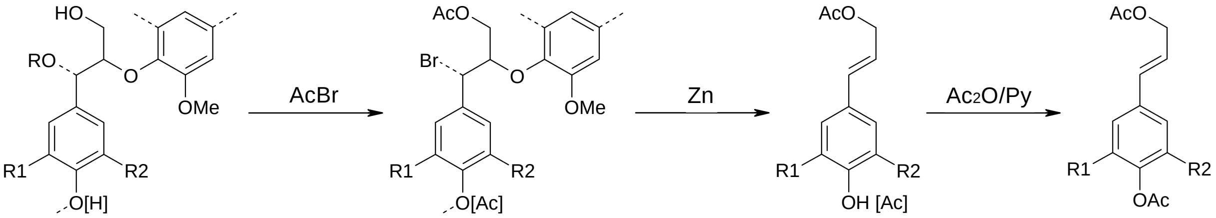

Figure 1: Selective ether cleavage in lignins by DFRC method

The final organo-soluble mixture was methylated with (TMS)diazomethane and fractionated by silica gel chromatography before analysis by Gas Chromatography – Mass Spectrometry.

3. Results and Discussion

The DFRC method allowed solubilizing in organic solvent about 64% of the lipid-free peat while humic substances seemed to be less reactive (about 15% for humic acids and 26% for humin). Expected monolignols were identified with several series of lipidic compounds such as *n*-alkanes, fatty acids, fatty alcohols, ketones, pentacyclic alcohols and sterols.

Monolignols were mostly vanillyl units showing the incorporation of gymnosperm lignin to humic substances. *n*-Alkanes of humic acids had an unusual distribution with a strong dominance of the even carbon numbered compounds which revealed a microbial contribution. Fatty acids, ranging from C_{12} to C_{32}, had a bimodal distribution, focused on C_{16} and C_{22}, with an even-over-odd carbon number predominance which is typical of higher plant input. A bacterial contribution (stronger for humic acids than for humin) has been revealed by the presence of *iso* and *anteiso* acids (C_{15} and C_{17}). Aliphatic alcohols, ranging from C_{22} to C_{32}, were even carbon numbered compounds characteristic of higher plant input and particularly of sphagnum species (dominance of the C_{22} and C_{24} members). Ketones, ranging from C_{17} to C_{33} and the 6,10,14-trimethylpentadeca-2-one (a degradation product of phytol) were also identified. Identified ω-hydroxyacids were even carbon numbered compounds with a predominance of C_{24}. These compounds were known to be cutin and suberin components.

4. Conclusions

Results show the capability of the DFRC method for tracing biopolymers (such as suberin and lignin) input in wetland. Monolignols, as vanillyl units, and several series of lipidic compounds were identified. The gymnosperm contribution to our sample is highlighted. Additionally, lipids from peat, humin and humic acids had qualitative and quantitative differences probably due to distinct vegetal and microbial origins.

Acknowledgements

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Identification of Abiotically-Produced Alkyl Amides in Humic Material in Anoxic Sediments: A Possible Source for N Immobilization

Georgina A. McKee* and Patrick G. Hatcher

Department of Chemistry and Biochemistry, Old Dominion Univ. Norfolk, VA, 23529, USA
E-mail: gmckee@odu.edu

1. Introduction

Nitrogen is a key nutrient for many aquatic organisms. When released into organic matter (OM) systems following the death of an organism much of the OM is remineralised back into inorganic forms. However, a small fraction, ~1 %, is released from the short-term N-cycle in water systems and is preserved in sediments as humic materials. The composition of this sequestered N is generally thought to be mainly in the form of amides [1, 2]. It has been generally assumed that these amides are entirely composed of peptides (proteins) or amino sugars which are well-known constituents of natural organic systems. A re-examination of humic matter from Mangrove Lake, Bermuda [3, 4], with modern NMR and mass spectrometric approaches, that provide molecular-level details, reveals the presence of other N-forms. The recent success of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) for the molecular-level characterisation of dissolved humic matter [5–7], convinced us that this technique could be used to investigate the nature of the amides in sediments. In addition to FT-ICR-MS, modern multidimensional NMR can be used to investigate the structural components of sediment OM (SOM). One- and two-dimensional liquids and one-dimensional solid NMR were utilized in this study to selectively examine the amide form of the sediment.

2. Materials and Methods

Mangrove Lake, Bermuda is a 300 × 400 m, brackish, Holocene, organic-rich, algal derived lake which has been used in several studies regarding its algal humic SOM [1,3–4,8–13]. The sediments used for this study were collected in 1982 using a piston corer, after which they were freeze dried and stored at room temperature [2]. For all liquid state NMR and FT-ICR-MS analyses, pyridine extracts of the whole sediment were obtained. Pyridine has been shown in coal to extract substantial portions of the OM [14], and so was used in this study due to similar results. The FT-ICR-MS is an ultra-high resolution technique resulting in highly accurate molecular formulae assignments (<1 ppm), identifying several thousand different formula types [6].

3. Results and Discussion

Figure 1 shows the resulting two-dimensional liquid state Heteronuclear Multiple Bond Correlation (HMBC) spectrum of ML depth 2.8 m pyridine extract. The amide region is shown as an insert to this Figure. As expected, peptides/proteins are assigned as contours correlating to 4.01/171, 2.7/171 and 2.58/171 ppm. However, another contour is observed which cannot be readily assigned to peptides/proteins; upon investigation with standards we assign 2.2/172 and 2.1/175 ppm as alkyl amide.

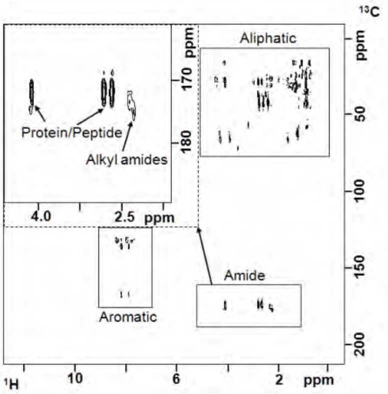

Figure 1: Liquid state HMBC NMR spectrum of ML pyridine extract of depth 2.8 m, showing the identity of amide compounds

Alkyl amides have not been considered as a possible source for the amide functionality in SOM previously, and therefore, warrants further investigation using molecular level techniques. The FT-ICR-MS spectrum of ML sediment pyridine extract from a depth of 2.8 m was analysed in both negative (Fig. 2a) and positive (Fig. 2b) ionisation modes. Examination of the individual molecular formulae assigned to these spectra, reveals homologous series of possible alkyl amides/diamides. These are shown in Fig. 2, in addition to other major peaks. Gas chromatography mass spectrometry confirms the structures of several of the assigned alkyl amides, verifying our initial assessment (Figure not shown).

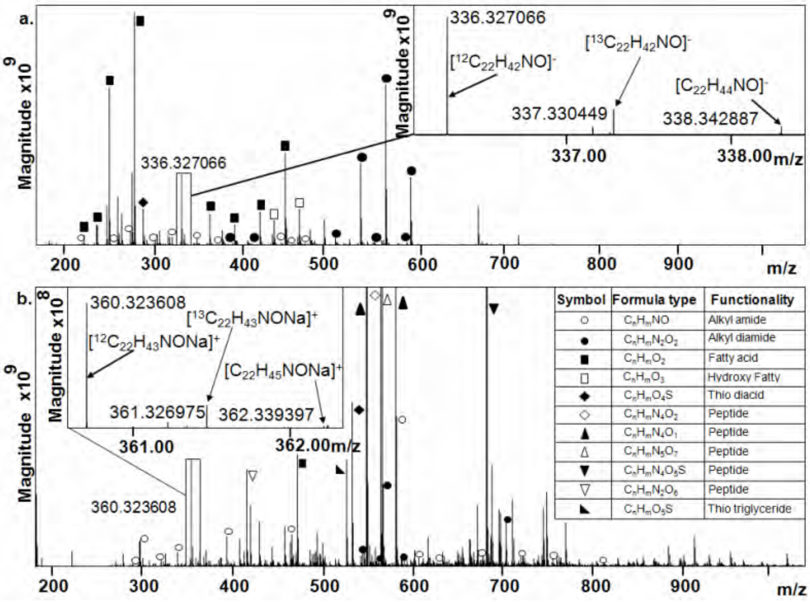

Figure 2: Resulting spectra from FT-ICR-MS analysis of ML sediment, pyridine extracted from a depth of 2.8 m. a. is the negative ion mode spectrum and b. is the positive ion mode spectrum. Major peaks and alkyl amides/diamides are identified

The identification of elemental formulae in an FT-ICR-MS spectrum provides much useful information. Figure 3 displays the magnitude-weighted spectral contributions of alkyl amides/diamides, as a percentage of CHNO formula types, in a 3-m core from Mangrove Lake. The general increase (except near the surface) of the magnitude weighted percentage with depth suggests that alkyl amides become a more dominant component of the CHNO formula types. This result is consistent with the selective preservation theory of sequestered OM [15] if one assumes that alkyl amides are refractory to biomineralisation. More labile N-bearing components such as proteins/peptides are selectively remineralised when moving to lower depths leaving the more recalcitrant alkyl amides to be preserved. In consideration of this result we suggest alkyl amides/diamides are selectively preserved in this sediment because traditional remineralisation routes (such as enzyme degradation) are not developed for these compounds. Further work indicates that these alkyl amides are produced abiotically by reaction of ammonia with triglycerides of fatty acids.

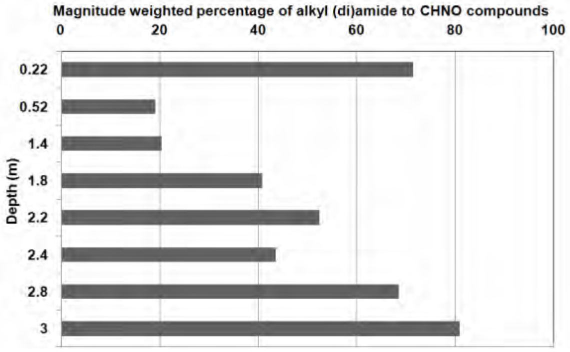

Figure 3: A graph displaying the magnitude weighted percentage of alkyl amides/diamides of CHNO containing compounds of ML pyridine extract analyzed using negative ion mode in FT-ICR-MS

2. Conclusions

The identification of alkyl amides as important contributors to sedimentary humic matter in Mangrove Lake and their probable formation through abiotic processes represents a major revelation about the manner in which N becomes sequestered in such sediments. If universal, then it also represents a new discovery on the role of abiotic processes during humification. Soil and sedimentary scientists have long sought an explanation for N-incorporation into humic substances. Reaction of ammonia with esters through an amidation reaction represents a new pathway for N incorporation. The relative enrichment with depth suggests that these alkyl amides survive degradation, possibly because the degrading organisms do not metabolically recognise them.

Acknowledgements

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Humin: The Simplest of the Humic Substances?

Michael H.B. Hayes[a]. Roger S. Swift[b], Corinna M. Byrne[a], Guixue Song[a], and Andre J. Simpson[c]

[a]Chemical and Environmental Sciences, University of Limerick, Ireland; [b]Faculty of Natural Resources, Agriculture and Veterinary Science, University of Queensland; [c]Department of Chemistry, University of Toronto at Scarborough, Toronto, Canada.
E-mail: Michael.H.Hayes@ul.ie; Corinna.Byrne@ul.ie; r.swift@uq.edu.au; andre.simpson@utoronto.ca

1. Introduction

Humin, the major component of humic substances (HS), makes up 50% and more [1] of the transformed or humified components (that bear no morphological resemblances to the structures from which they were derived) in soil organic matter (SOM). Based on the classical definitions, any humified materials remaining, following exhaustive extractions in aqueous basic media, might be regarded as humin. [2].

The present study involved an exhaustive extraction of the classical humic components of SOM, including the isolation of humin residues by novel procedures from a Brown Earth and a Grey Brown Podzol soil. ^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectroscopy techniques were employed to show differences in the compositions of the different isolates.

2. Soils and Methods

Soil samples were taken from t6he A horizons of a well-drained calcareous coarse gravely Brown Earth (GBE), (67% sand, 22% silt, and 11% clay, 1.4% organic C) that had been in long term cultivation for 30 years, and from a well-drained Grey Brown Podzolic (GBP) grassland soil (45% sand, 20% silt, and 12% clay, 5.3% organic C). The H^+-exchanged soils were exhaustively extracted in 0.1 M NaOH adjusted to pH 7, then to pH 10.6, and to pH 12.6. Following removal of t6he HAs and FAs the residual materials were exhaustively extracted in 0.1 M NaOH + 6 M urea. The urea was washed out, the silt/clay fraction was isolated, air dried, and exhaustively extracted with dimethylsulphoxide (DMSO) + concentrated H_2SO_4 (6% v/v). The HAs and FAs were recovered as described by [3], from the materials sorbed on XAD-8 resin. The DMSO/H_2SO_4 isolate from the silt/clay-size fraction **(**< 63 μm) was poured into water; and the precipitate formed was washed with water and freeze dried [4]. The residual organic materials remaining on the clay fraction (DMSO insoluble humin, or DIHU) after the DMSO/acid extraction were recovered by dissolving the silicates in 10% HF, dialysing the residual materials, and freeze drying. Instrumental analysis using CPMAS 13C and proton nuclear NMR spectroscopy followed the procedures outlined elsewhere [4,5].

3. Results and Discussion

Figure 1a shows the VACP/MAS-TOSS (thin line) ^{13}C NMR and the corresponding DD spectra (thick) of the humic acids (HAs) isolated at pH 12.6 from the GBE soil and Fig. 1b shows the same spectra for the HAs subsequently isolate in the 0.1 M NaOH + 6 M urea solvent system. Note the close similarities between the spectra. The 162 ppm resonance is from urea that was not removed in the washing process. Both spectra differed from the isolates at pH 7 and at pH 10.6 (not shown) in the strong resonances characteristic of lignin (*O*-aromatic at 153 ppm and the methoxyl at 56 ppm). It is clear, however, that the base/urea isolates could be regarded as HAs, and it is likely that these were released by breaking hydrogen bonding mechanisms between these acids and the humin/mineral matrix, or by causing conformational changes to component structures that allowed the HAs to be set free.

Figure 1c presents spectra of humin materials isolated in the DMSO/H_2SO_4 system. The isolates in that solvent system can be considered to satisfy the criteria for humin. The DMSO + H_2SO_4 solvent extracted about 64% of total residual organic carbon from the silt/clay fraction. (after the sequential extractions with the base and base-urea systems) from the organic silt/clay-size-fraction. More than 80% of the SOM was extracted in the solvent sequence described. Spectra for this humin and for the residual DIHU material (Fig. 1d) recovered after the HF treatment are very similar. The spectra show several well-resolved, though generally broad resonance bands centred around 15, 33, 56, 74, 104, 129, and 173 ppm. The predominant resonance at 15–45 ppm emphasizes the aliphatic nature of these humin fractions. Note also the significant carbohydrate resonances at 74 and 104 ppm. There is little evidence in the aromatic resonance to suggest the presence of significant amounts of HAs, and the shape of the resonance at 56 ppm in the case of the DIHU sample suggests origins in peptide materials, although resonances at ca 145–150 ppm and at 56 ppm could suggest some contributions from altered lignin in the case of the DMSO humin (Fig. 1c)

Figure 2(a), the liquid state proton NMR spectrum for the humin isolated from the GBP, is dominated by resonances indicative of aliphatic hydrocarbon-type structures, and more extensive investigations from other studies of this resonance region [5] would suggest that the aliphatic components consisted mainly of lipids, waxes, and various hydrocarbon-type structures. There is evidence also for protein/peptide materials, and there is some evidence for minor components with origins in lignin, although there is not convincing evidence for aromaticity. The solid state spectrum, Fig. 2(b), indicates also the lack of significant aromaticity, but shows significant carbohydrate, and the resonances at 50–60 ppm is more likely to represent protein/peptide functionality than the methoxyl of lignin. The dominance of the 20–35 ppm region emphasizes the contributions of functionalities with aliphatic hydrocarbon components. The carboxylic acid resonance is likely to arise from lipid and other aliphatic structures.

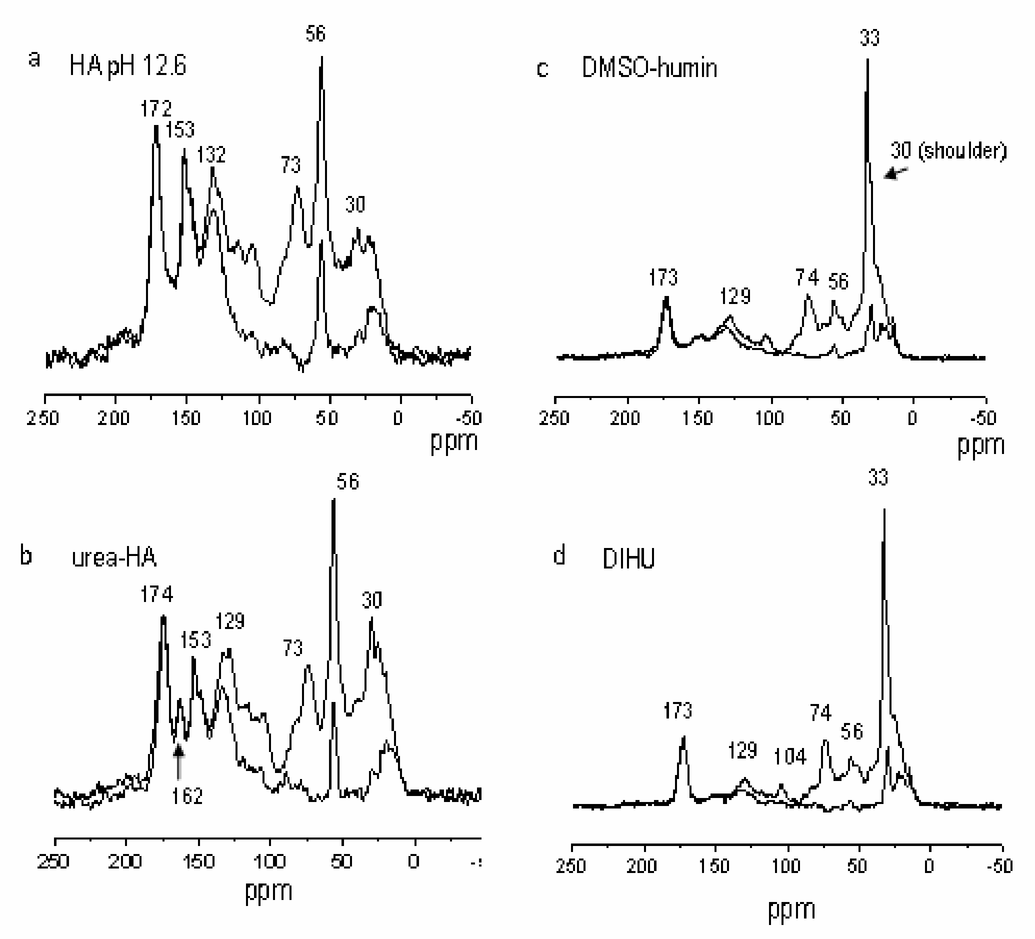

Figure 1: Full VACP-MAS/TOSS (thin line) ^{13}C NMR and the corresponding DD spectra (thick line) of: (a), HAs isolated at pH 12.6 (after prior exhaustive extractions at pH 7 and pH 10.6) from the GBE soil; (b), HA isolates from the same soil using 0.1 M NaOH + 6 M urea; (c), humin isolated in DMSO + 6% (v/v) H_2SO_4; and (d), residual materials following treatment with HF

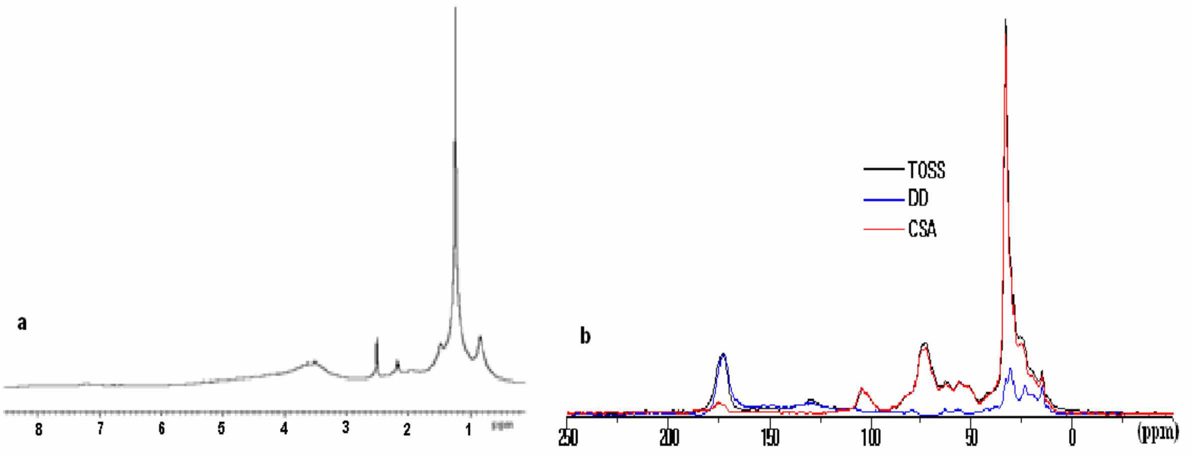

Figure 2: ^{1}H NMR spectrum in DMSO-d_6 for humin from the Grey Brown Podzolic soil (a); and (b), the TOSS, Dipolar Dephasing (DD) and Chemical Shift Anisotropy (CSA) ^{13}C NMR solid state spectra for the humin isolated from the same soil

4. Conclusions

The composition of humin has baffled soil scientists in the past. This study shows that applications of modern NMR spectroscopy techniques prove convincingly that, as others have indicated [6,7] humin is a mixture of materials with origins in plants and microorganisms and whose general structures are well known. The most abundant components have significant aliphatic hydrocarbon functionalities with major contributions from materials such as waxes, lipids, long chain fatty acids and esters, cutins, cutans, suberins, with significant amounts of carbohydrates and peptides, and with only traces of lignin-derived substances (which predominantly occur in the HAs and FAs). The carbohydrates and peptides would be expected to be readily degraded. Their preservation as components of humin can be attributed to their associations with and steric protection by the largely non-polar components of humin that have a high degree of resistance to microbial degradation. The protection is enhanced by affinities of the non-polar materials to the soil inorganic colloidal components.

Humin should no longer be regarded as the ‘mystery component’ of SOM. Based on the evidence we now have it is clear that most of the humin components do not satisfy the classical definitions for humic substances. These components should be regarded as a mixture of largely identifiable biological molecules derived from plant materials composed predominantly of resistant non-polar moieties in intimate associations with, and protecting some biodegradable biomolecules, all in intimate associations with the soil mineral colloids. On the basis of this evidence it is now appropriate to reconsider the inclusion of humin in the classical definitions of soil humic substances.

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Past Human Activities and Environmental Changes as Recorded by Humic Acids from Raised Bogs

Claudio Zaccone[a*], William Shotyk[b], Teodoro M Miano[c]

[a] Dept. of Agro-Environmental Sciences, Chemistry and Plant Protection, University of Foggia, via Napoli 25, 71100 Foggia, Italy; [b] Inst. of Earth Sciences, University of Heidelberg, Im Neuenheimer Feld, 236, 69120 Heidelberg, Germany; [c] Dept. of Biology and Chemistry of Agro-Forestry and Environment, University of Bari, via Amendola 165/A, 70126 Bari, Italy
E-mail: c.zaccone@unifg.it

1. Introduction

Peat soils play a crucial role in the carbon cycle as they are generally net sinks for atmospheric carbon dioxide and net sources of greenhouse gases on a long term basis. Among peatlands, ombrotrophic bogs are also considered unique archives of past environmental conditions because their genesis is directly linked to the atmospheric conditions occurring during peat formation [1]. Although several studies have been carried out in the last decades using ombrotrophic bogs in order to reconstruct the historical trends of heavy metal pollution due to anthropogenic activities [2], scientific literature is still rather controversial about the role of ombrotrophic bogs as reliable record of past climatic and environmental changes.

To answer such a nodal point, it is extremely important to better understand the process of decomposition/humification in these ecosystems. In fact, understanding whether all these "information" are effectively preserved in peat deposits during humification becomes an essential aspect to be tested before using bogs as natural archives. Consequently, in order to better understand if bogs are consistent archives, all the information about past human activities and environmental (climatic and vegetational) changes should be recorded into the fraction of peat more recalcitrant and refractory to the degradation, i.e., humic substances.

2. Materials and Methods

A 81 cm peat core was collected in 1991 from the Etang de la Gruère bog (Jura Mountains, Switzerland), cut into 3 cm slices, and age dated; after that, humic acids (HA) were isolated from each layer according to [3]. The whole core, corresponding to ca. 2,100 years of peat formation, and the HA samples were characterized using several molecular spectroscopic methods (Ft-IR, UV-Vis, DSC, Fluorescence). Furthermore, both peat and HA samples were analyzed by XRF (for major and trace elements), Low Background γ-spectrometry (for ^{137}Cs and ^{241}Am), and Isotopic Ratio Mass Spectrometer coupled with an Elemental Analyser (for isotope ratios, δ^{13}C and δ^{15}N).

3. Results and Discussion

In general, Ft-IR, UV-Vis and fluorescence results, together with main atomic ratios, suggest significant variations of the molecular composition and chemical structures of the peat samples along the profile, underlining an increase of the humification degree with depth. In fact, the HA fraction accounts for 65.6 to 154.5 g kg^{-1} of bulk peat in the upper, poorly decomposed layer (from the living layer to ca. 24 cm), and for 200.5-327.3 g kg^{-1} of bulk peat in the bottom section.

Among the studied major and trace elements, Br, Cu, and Hg showed a conservative behaviour as their distribution in HA mirrored that in peat. Actually, some authors suggested that the concentration of these elements in peat increases as result of mass losses during humification. Mercury data (Fig. 1a), on the contrary, clearly underlined that: *i)* the magnitude of changes in Hg concentrations in peat samples along the profile is not directly correlated to their corresponding humification degree; *ii)* expressing both Hg concentrations per mass unit of peat, it is possible to identify a similar trend ($R^2 = 0.84$, $p<0.001$); and *iii)* most of the total Hg present in peat (66%, average value) is retained by HA molecules. This complexing behaviour may greatly limit the Hg mobility along the profile, thus suggesting a prevailing Hg immobilization that may reflect the different Hg deposition rates [4].

On the contrary, other elements (e.g., Ca, Fe, Mg, Mn, Rb, Sr, Zn) showed a certain mobility along the profile. Finally, Pb, although is considered a “soft” cation able to form inner-sphere complexes with the organic matter (OM), seems to behave similarly to Ti and Zr. Since the latter ones are known to be associated almost exclusively with dense accessory minerals (such as rutile and zircon) which are resistant to chemical reaction, even at the low pH (4) which is typical of ombrotrophic bogs, the absence of these elements in HA suggests that, during humification, the mineral phases bearing these elements are unaffected, and therefore that these metals are not available for chemical reaction [5].

The occurrence of ^{137}Cs (Fig. 1b) can be reasonably related to the 1986 Chernobyl disaster, even though its trend in the upper profile could be affected by plant uptake. The ^{137}Cs activity along the peat profile shows also additional peaks, one of which corresponding to the ^{241}Am peak, ascribed to nuclear explosions. The occurrence of ^{137}Cs in HA, although suggested a significant downward movement of ^{137}Cs along the profile, clearly remarks that the Chernobyl disaster is recorded also into the recalcitrant HA fraction.

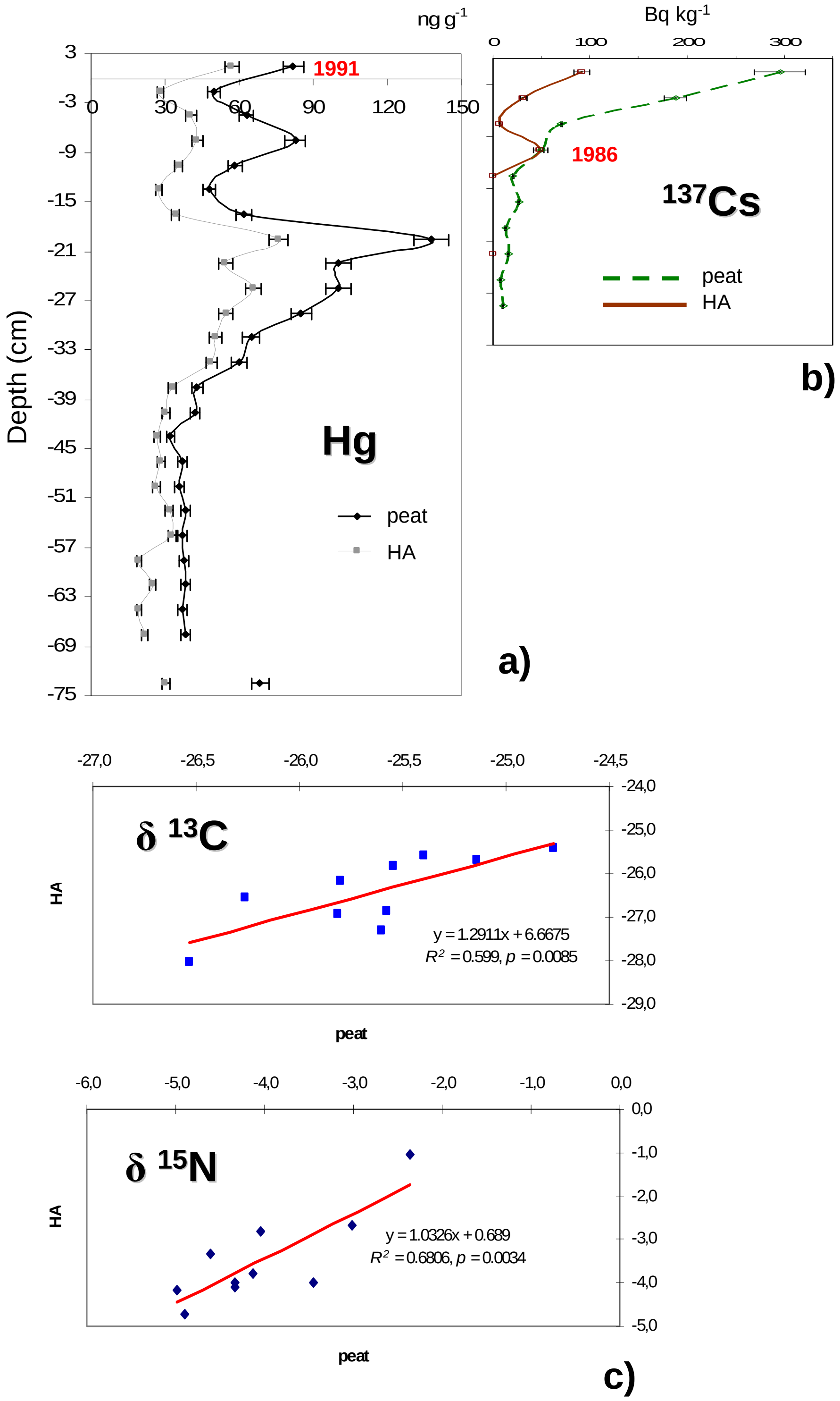

Figure 1: Hg distribution (a), ^{137}Cs activity (b), and δ^{13}C and δ^{15}N (c) in peat and corresponding HA samples

Finally, although big differences in the OM quality were observed along the peat profile, also isotopic ratios seem to have a certain conservative behaviour (Fig. 1c). In detail, the δ^{13}C ranges between -26.53±0.01 ‰ (in the living layer) and -24.77±0.05 ‰ (at 55-58 cm) in peat samples, and between -28.03±0.05 ‰ and -25.42±0.05 ‰ in corresponding HA, underling a greater “depletion” of ^{13}C in the latter fraction. Anyway, the δ^{13}C recorded both in peat and in HA samples shows a significantly similar trend with depth ($R^2 = 0.60$, $p < 0.01$). Also the δ^{15}N features similarly in peat and HA throughout the profile ($R^2 = 0.68$, $p < 0.01$), showing values between -4.99±0.21 ‰ (in the living layer) and -2.36±0.06 ‰ (at 18-21 cm of depth) in peat samples, and between -4.15±0.05 ‰ and -1.06±0.24 ‰ in corresponding HA.

4. Conclusions

Can HA constitute the “memory” of past human activity and environmental changes? Obviously, HA from bogs, and peat bogs themselves, cannot serve as archive of all these information.

About major and trace elements, it will depend mainly on their nature and affinity for the OM. For example, data clearly showed that humification processes did not affect the Hg distribution along the profile. In fact, because the zone of elevated Hg concentrations seen in the surface layers of the Swiss peat core corresponds to the decades of greatest industrialization, the accumulation of this element is probably the result of both natural processes as well as anthropogenic inputs, with the latter clearly dominant during the past few centuries. The same behaviour was observed for Br.

Moreover, HA are also witnesses of the Chernobyl disaster, as well as of nuclear explosions.

About isotopic ratios, data apparently suggest that they are quite conservative in HA from peat, thus supporting on one hand the role of HA as recalcitrant, stable molecules with a long-term residence time, on the other hand the potential of ombrotrophic bogs to be used as “reliable archives” of climatic and vegetational changes occurring in the last 2,000 years.

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Influence of Natural Organic Matter on Transport of Soot Black Carbon in Saturated Porous Media

Jenyuk Lohwacharin*, Kumiko Oguma, Satoshi Takizawa
Department of Urban Engineering, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
E-mail: jenyuk@env.t.u-tokyo.ac.jp

1. Introduction

Incomplete combustion of fossil fuels and biomass generates soot black carbon, typically associated with polycyclic aromatic hydrocarbon. Atmospheric deposition, soil erosion, and surface run-off create distribution of soot in oceans, rivers, and subsurface [1]. Soot undergoes transformations that will modify surface chemistry and thereby affecting transport behavior in aquatic environment. In aquatic environment, soot interacts with natural organic matter (NOM), i.e. humic substances and polysaccharides. The aqueous dispersion of soot is affected by physico-chemical ambient conditions e.g. pH, ionic strength, divalent cations. These factors govern charge behavior, dispersion stability, and soot transport in aquatic environment [2, 3]. Therefore, there is a need to understand influence of natural components and ambient conditions on fate and transport of soot in the aquatic environment.

Here we report laboratory assessment of soot transport in saturated sand media to simulate groundwater exposure. The deposition kinetics of soot in sand media under different conditions were quantified using a particle transport model. Influence of NOM and solution pH on soot transport in sand media were investigated using the same hydrodynamic condition. This study reports engagement of NOM in environmental transformations of soot particles and thereby elaborating role of NOM in ecosystems.

2. Materials and Methods

A microporous carbon black (CB, Black Pearls 2000, Cabot Corp., USA) was used to model environmental soot particles. Suwannee River aquatic NOM (SRNOM, IR101N, International Humic Substances Society) and alginic acid sodium salt (A2158, Sigma Aldrich, USA) were used as models of humic substances and polysaccharides, respectively.

Sand filtration was performed on a 3.07 cm I.D. glass column. Each column was packed with virgin quartz sand under wet condition at a bed depth of 13 cm and bed porosity of 0.36. The column was first saturated with 10 mM NaCl solution for 2 h. Soot solutions were prepared by agitating CB for 60 h in Milli-Q water (dissolved organic carbon, DOC < 5μg/L), solution containing 5 mg-C/L SRNOM or 5 mg-C/L alginate at the NaCl concentration of 10 mM and pH 5.0 or 8.0. Sand filtration was performed at a flow velocity of 1.69 m/h. The effluent (C) and influent (C_0) soot concentrations (mg/L) were estimated using a multiplying factor of 79.8 per a unit visible-light absorbance at 400 nm (Vis_{400}).

The DOC concentrations were analyzed with a non-purgeable organic carbon method employing a TOC-5000A analyzer (Shimadzu, Japan). The Vis_{400} absorbance was measured using a UV/Vis spectrophotometer (U-2010, Hitachi, Japan). Particle size was analyzed using a Nanotrac particle analyzer (Nikkiso, Japan) based on the dynamic light scattering method. A Photal ELS-8000 (Otsuka Electronics, Japan) analyzer equipped with an ELS standard cell was used to monitor the zeta potential of soot particles.

3. Results and Discussion

Interactions of soot particles and NOM. Aqueous soot dispersion showed a volumetric mean size in a submicron range (Table 1). Increasing pH did not significantly affect the size of particles in Milli-Q water and SRNOM solution while increasing pH induced a formation of larger particles in alginic solution.

Table 1: Mean size of soot particles and adsorption amount of NOM by soot after 60-h equilibration

Solution	Mean soot particle size (micron)		Adsorption amount (mg-C/g-soot)	
	pH 5	pH 8	pH 5	pH 8
Milli-Q water	0.173 ± 0.083	0.163 ± 0.048	-	-
SRNOM	0.189 ± 0.064	0.192 ± 0.091	237 ± 95	197 ± 59
Alginate	0.133 ± 0.040	0.211 ± 0.145	42 ± 18	47 ± 18

Adsorption amount of SRNOM by soot was larger than that of alginate (Table 1). This would be due to low molecular weight of SRNOM (2.7 kD [4]) which could assess micropores (c.a. 0.8 nm) of soot surface and hydrophobic interaction between hydrophobic graphitic surface of soot and aromatic surface groups of SRNOM. Adsorption mechanism was also influenced by electrostatic interactions since a lower adsorption amount was observed at pH 8 in the SRNOM solution. In contrast, adsorption amount of alginate was slightly higher at pH 8 than that at pH 5. This suggests that adsorption mechanisms of SRNOM and alginate by soot were different.

Soot transport in saturated sand media. The deposition rate coefficient (*k*) was calculated using the particle transport model [5]:

$$k = -\frac{v}{L}\ln\frac{C_f}{C_0} \qquad (1)$$

where v is the average travel velocity of soot particles and L is bed effective depth. C_f is the effluent soot concentration after the breakthrough curve has reached the plateau.

The *k* values of soot particles in the sand media after equilibrated with NOM are presented in Fig.1(a). Soot particles were deposited with a higher rate at pH 8 than pH 5 although larger charge repulsions at pH 8 (Fig.1(b)) was expected to prevent attachment between soot particles and sand matrix. The *k* values were reduced to 26% or 48% at pH 5, but reduced to 33% or 66% at pH 8 compared to those in Milli-Q water, for soot equilibrated with SRNOM or alginate, respectively. The result indicates that SRNOM was more effective than alginate in reducing soot deposition under both acidic and basic conditions.

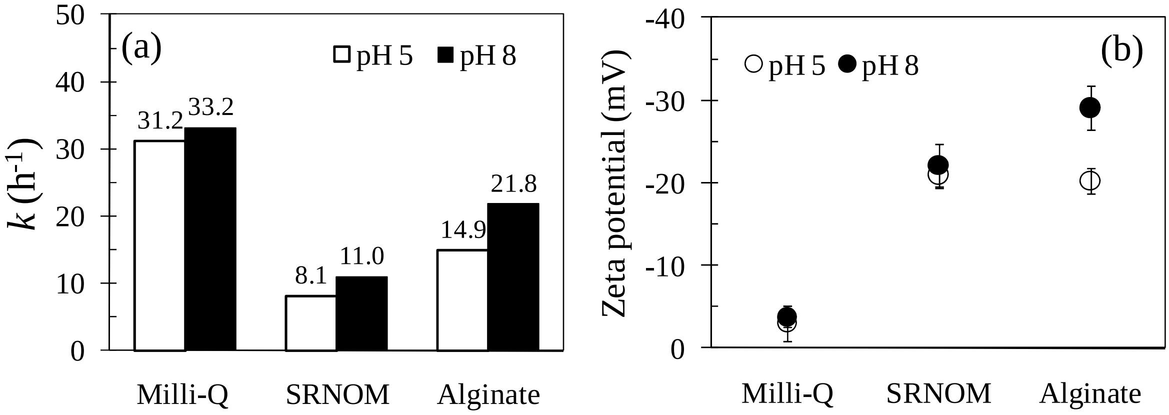

Figure 1: (a) The deposition rate coefficients (*k*) determined by the particle transport model (b) Zeta potentials of soot particles after equilibrated with Milli-Q water, SRNOM, or alginate at pH 5 or 8

In Fig.2(a), breakthrough curve reached the plateau differently between soot equilibrated with alginate and SRNOM. After soot injection, loading a column with the NaCl solution caused elution of SRNOM-equilibrated soot, but not alginate-equilibrated soot. This suggests that soot was not readily desorbed from the sand surface possibly due to sticky attachment induced by alginate. The mass distribution of the alginate-equilibrated soot particles shows that soot equilibrated at pH 8 were mostly accumulated near the inlet of soot injection, whereas soot mass was distributed uniformly along the bed for soot equilibrated at pH 5 (Fig.2(b)). Considering a relatively large particle size formed at pH 8 (Table 1), the large particles might be entrapped in the sand matrix and accumulated at the 0–2 cm depth. The results suggest that not only electrostatic repulsions, but also other factors, e.g. soot particle size and sand surface chemistry, govern soot attachment to the sand surfaces.

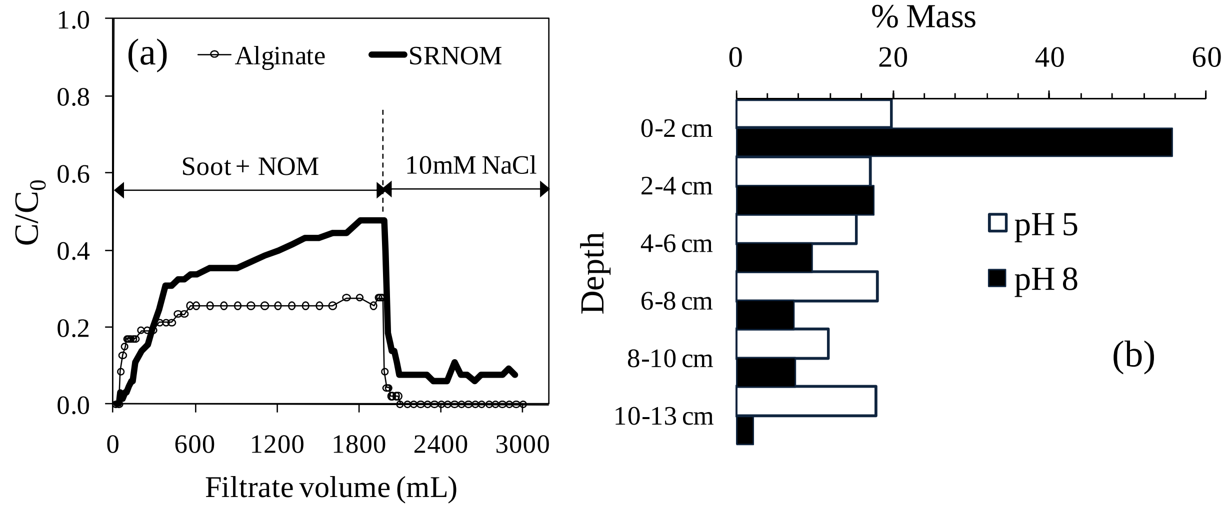

Figure 2: (a) Breakthrough curve of soot equilibrated with alginate or SRNOM at pH 5 (b) % mass distribution of soot equilibrated with alginate at pH 5 and 8 in the saturated sand column

4. Conclusions

The experimental results demonstrate that NOM plays important roles in ecosystems by modifying surface chemistry of the soot particles and/or sand surfaces. The environmental transformations resulted in the increased transport of soot particles, particularly under acidic condition, in the saturated sand media. Charge repulsions, soot particle size, and type of NOM were factors determining the deposition rate coefficient of soot particles in the sand media. This study suggests potential of soot-facilitated transport of hydrophobic organic contaminants into a groundwater aquifer.

Acknowledgements

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Elemental Composition of Humic Acids of Automorphic Forest Steppe and Steppe Soils of Northern Eurasia

Galina Chimitdorzhieva[a], Boris Klenov[b]
[a] Inst. of General & experimental Biology of SB RAS, Ulan-Ude, Sakhianova St 6, Russia; [b] Inst. of Soil Science & Agrochemistry of SB RAS, Novosibirsk, Sovietskaya St 18, Russia
E-mail: galdorj@gmail.com; klenov@iss.nsc.ru

1. Introduction

Humic acids (HA) and fulvic acids (FA) represent an irreplaceable component of soil which stipulates for its sustainable environmental functioning. Now it is generally known that HA are the most sustainable soil organic compounds in ecological respect therefore their elemental composition is more in accordance with soil and humus forming conditions than FA. Furthermore, as distinct from HA, FA are practically not changed depending on soil type, therefore they, as a rule, are not taken into consideration in comparative geographical studies of soil organic matter. Different insolation at vast areas of Russia, i.e. the most part of Eurasia, calls forth here the formation of various natural bioclimatic belts with diverse types of soils and respectively with diverse specific features of nature. Hence, this communication deals with elemental composition of HA of automorphic soils formed under continental and sharply continental climate of Siberia as compared to that of European part of Russia which is characterized by softer moderately continental climate.

2. Materials and Methods

Elemental composition of HA was studied in three types of virgin automorphic soils such as gray forest, chernozems and chestnut soils. In the northern hemisphere these soils are formed, as a rule, in latitudinal direction from the North to the South within the limits approximately of 55 and 62^{o}. In longitudinal direction this belt with the above mentioned soils passes through European part of Russia (EPR), Western Siberia (WS) and Eastern Siberia (ES, Buryatia and Transbaikal area, in particular). Within the limits of these areas the climate varies from moderately continental in EPR to continental in WS and to sharply continental in ES. Different degree of continentality in meridian direction is clearly evidenced by earlier determined factor of continentality. This factor being equal to 130–140 in EPR varies to 170–180 in WS and amounts to practically extreme value in ES 190–200 [1]. The increase in continentality of climate is accompanied in meridian direction by increase in “coldness” of climate which, in its turn correlates, with the depth of soil freezing. During the coldest winters the depth freezing reaches in these three areas to 0.5–0.8 m. 1.0–1.5 m and 2.5–3.0 m respectively. Taking into account the specificity of bioclimatic conditions these soils should be classified as the cold ones (i.e. longely and deeply frozen) soils [2]. The average typical data on elemental composition were taken from the material published elsewhere [3,4]; as for ES the recent data were obtained in the Buryat Research Center by the first coauthor. The preparations of HA were obtained by generally accepted method [5]. The determination of elemental composition was conducted by method of dry combustion with gas-chromatographic termination. The account of degree of aromaticity (benzoidicity) (AD) was fulfilled by modified formula by Van-Krevelen [3].

3. Results and Discussion

The interpretation of elemental composition from the viewpoint of soil genesis is always difficult because of diversity of the reasons of its variation. As for the content of structural elements of HA the soils under study would seem to differ slightly from one another but in general their content is characteristic of this group of compounds (Table). In our case the results can be considered in comparison between soil types as well as between the areas under study. Thus, in elemental composition of gray forest soils as compared to that of the analogous of EPR an increase in C content to some extent and at the same time the decrease in H is observed. Therefore the H/C value decreases and simultaneously the AD value increases. This fact is interesting per se and should be studied subsequently. As a result, the HA of Siberian gray forest soils proved to be more aromatic in their nature than their European analogues. It should be noted that the classification of HA according to AD has not yet elaborated so that the HA of these soils can de assumed to be within the limits of the same classification. As for elemental composition of HA of chernozems, one should note the gradual increase in H/C and decrease in DA as the continentality of the climate increases. Chernozems of EPR are formed under conditions of soft climate, under favorable microbiological conditions when the removal of lateral carbohydrate and amino acid constituents occurs. Under such conditions the HA with more condensed molecule are formed. But in case of deeply frozen soils the microbiological processes are hindered so that low AD is to be as a result.

Elemental composition of HA of humus horizons of automorphic soils (percentage based on dry free ash substance)

Soil	Area	Contents of elements, %				Atomic ratios			AD
		C	H	O	N	H/C	O/C	C/N	
Gray forest (Albic Luvisol)	EPR	38.1	40.3	19.2	2.4	1.06	0.50	15.9	17
	WS	40.4	38.1	19.0	2.4	0.94	0.47	16.7	22
	ES (Transbaikal Area)	41.0	37.0	21.1	0.9	0.90	0.51	15.5	24
Chernozem (Luvic Chernozem)	EPR	42.5	35.2	19.9	2.4	0.83	0.47	17.7	37
	WS	40.7	39.3	17.0	2.4	0.96	0.43	17.0	28
	ES (Transbaikal Area)	39.2	37.4	20.7	1.6	0.95	0.53	24.5	21
Chestnut (Kastanozem/Calcisol)	EPR	37.7	42.1	17.4	2.8	1.12	0.46	13.5	16
	WS	41.4	36.1	19.9	2.6	0.87	0.45	15.9	32
	ES (Buryatia)	38.7	40.8	17.3	3.0	1.05	0.45	12.9	22

In EPR the HA of chestnut and gray forest soils as compared to corresponding Siberian analogues proved to be with slightly developed aromaticity but as a whole all these data seem to be within the limits of the same scale. While comparing elemental composition of three regions under study one should note that AD cannot be accepted as a distinctive universal sign on the level of soil type. The statement of Orlov [3] seems to be reasonable for EPR only. In WS the value AD differs only in soils which are contrast by their genesis, for example in soils of taiga and steppe [4], in our case these data account for 22, 28 and 32 respectively. The data on elemental composition of HA of EA soils indicate that AD of HA varies insignificantly in the studied soil sequence and ranges around 20. It should be noted that this phenomenon is regular. It is worth to note that the soils of ES do not form continuous belts and occur as individual spots or islets owing to mountainous relief. Here the vertical zonality is more revealed with hollow effect. Furthermore, the climate is here formed under influence of three contrast components such as dry and cold climate of the northern areas of Siberia, hot and dry climate of Mongolian steppes, warm and humid climate of the Pacific. Therefore, in Transbaikal area very contrast belts are in contact such as dry steppe and taiga where cryogenic processes become apparent that results in smoothing effect on the nature of HA.

4. Conclusion

AD of HA is a distinctive sign of soil type only in those areas of the Northern Eurasia which are characterized by soft moderately continental climate. With increase in continentality the AD exhibits a tendency to decrease, in soils of chernozem type especially. Moreover, with increase in continentality the differences in AD at the level of soil type are practically obliterated.

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SESSION 4: HS AND NOM IN AGRICULTURE AND ENVIRONMENT REMEDIATION

Photonics Technologies for Analysis of Organic Matter and Metal in Soils

Débora M. B. P. Milori[a], Ladislau Martin-Neto[a,b], Edilene C. Ferreira[a], Cleber H. dos Santos[a],
Ednaldo J. Ferreira[a]

[a]Embrapa Agricultural Instrumentation, Rua XV de novembro, 1452, CEP 13560-970, São Carlos-SP, Brazil; [b]Embrapa Labex USA, 5601 Sunnyside Avenue, Beltsville, MD 20705, USA
E-mail: debora@cnpdia.embrapa.br

1. Introduction

Soil tillage intensity and crop residue management affect soil organic matter (SOM) and, are considered to be the key factors in regulating soil carbon (C) balance. Thereby, agricultural and degraded soils have significant potential sinks for atmospheric carbon. However, soil C accumulation is not linear with increasing of C inputs. Depending on the situation, soil C may until decrease despite of residues addition. Continued research on chemical and biological mechanisms in soil is needed if soils are to be relied upon for food and fiber production and for the mitigation of climate change. The scenario makes clear that in order to advance in this scientific area, more research about low cost and quick methods, which make possible a high number of analyses, is immensely coveted. On the other hand, information obtained in conditions near of field soil conditions, without chemical or physical treatments, also are a demand of soil scientists. Photonic techniques have high potential of miniaturization and frequently are applied in equipments on the several areas of knowledge, such medicine and odontology. Usually, they show high potential to perform analysis *in situ*. This study consists in to apply two photonic techniques to assess carbon and metals in whole soil samples.

Laser-induced breakdown spectroscopy (LIBS) is an emerging technique for simultaneous multi-elemental analysis of solids, liquids and gases with minute or no sample preparation and thus revolutionized the area of on-line analysis technologies. During the LIBS analytical process the sample is irradiated by a highly energetic laser pulse and absorbs this energy. The high temperature of ablated material generates a small plasma plume. During the plasma cooling, the excited species return to their ground state emitting electromagnetic radiation in characteristic wavelengths. In this sense, analysis of sample emission spectra gives a qualitative view of sample elemental composition. Nevertheless, to accomplish LIBS quantitative analysis is not so simple, because elements emission lines in LIBS spectrum are closely related with the matrix in which they are embedded. This feature of the signal becomes very difficult to find appropriate calibration standards for LIBS methods. In some cases, the appearance of so-called matrix effects could be reduced by a multivariate calibration. However, many efforts have been dispended to adapt univariate calibration models.[2] Artificial Neural Network (ANN) is a kind of multivariate technique which due to its superior classification and prediction capabilities, has found their impact in spectral analysis and has gained space in LIBS spectroscopy.[3,4]

Fluorescence of soils is originated mainly from organic matter (OM), since it has several functional groups containing unsaturated bondings in rigid systems. Such groups are present mainly in more humified OM, such as aromatic rings, phenolics, quinone structures and carboxylic groups.[5] In general, the more complex molecular structure of humic substances, the higher their stage of humification. For this reason, when soil samples are illuminated with near-ultraviolet or blue laser, mainly the more complex structures are excited, and the area under the fluorescence emission, normalized by C content, provides a parameter proportional to humification degree of SOM.[6] This method is interesting because allows analysis without chemical treatment in a very precise and quick way.

The goal of this work is to evaluate LIBS and LIFS portable systems for non-destructive analysis of organic matter and metal in whole soils.

2. Materials and Methods

The total carbon content in the samples was determined by the method of dry combustion using a Total Organic Carbon (TOC) (Shimadzu TOC - V).

Before LIBS and LIFS analyses, around 0.5 g of grinded soil was pressed into a pellet during 10 min using a force of 14 tons. For each soil sample two pellets were done.

The spectra the samples were obtained using a portable LIBS system from StellarNet (model PORTA-LIBS-2000) fitted with Nd:YAG laser of 25 mJ with pulse duration of 4 ns, repetition rate of 1Hz; spectrometric range of 190-1000 nm and optical resolution of 0.2 nm, with the fixed delay time of 2 μs. To improve the relation signal/noise, 60 spectra were obtained from each sample, each corresponding to one shot in different areas of the pellet. The final spectrum of each sample was the average of 60 spectra. Calibration models using ANN for LIBS quantitative measurements were evaluated for determination of C and some metals, such as Ba, Co, Cu, Cr, Mn, Ni, V and Zn.

Also it was assessed a portable LIFS system, built by Embrapa Agricultural Instrumentation, to estimate humification degree of soil organic matter. The bench system, described by Milori and co-workers[6], was used as reference technique.

3. Results and Discussion

The results show that concentration values determined by LIBS proposed method presented good agreement with reference values obtained by ICP OES. The correlation coefficient for the comparisons between LIBS values and reference values were all above 98%.

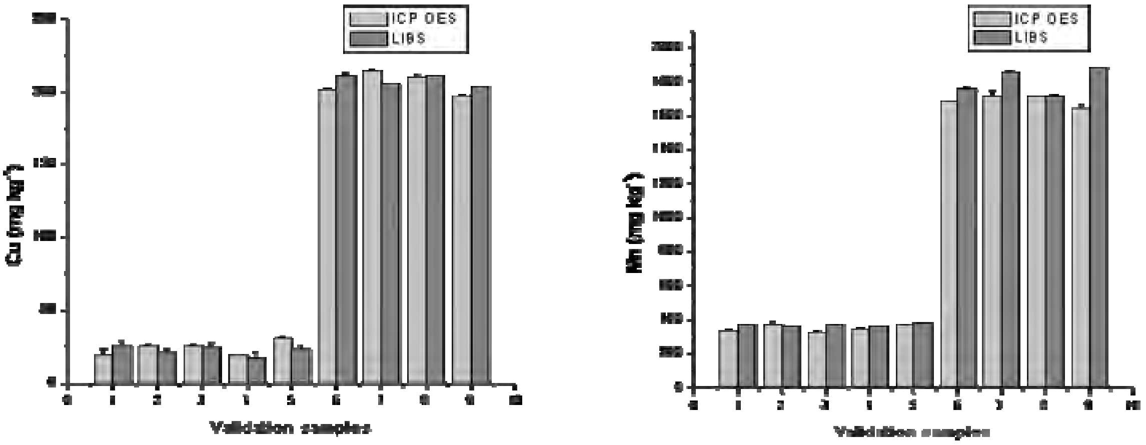

Figure 1: Correlation among ICP OES reference concentrations and LIBS determined concentrations for Cu and Mn, two important plant micronutrients

Although the LIBS proposed method presented higher limit of detection (LOD) when compared to ICP OES reference method, the obtained results were considered satisfactory for direct evaluation of soil fertility or contamination. Moreover the proposed method practically avoid sample preparation step, taking only 3 minutes to prepare a pellet.

The results for C determination were similar to metals. The calibration model obtained presented a correlation coefficient of 0.94. By using this model, the predicted values presented 93% of correlation with reference values determined by TOC. The absolute medium error was 0.08% for almost all samples in the validation set and the LOD was around 0.3%.

Table 1: Validation results of LIBS using calibration method based on ANN

C reference concentration (%)	C predicted concentration (%)	Absolute Error
0.51	0.43 (±0.04)	-0.08
0.79	0.94 (±0.08)	0.15
0.62	0.78 (±0.15)	0.16
0.48	0.56 (±0.09)	0.08
0.65	0.76 (±0.10)	0.11
0.36	0.44 (±0.07)	0.08
0.45	0.51 (±0.07)	0.06
0.72	0.76 (±0.07)	0.04

The portable LIFS system to evaluate chemical properties of SOM related to more humified structures also presented interesting results. The correlation obtained between the portable and bench equipments was around 94%, with P <0.0001. This high value shows that the prototype reached the expectations and reduced the measurement time from 1 min. to 5 sec. per sample.

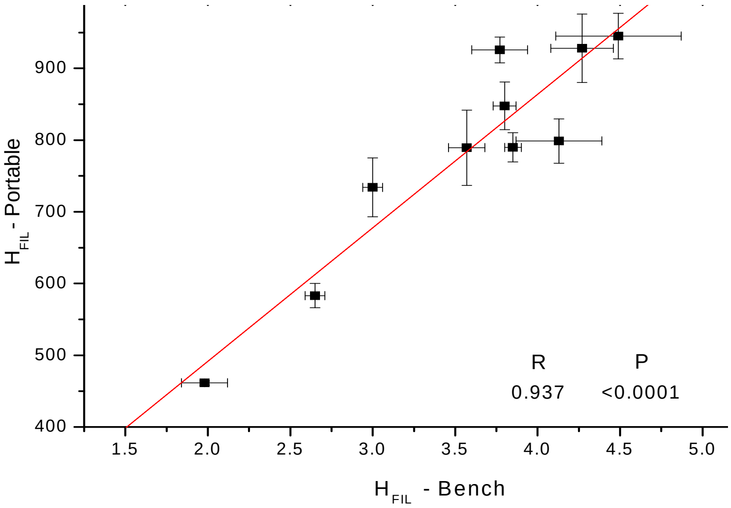

Figure 2 Correlation between humification degree determined by LIFS portable and bench systems

4. Conclusions

These results highlight the high potential for future applications as field sensor oriented for precision agriculture and studies directed to soil carbon sequestration in projects about climate changes. Also many others opportunities to more detailed studies on soil organic matter dynamics and reactivity, without risks of artificial laboratory or chemical preparations, could be addressed with important impact in this research area.

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Interactions between Organic Pollutants (OPs) and Soil Organic Matter: The Crucial Effect in Determining OPs Behavior in Soils

Haizhen Wang[a, b, c, d], Yan He[a, b], Jianming Xu[a, b*], Scott R. Yates[c, d], Jay Gan[d], Jincai Ma[c, d]

[a]Institute of Soil and Water Resources and Environmental Science, Zhejiang Univ., Hangzhou 310029, China; [b]Zhejiang Provincial Key Lab of Subtropical Soil and Plant Nutrition, Hangzhou 310029, China; [c]USDA-ARS, U.S. Salinity Laboratory, Riverside, CA 92507, USA; [d]Department of Environmental Sciences, Univ. of California, Riverside, CA 92521
E-mails: jmxu@zju.edu.cn

1. Introduction

There are quite number of organic pollutants (OPs), in particular persistent organic pollutants (POPs), in the environment. The adverse effect of OPs on environment safety and human health had caused an increasing concern in the world. Researches on OPs regarding their environmental behavior and the relevant effect have already become one of the hot topics of environmental science in this century. Many reports have demonstrated the crucial effect of soil organic matter (SOM) in determining the environmental fate of OPs. In this paper, we discussed some important aspects of interactions between SOM and OPs including sorption, binding, degradation, and etc. mostly based on our previous and current research results, giving an improved understanding for the environmental fate and the potential ecological risk of OPs in soil.

2. Sorption of OPs by SOM

SOM is one of the most important factors to affect OPs sorption by soils. Many researchers have conducted experiments to explore OPs sorption using the separated SOM fractions, such as water dissolved organic mater (WDOM), fulvic acid (FA), humic acid (HA), and humin [1-8]. Ling et al. reported that the effects of WDOM on the atrazine sorption by soils were WDOM-concentration dependent and dominated by the interactions among atrazine, WDOM, and soil solids [1-2]. The sorption of hydrophobic WDOM fraction (Ho) on soils promoted the atrazine sorption by soil, while the release of SOM by hydrophilic WDOM fraction (Hi) and the competitive sorption between Hi and atrazine on soil surface led to the decrease of atrazine sorption. The total WDOM sorption on soils described by the Langmuir Equation reached saturation as the WDOM concentration increased, and the sorption of Ho fit the Freundlich model best[1]. The other investigations showed that HAs was a crucial factor that controlled the process of pentachlorophenol (PCP) and butachlor sorption [3-5]. The typical sorption isotherms of PCP and butachlor on HAs were also fitted well with the logarithmic form of the Freundlich model. Moreover, for an adsorbent with a very low SOM content, mineral colloids could enhance butachlor adsorption because of their high exposed mineral surface. The ratio of clay content to total organic carbon content is a useful parameter to predict butachlor adsorption in soils [5].

3. Binding of OPs with SOM

A large number of researches revealed that a substantial percentage of the total amount of OPs may persistent in soil through their interactions with SOM. However, the binding ability of SOM for Ops was dependent on both the properties of OPs and SOM fractions [9-11]. Wang et al. reported that the proportions of metsulfuron-methyl (MSM) ^{14}C-bound residues in soil humus components increased in the order of FA>> humin > HA [10]. Other researches also showed that humin-bound-PAHs was the main component in total non-extractable PAHs, matching the content of the fractions of organic matter in most soils [11]. Our latest experiments indicated the affinity of SOM fractions for ^{14}C-PCP varies greatly (Figure 1). The amounts of bound-^{14}C-PCP residues in SOM fractions increased in the order of humin >> FA, HA>WDOC. Compared with MSM, more PAHs and PCP residues were bound with soil humin. It is the most important factor to dominate the persistence of PAHs and PCP in the environment.

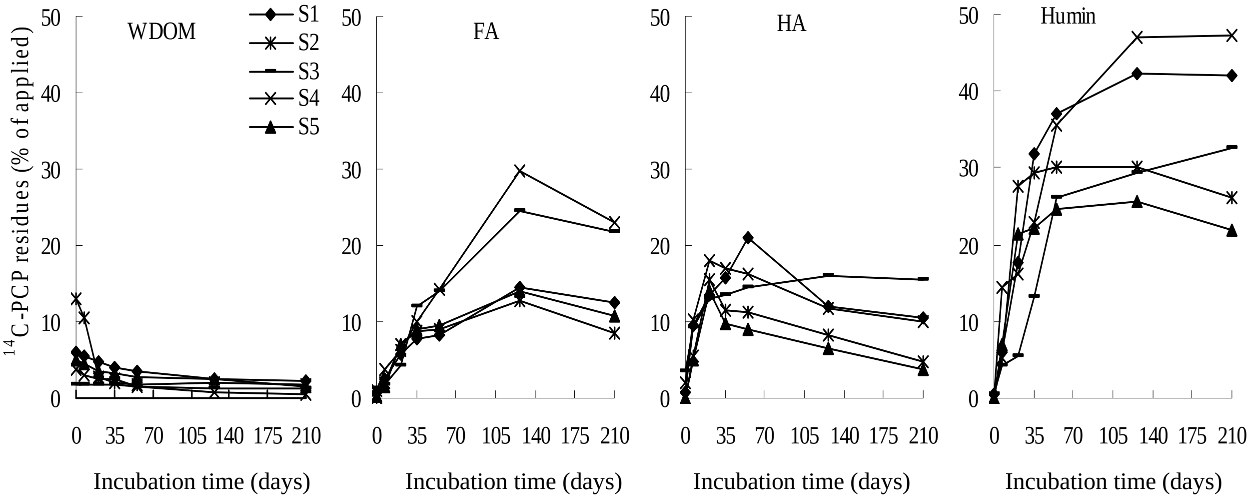

Figure 1: ^{14}C-PCP residues in SOM fractions

Additionally, the bound OPs residues in SOM were controlled by different soil properties. Stepwise multiple regression analysis denoted that the distribution of ^{14}C-PCP residues in WDOC fraction was positively correlated with soil pH, sand content and WDOC content. Free Fe_2O_3, WDOC, and SMBC (soil microbial biomass carbon) played an important role in the distribution of ^{14}C-PCP residues in FA fraction; while soil pH, amorphous Fe_2O_3, and HA were major factors affecting the bound ^{14}C-PCP residues in HA fraction. The bound ^{14}C-PCP residues in humin fraction were significantly influenced by TOC, amorphous Fe_2O_3, and surface area.

4. Degradation of OPs in soils as affected by SOM

It is reported that there are different effects of SOM on OPs degradation. The sorption or binding of OPs by SOM induced OPs less available to organisms, and slowed down their biodegradation in soils [12]. On the other hand, SOM, especially organic amendments, may accelerate OPs degradation through a general stimulation of soil microbial activity [13-15]. Zhang et al. reported that SMBC significantly increased with the increasing application of organic amendments in soils. The average half-life of atrazine in soil reduced by 1.4~2.0-fold after the application of organic amendments [13]. And He et al. pointed out that the residual dissipation of PCP in soil differed with the addition rates of root exudates [15]. SMBC showed the largest responses at the lowest addition rate (13.38 TOC mg kg^{-1}) of root exudates. PCP residues in soils were the smallest and the remediation was the most effective at this addition rate.

The bioavailability or degradation of SOM associated OPs may differ in different SOM fractions that have contrasting physico-chemical properties such as chemical composition, physical structure, and bioactivity [16]. Lower bioactivity and longer persistence of soil bound OPs were observed bound with soil humus, especially the soil humin fraction. The ^{14}C-MSM bound residues in FA increased during the initial 28 days, and then decreased. However, the amounts of ^{14}C-MSM bound residues in humin did not change significantly with time from the 28-th to the 224-th day after application [10]. As shown in Figure 1, the ^{14}C-PCP residues in WDOC decreased rapidly over the incubation period of 210 days, while the amount of ^{14}C-PCP residues in humin increased significantly within the same incubation time. The amounts of ^{14}C-PCP residues in FA steadily increased until 126 days followed by a slight decrease thereafter. The highest amounts of ^{14}C-PCP residues in HA were observed at the 21–56 days after application. It is quite obvious that MSM and PCP degraded slowly after bound into soil humin.

5. Conclusions

The interactions between OPs and SOM will directly affect the behavior of OPs in soil. The stronger binding capacity of OPs with humin and the longer persistence of OPs residues in humin would enhance the accumulation of OPs residues in soil and increase their potential ecological risk in the environment.

Acknowledgements

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Properties of Biochar Produced from *Miscanthus* x *giganteus* and its Influence the Growth of Maize (*Zea mays* L.)

R. McInerney[a], W. Kwapinski[a*], P. Wolfram[a], C. Byrne[a], F. Melligan[a], E.H. Novotny[b], J.J. Leahy[a], M.H.B. Hayes[a]

[a]Department of Chemical and Environmental Sciences, University of Limerick, Limerick, Ireland; [b]Embrapa Solos, Rua Jardim Botânico 1024, Brazil
E-mail: witold.kwapinski@ul.ie

1. Introduction

In technical terms, biochar, a carbon-rich product, is produced when biomass (e.g. wood, organic manures, and bio-waste materials) is heated (pyrolysed) at temperatures below 700 °C in a limited air supply [1, 2]. Pyrolytical-oil (bio-oil), combustible gases, and biochar are formed during pyrolysis. Our investigations focus on the upgrading of bio-oil (not discussed in this communication), and on the production and characterisation of biochar, and its influences on plant growth when used as a soil amender.

Slow, intermediate and fast pyrolysis processes are relevant for the production of biochar [2]. In the slow process (450–650 °C) vapour slowly evacuates from the porous structures of biomass, and the yields of the three products are relatively similar.

2. Materials and Methods

Biochar, from *Miscanthus x giganteus* chips (max. size 1 cm) was prepared in a lab-scale pyrolyser (1 dm^3) at: (a) 400 °C for 10 min; (b) 500 °C for 30 min; and (c) 600 °C for 60 min. A shallow calcareous brown earth loam soil (20% clay, pH 7.5) from the Kinvara series in the Burren area (N. Clare, S. Galway, Ireland) was amended with biochar (3% w/w), and 10 maize (*Zea mays* L.) seeds were planted per pot (13 cm). The system was incubated in a growth chamber for 21 days and artificial light was provided for 13 h/day. The pots were watered (50 cm^3) every fourth day. After 10 days the weakest five plants were removed from each pot. At the end of the growing period all plants were cut at the soil level, weighed, and oven dried at 60 °C until a constant weight was reached.

Surface area measurements (BET), using a Gemini Micromeritics apparatus, high heating values (HHV), using a Paar calorimeter, and C, H and N contents (Elementar) were determined for each biochar sample. Scanning electron microscopy (SEM) was used to observe the morphologies of the biochars. Volatile material associated with the biochar was determined from weight loss on heating for 7 min at 900 ^{o}C, according to the standard procedure CEN/TS 15148:2005.

3. Results and Discussion

Results of growth experiments. Table 1 indicates that favourable plant responses were not obtained for all of the biochar samples. Best results were obtained for the biochar heated at 600 °C for 60 min. Biochar heated for 10 min at 400 °C suppressed plant growth.

Table 1: Mass of maize seedlings (as a percentage of the controls, where biochar was not added) from soils amended with biochars (3% w/w)

Preparation method	400 °C, 10 minutes	500 °C, 30 minutes	600 °C, 60 minutes
Yield of dry matter (as wg.% of control)	76.6	135	165

Biochar prepared at 400 °C for 10 minutes had a significant lower surface area than that prepared at 600 °C for 60 minutes (Table 2). This could be important for microbial associations that could influence plant growth.

Table 2: Analytical data for biochars prepared under different conditions

Preparation method	400 °C, 10 minutes	500 °C, 30 minutes	600 °C, 60 minutes
Surface area, m^2/g	1.40 – 1.73	3.87 – 7.87	50.9 – 51.1
HHV, MJ/kg	29.4 – 30.3	30.9 – 30.9	31.5 – 32.5
C	74.76	79.67	85.10
H	4.33	3.16	2.40
N	0.39	0.50	0.55

Calorimetry investigations (Table 2) show that the HHV of biochar produced at 600 °C for 60 min had the highest heating value; that is confirmed by the carbon and hydrogen contents.

The ^{13}C NMR spectrum of untreated *Miscanthus* (not presented in this communication) showed significant resonances attributable to cellulose and hemicelluloses. There was clear evidence for carbohydrate structures in the 65 to 110 ppm range of resonances. C-4 carbons of amorphous cellulose and hemicelluloses gave resonances at 83 ppm; shoulders at 65 and 89 ppm corresponded to the C-6 and C-4 carbons of crystalline cellulose, respectively; and peaks at 73 and 75 ppm represented overlapping signals for the C-2, C-3, and C-5 carbons of polysaccharides. The anomeric carbon (C-1) carbohydrate resonance was clearly evident (sharp peak at 105 ppm). Signals at 56 ppm, at 130-155 ppm, and at 168-178 ppm could be associated, respectively, with the methoxyl, aromatic, and the carbonyl groups of carboxylic acids and ester functionalities of hemicelluloses and lignin. The peak at 20 ppm could be attributable to the acetyl groups of hemicelluloses [3].

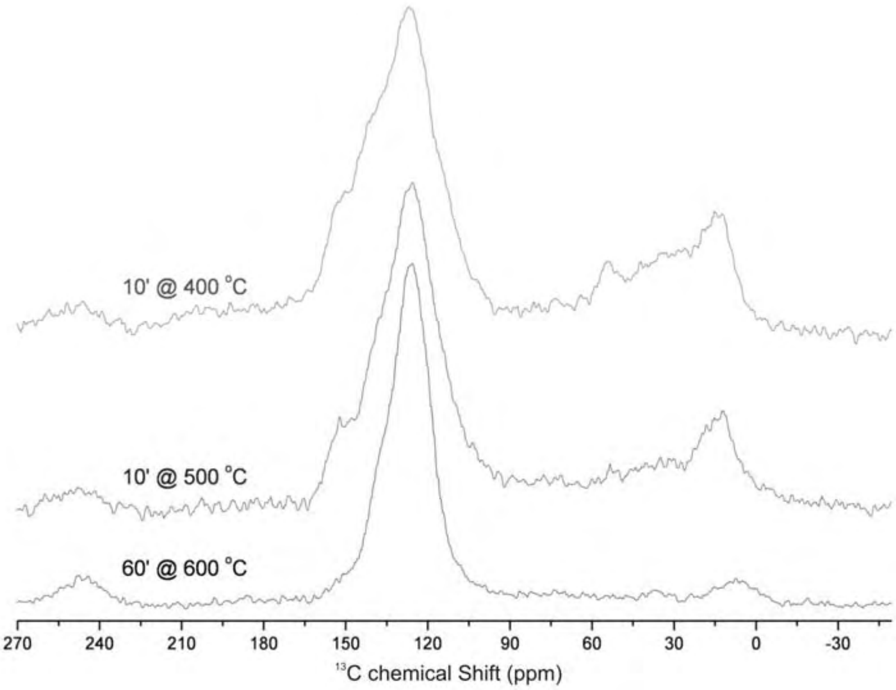

Figure 1: ^{13}C NMR spectra of pyrolysed *Miscanthus*

The spectra for the biochars (Fig. 1) are vastly different from that of the Miscanthus starting material. The ^{13}C NMR patterns emphasize how the structural compositions of the biochars change during pyrolysis. Pyrolysis at 400 ºC and 500 ºC caused biochars to retain their volatile materials, mostly aliphatic-C structures. In particular Miscanthus pyrolysed at 400 ºC shows significant peaks in the aliphatic regions 25 to 35 ppm. The resonance at ~15 ppm is from terminal methyl groups. Raising the pyrolysis temperature to greater than 400 ºC, and lengthening the pyrolysis time caused losses of aliphatic-C moieties and an accumulation of largely poly-condensed aromatic-C type compounds dominated by the 130 ppm resonance [4]. The spectrum of the biochar from the 400 ºC reaction has some evidence for lignin contributions, as indicated by the peak at 56 ppm for methoxyl, and the shoulder at ~150 ppm typical of O-aromatic carbons. As the temperature is raised from 400 ºC, through 500 ºC to 600 ºC, these lignin signals diminish. Increased heating also resulted in a significant peak shift in the aryl region from 131 to 127 ppm, considered to be indicative of the formation of fused aromatic structures [5, 6]. That signifies the transformation of labile compounds into environmentally recalcitrant forms with important biochemical implications with regard to greater resistance to microbial transformations.

Because the chars produced at 400 ºC and 500 ºC and at short reaction times (10 min and 30 min) contained recognisable fragments of feedstock biopolymers (lignin signals) it can be concluded that the reaction time was insufficient for complete conversion to char. On the other hand, the char produced at 600 ºC for 60 min contained no recognisable feedstock components, indicating complete conversion to char.

The SEM analysis showed that that the plant cellular morphology was preserved after the biomass was pyrolysed. However, the SEM device does not allow observation of difference in surface area or structure between the samples made under the different conditions.

It is plausible to consider the effectiveness that biochar will have for carbon sequestration leading to the reduction of atmospheric CO_2. The volatile residue after outgassing at 900 ^{o}C amounted to maximum 40% of the mass of the biochar.

4. Conclusions

In order to obtain the optimum value for soil applications of biochar it is important to establish the preparation criteria that will give rise to properties that will have desired effects. This study shows that at the higher temperature a porous biochar was obtained which can be used to store air and water, and provide a refuge against predators for soil microorganisms. It also has value as a high energy biofuel because of its high carbon content and high HHV.

Pyrolysis and biochar production provides an attractive process for carbon sequestration in soil because of its long term stability in soil.

There is, however, a need to establish a classification system that will define the values of biochar for different applications. NMR, BET, mineral matter (ash) and volatile content determinations would seem appropriate for the provision of data for standardisations.

Acknowledgements

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Effect of Biochar on the Emission of CO_2 and N_2O After Soil Application

Jürgen Kern[a*], Jan Mumme[a], York Neubauer[b], Mike Kleinert[c]
[a]Leibniz-Institute for Agricultural Engineering Potsdam-Bornim e.V., 14469 Potsdam, Max-Eyth-Allee 100, Germany; [b]Berlin Institute of Technology, Fasanenstr. 89, 10623 Berlin, Germany; [c]SunCoal Industries GmbH, 15711 Königs Wusterhausen, Hafenstr. 18, Germany
E-mail: jkern@atb-potsdam.de

1. Introduction

Soil fertility is expected to decrease by accelerated decomposition of soil organic matter as a result of increasing mean annual temperatures. One way to counteract the expected loss of soil organic matter may be the application of biochar, which is produced by combustion of different types of biomass under oxygen-limited conditions (pyrolysis, hydrothermal carbonisation = HTC). Its carbon content is up to 80%, which is considered to sequester carbon in the soil for a long term. Some positive effects have already been found after application to soil as for example the improvement of soil structure and water retention and the reduction of greenhouse gas emissions [1, 2]. Although research about biochars has increased during the last years [3], results about carbon stability and environmental effects of such new soil conditioners are still limited. The objective of this study is to compare two different carbonaceous products from thermal conversion in terms of carbon stability and emission of nitrous oxide (N_2O).

2. Materials and Methods

This study included three carbonaceous substrates. First a sandy soil (air dried) from a poplar plantation relatively poor in organic matter, second a biochar, which derives from gasification of poplar wood in a fluidised bed after treatment at 850-900°C and third a product from hydrothermal carbonisation of hardwood after treatment at about 200°C. Chemical characterisation of the substrates was performed according to standard methods. CO_2 and N_2O release from the rewetted substrates mixed with the sandy soil were measured by gas chromatography after incubation in an atmosphere of air for 48 hours in the laboratory [4].

3. Results and Discussion

Carbonisation of wood resulted in a carbon content of 55% in the HTC biochar and 75% in the gasifier biochar (Table 1). The nitrogen content was ten times higher in the gasifier biochar compared to the HTC biochar. The same pattern was found for the oxidised extractable ($CaCl_2$) nitrogen compounds NO_2 and NO_3. These substrates for denitrification might enhance N_2O release, which however could not be observed within our 48 h incubation experiment. Most remarkable are the low N_2O emission rates of both biochars mixed with the sandy soil. They were significantly reduced by one order of magnitude compared to the pure soil. Since N_2O has a global warming potential 298 times higher than that of CO_2, this effect of biochars may play an important role in mitigating CO_2 equivalents. At the same time CO_2 emission increased considerably in case of HTC biochar and reflects relative low carbon stability. Assuming a constant decomposition rate, after one year the sandy soil, the HTC biochar and the gasifier biochar would have lost 76%, 43% and 1% of their carbon content, respectively.

Table 1: Chemical characterisation of sandy soil and two biochars. CO_2 and N_2O emission rates derived from 48 h incubation of biochars mixed with the sandy soil. All values are related to dry weight (105°C) of the substrates.

	C	N	NH_4-N	NO_2-N	NO_3-N	CO_2	N_2O
	%	%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg C kg^{-1} h^{-1}	µg N kg^{-1} h^{-1}
Sandy soil	1.29	0.13	3.34	0.16	8.62	1.12 ± 0.27	34.20 ± 27.83
HTC biochar	55.08	0.08	4.94	0.07	0.73	27.19 ± 3.53	3.04 ± 1.05
Gasifier biochar	75.40	0.78	0.45	33.53	90.94	1.16 ± 0.76	4.99 ± 3.75

4. Conclusions

It was shown that biochars applied to soil reduce the emission of N_2O significantly. This positive effect may be reduced by simultaneous emission of CO_2, which has to be taken into account for a complete balance of greenhouse gases. These first results indicate the importance of the biochar’s properties, which will be subject to future studies.

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Porosity in Wet-sieved Aggregates and its Effects on SOC Protection

Gianluca Simonetti[a], Emanuele Lugato[a], Serenella Nardi[b], Ornella Francioso[c],
Antonio Berti[a]*, Luigi Giardini[a], Francesco Morari[a]

[a] Dip. di Agronomia Ambientale e Produzioni Vegetali and [b] Dip. di Biotecnologie Agrarie,
V. le dell'Università 16, 35020 Legnaro (PD), Italy; [c] Dip. di Scienze e Tecnologie
Agroambientali, V.le Fanin 40, 40127 Bologna, Italy
E-mail: antonio.berti@unipd.it

1. Introduction

A complex feedback involves the soil structure evolution and SOC turnover (Christensen, 2001), since the organic matter is a binding agent promoting the aggregation, but it is also protected by porosity. The soil pore distribution controls the spatial inaccessibility to microorganisms and the low O_2 diffusion, that are very important factors in SOC protection. The smaller pores internal to microaggregates (53–250 µm) are more likely to exclude biota and their enzymes than those of macroaggregates. The aim of this study was to investigate the chemical and physical mechanisms of SOC protection in a long-term experiment established in the early 1960s in North-eastern Italy.

2. Materials and Methods

The experiment considers the factorial combination of three types of soil (clay-A, sand-S and peaty-P) with six types of mineral, organic or mixed fertilization, organized in two randomized blocks (36 lysimeters). Fertilization treatments considered for this study were as follows: no applications (O); farmyard manure – F2 (40 t ha^{-1} yr^{-1}); mineral fertilizer – M2 (200 kg ha^{-1} yr^{-1} N – 100 P_2O_5 – 240 K_2O). The top soil layer (0–20 cm) was sampled in June 2008. Large macroaggregates (from 1 to 2 mm) were isolated by dry-sieving. These aggregates were then separated into three aggregate size fractions (WSA, water stable aggregates) by wet-sieving (2000–250 µm, 53–250 µm and <53 µm), using an automatic machine oscillator. The WSAs and the starting bulk macroaggregates (1000–2000 µm) were analyzed by MIP Mercury Intrusion Porosimetry for total porosity (TP) and pore size distribution (PSD), within the range of 0.0035–75 µm (from cryptopores to mesopores, Cameron and Buchan, 2006). Humic substances (HS) were extracted and characterised by using elemental analysis, molecular weight distribution (nominal molecular weight: >100 kDa – HF1 , 100-10 kDa – HF2 and <10 kDa – HF3) and solid state ^{13}C-NMR spectroscopy. The integration areas for different 13C resonances were used for statistical analyses.

3. Results and Discussion

Total porosity and pore size distribution varied according to aggregate fraction while no significant difference was observed between treatments. Micro and silt clay aggregates had the higher total porosity, mainly represented by micro and ultra-micro pores. In sandy soils the meso-pore class showed significant differences (P<0.01) between fertilizations, with higher values in M2 (0.20 cm^3 cm^{-3}) than in F2 (0.13 cm^3 cm^{-3}) and O (0.15 cm^3 cm^{-3}).

The application of principal component analysis (PCA) to soil data allowed four factors to be extracted, explaining the 89% of the total variance. The first and second principal component (69% for the total variance) showed a strong relationship with the majority of the HS quali-quantitative parameters (Humic C, HF2, HF3 and Aryl C) and crypto-pores, respectively. The third factor (14% of variability) was correlated with meso-pores, ultramicro-pores, with the carbonyl C and anomeric C of HS. The fourth factor (10%) was correlated with the amount of micro-pores. The plots according to factors 1 and 3 (Fig. 1) allowed to identify the interaction between carbonyl-C of HS and mineral surfaces in silty clay fractions of clay soil (A-53 μm) and the anomeric and *O,N*-alkyl C in sandy soil (S-250 and S53–250 μm). The strong relationship between carbohydrates and sandy soil fractions indicated that some undecomposed organic matter, rich in cellulose and hemicellulose, was still persistent (Turchenek and Oades, 1979).

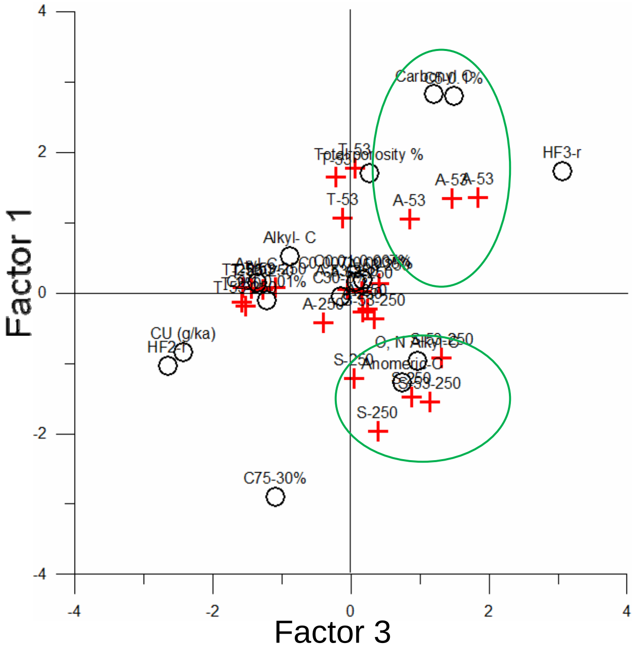

Figure 1: PCA plots of the variables and cases: factors 1 and 3

4. Conclusions

Soil porosity distribution might be a valuable indicator of the soil capacity to sequester organic carbon. The SOC stock can be efficiently enhanced and protected in clay, particularly in silt clay fraction and in microaggregates.

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Proposal of Biogeochemical Indicators for the Ecosystem Services Supported by Volcanic Ash Anthrosols in Tenerife Island (Spain)

Z. Hernández[a*], G. Almendros[a], A. Gómez Sal[b]
[a]Centro de Ciencias Medioambientales–CSIC, Serrano 115B, 28006, Madrid, Spain;
[b]Department of Ecology, University of Alcalá, Alcalá de Henares, 28871, Madrid, Spain
E-mail: zulimar@ccma.csic.es

1. Introduction

Soils hold the basic support functions for the services supplied by ecosystems, such as food production, micro-climatic regulation, control of erosion, retention and quality of water. These functions and services are receiving a renewed attention because of their links with the human well-being's components [1]. Many authors consider soil as an integrated system, where dynamic organomineral interactions are in part responsible for its regulating role as regards the flow of C, nutrients and water throughout the whole ecosystem [2]. However, one of the main problems to evaluate the ecosystem services of a local soil under different management regimes is the lack of specific indicators to be used. One part of the problem consists of the variable meaning of the same descriptor in the different soils (e.g. C/N ratio is not a suitable indicator of the quality of humification in agriculture soils, mainly when values are lower than 7 which could be a symptom of chemical overfertilization).

However, some soil variables could be used to obtain information about the supporting and regulating services supplied by the ecosystems. Among them, the content of soil organic carbon (SOC) is frequently used as a measure of actual soil C sequestration as well as changes of land use [3]. Apart from this, the stable forms of C strongly linked to the mineral fraction, accumulated in the soil for long periods of time (mainly the fraction of humic acids, HAs), could be used to define a molecular signature responsive for the extent of environmental impacts [4]. This is also particularly important when we are dealing to establish the different biogeochemical processes and microbial metabolic pathways that make possible the accumulation of a distinctive molecular pattern in the soil [5].

Although the food production is mainly supported by agricultural soils, these soils could carry out other services different than provisioning, such as local climate regulation or water retention (regulating services in the same way than the natural soils). In this sense, some authors have suggested that this quality depends on the type of soil and its specific agricultural management [6]. It has also been observed that intensive land use tend to decrease the content of SOC whereas this does not necessarily occur in traditional or ecological crops.

This is the case of Mediterranean soils, where agricultural practices (mainly tillage) can lead to a progressive loss of total SOC [7]. On the other hand, in volcanic ash soils, it has been largely discussed the stabilizing role of short-range minerals which promote stable organomineral interactions at the level of the soil matrix, avoiding the loss of SOC, even after being cultivated for long time [8].

This paper focuses on identifying a set of biogeochemical descriptors to assess the ecosystem services supplied by Anthrosols in Tenerife Island, mainly food provision and micro-climatic regulation. This research is supported by analyses of hydrophysical and agrochemical properties, as well as by analysis of soil organic matter constituents including the molecular characterization of HAs which could represent a source of biogeochemical proxies on the structure and function of the ecosystems on these volcanic soils.

2. Material and methods

The experimental design includes different agricultural practices (minimal tillage, use of mineral and vegetation covers, organic amendment) in vineyards from Tenerife (Canary Island, Spain). A set of soil physical and chemical variables were determined, such as: SOC, nitrogen and C/N ratio, amorphous oxides ($Al_o+{}^1/_2\ Fe_o$), pH, exchangeable bases (sum of Na, K, Ca and Mg), micronutrients (Fe, Mn, Cu and Zn), available phosphate, retention of P, porosity, water holding capacity at atmospheric pressure and at -1500 kPa (WHC_1 and WHC_{15}), texture (clay, silt and sand), water percolation and aggregate stability [9]. The content of soil lipids was determined after extraction with dichloromethane-methanol 3:1 (v:v). The HAs and extractable insolubilized humins (soil-matrix fixed HAs) were isolated by successive alkaline extractions with 0.1M $Na_2P_4O_7$ and 0.1 M NaOH; in the latter case treating previously the soil with 60 mM $Na_2S_2O_4$ and 1 M HF-HCl mixture [9]. The characteristics of HAs were studied using visible and infrared spectroscopy, elementary analysis and analytical pyrolysis. The visible spectroscopy was used to measure the optical density at 465 nm (E4) and the second derivative spectra were obtained in order to check and to quantify fungal quinoid pigments (dihydroxyperylenequinones, DHPQs). Derivative infrared spectroscopy was especially useful to identify the "lignin fingerprint" which is a characteristic pattern of bands at *ca.* 1640, 1620, 1540, 1510, 1460, 1330, 1260 and 1030 cm^{-1}. Analytical pyrolysis was carried out in a Pyrojector (SGE instruments) connected to a GC/MS system Finnigan Trace GC Ultra with a Trace DSQ mass spectrometer.

Several statistical treatments were used to analyze the information obtained: Non-linear multidimensional scaling was used to classify biogeochemical indicators using the 1-Pearson correlation coefficient as similarity index. The plot obtained illustrated the mutual relationships between variables to greater extent explaining the accumulation of SOC. Discriminant analysis is used to assess whether soil samples are properly grouped in terms of soil management practices previosly designed to optimize either food production or soil C sequestration.

3. Results and discussion

The results of non-linear multidimensional scaling (Fig. 1) showed an arrangement of the variables showing more or less defined clusters corresponding to the variables which tend to be correlated each to other.

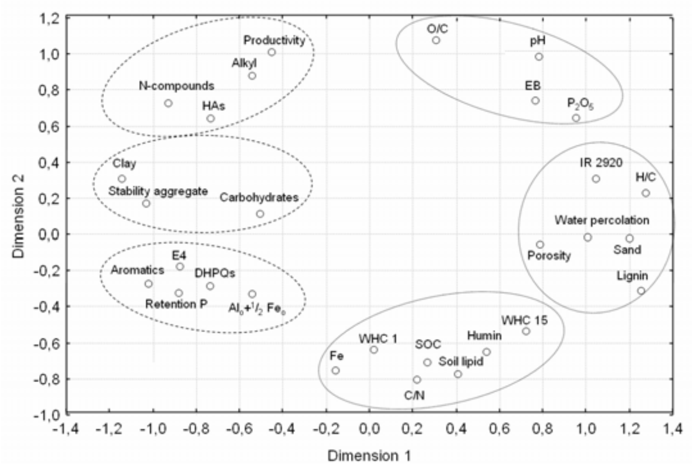

Figure 1: Automatic non-linear classification of soil physical and chemical properties by multidimensional scaling. SOC: Soil organic carbon, HAs: humic acids, H/C and O/C: atomic ratios of HAs, WHC1 and WHC15: Water holding capacity at atmospheric pressure and at –1500 kPa respectively, BS: Available bases, E4: optical density at 465 nm, DHPQs: dihydroxyperylenequinone content, IR 2920: intensity 2920 cm^{-1} infrared band. Pyrolytic products: lignin (methoxyphenols), aromatics (methoxyl-lacking aromatics), carbohydrates, N-compounds and alkyl constituents of the Has

In the case of SOC, it was found that its accumulation was correlated with the content of soil lipid, WHC_1, WHC_{15} and the C/N ratio, as correspond to its significant bearing on soil hydrophysical properties (porosity, texture, water percolation). This concurs in samples with HAs of a predominant aliphatic character where the high atomic H/C ratio, the intense 2920 cm^{-1} infrared band and the conspicuous accumulation of methoxyphenols, pointed to preservation of young organic matter. In an independent cluster, a series of organic matter descriptors suggested resilience and advanced humification stages (high E4, abundance of aromatic compounds and DHPQs) associated with indicators of the andic character (amorphous materials and retention P). This indicates intense organomineral interactions (high aggregate stability and content of clay sized minerals) in the corresponding soils. Concerning soil agrochemical fertility, most of the indicators (pH, base saturation) tend to be correlated mainly with the oxidation level of the organic matter (atomic O/C ratio).

The results of discriminant analysis after backward variable selection, using as classification factor the different levels of annual grape production (Fig. 2), revealed differences in crop yields for each type of soil, showing the lowest production in soils with andic character (high content of amorphous oxides and porosity) located on the Northern side of the Island and the highest production in soils built by the farmers carrying the volcanic materials from upper zones to lowlands (high aggregate stability and agrochemical fertility).

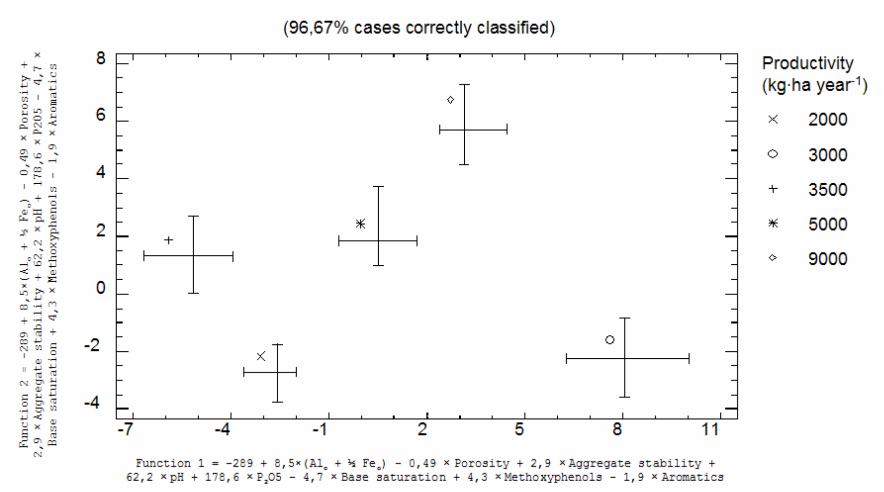

Figure 2: Representation of points corresponding to soil samples in the space defined by the two first functions obtained by discriminant analysis using the productivity (kg grape · ha year^{-1}) as classification factor. Group labels are located near the cluster centroids and error bars are also shown

In these agroecosystems, it is necessary to differentiate between the agronomic and biogeochemical quality, i.e., that some biological processes that could reach their maximum development in natural ecosystems (such as SOC sequestration) could be meaningless in systems oriented to food production. In addition, biogeochemical quality allows us to explore a new quality concept linking between of ecosystem services and the components of human well-being [1].

4. Conclusion

A series of biogeochemical indicators could be used to assess the extent of the interaction between SOC and other soil matrix constituents (minerals, metal oxides or soil aggregates). In most cases, these descriptors could indicate emergent properties of the ecosystems related with the support of services for human well-being. Of the mentioned descriptors, the total amount SOC by itself should not be sufficient to predict the agroecological success of the sequestration C in agroecosystems on volcanic ash soils. In fact, SOC amount was found unrelated to HA maturity, being necessary to know the residence time of C on the soil or, conversely the quality of the C forms. In this work, the optical density and the content of aromatic-compounds of the HAs—that were not highly correlated with the SOC—were found to be suitable indicators of the quality of organic matter in soils.

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Interactions Between Hanford Colloids and Humic Substances: Sorption Mechanisms and Colloid Stabilization

Joanna Cloy[a*], Markus Flury[b], James Harsh[b], Jeff Boyle[b]

[a]School of GeoSciences, University of Edinburgh, UK; [b]Department of Crop and Soil Sciences, Washington State University, USA
E-mail: Joanna.Cloy@ed.ac.uk

1. Introduction

Colloids are an extremely active portion of soil and they play a major role in environmental processes such as contaminant fate and transport. Colloids from the radioactive waste repository at Hanford in Washington State have been characterized and extensively studied in terms of their role in colloid-facilitated transport of radionuclides [1, 2], but the effects of organic matter (OM) on the stability and transport of these colloids are still not fully understood. This work aims to study the interactions between humic substances (HS) and Hanford colloids through the investigation of fulvic acid (FA) and humic acid (HA) sorption mechanisms and the role of these HS in the stabilization of Hanford colloids.

2. Materials and Methods

Colloids (operationally defined in this work as particles with diameters < 2 μm) were isolated from fine Hanford sediments representative of those found underneath the Hanford radioactive waste tank farms. An aliquot of the prepared colloid stock solution was treated with 30% vol/vol H_2O_2 to serve as an OM-free control suspension. Total metal e.g. Al, Ca, Fe, Mg, Mn (microwave-assisted HNO_3/HF digestion followed by ICP-OES), C and N (C/N analyser) concentrations were determined for the isolated colloids.

Sorption isotherm experiments were carried out using Suwannee River FA and Aldrich HA standard materials. Sorption experiment solutions (pH 7, 1 mM $CaCl_2$ background) with colloid concentrations of 1 g L^{-1} and FA or HA concentrations ranging from 0 – 96 mg L^{-1} were used. No-colloid control solutions were also used. Sorption experiment suspensions were equilibrated on a shaker (17 hours, room temperature) and aliquots of reacted suspensions were used for characterization. Colloids were then centrifuged out of solution and metal (ICP-OES), C and N (TOC analyzer) concentrations in the equilibrium aqueous phases were determined.

Natural, FA/HA-reacted and H_2O_2-treated colloids were characterized using FTIR spectroscopy and SEM-EDAX. Changes in colloid particle size (at pH 7 and 1 mM $CaCl_2$) and electrophoretic mobility at different values of pH (4, 5, 7, 8, 9, 10) and sorption experiment solution ionic strength (0.01, 0.1 and 1 mM $CaCl_2$) were measured using dynamic light scattering.

3. Results and Discussion

Results from this investigation indicated that the maximum sorption capacities of Suwannee River FA and Aldrich HA on Hanford colloids were ~ 10 mg C g^{-1} and ~ 15 mg C g^{-1}, respectively. Langmuir type adsorption isotherm shapes were observed, suggesting monolayer sorption of FAs and HAs on these colloids.

At pH 7 and 1 mM $CaCl_2$ ionic strength, no differences in particle size were observed between the natural colloids and FA-reacted colloids but a greater particle size was found for the H_2O_2-treated colloids, suggesting aggregation of particles in absence of organic matter coatings. Also, under these conditions, the natural and FA-reacted colloids had more negative electrophoretic mobility values than the H_2O_2-treated colloids. Furthermore, electrophoretic mobility values for the FA-reacted colloids generally became increasingly negative with increasing FA reaction concentration. These findings suggest that OM stabilizes the Hanford colloids.

FA and HA sorption isotherm and colloid characterization results will be discussed further and linked with findings from previous Hanford colloid investigations. The chemical characteristics of the colloids and potential FA and HA sorption mechanisms, as indicated by FTIR spectroscopy will also be discussed.

4. Conclusions

Initial results from this investigation of the interactions between Hanford colloids and HS indicate that natural OM and HS do have a stabilizing effect on colloids from the Hanford radioactive waste repository. The impact of these findings is that OM has the potential to increase the reactivity of Hanford colloids towards contaminants and ultimately influence their participation in colloid-facilitated contaminant transport.

Acknowledgements

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Bio-surfactant molecule from organic wastes: surfactant properties and ability to solubilise tetrachloroethene (PCE).

Fabrizio Adani[a*], Fulvia Tambone[a], Enrico Davoli[b] and Barbara Scaglia[a]

[a]Dipartimento di Produzione Vegetale, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy; [b]Istituto di Ricerche Farmacologiche “Mario Negri”, Via La Masa 19 - 20156 Milano, Italy.
E-mail: fabrizio.adani@unimi.it

1. Introduction

Surfactants are molecules able to solubilise xenobiotic in the environment. Synthetic surfactants are usually employed to perform decontamination process , however some natural substances included, also, humic acid (HA) show surfactant properties due to their amphilic composition [1].

Therefore the availability of a wide range of organic matrices with different compositions and of biotechnologies used to transform them (e.g. composting) offers the opportunity to obtain, theoretically, infinite HAs with different chemical characteristics and surfactant properties at reasonable yield and low cost. Municipal solid wastes surely offer a great amount of potential raw material to extract HA-surfactant molecules [2]. On the other hand, agriculture residue could also be considered as a good source of raw material to recover natural surfactant, if the HA extracted has surfactant properties. As consequence, the first aim of this work was to test the surfactant properties of HA-like extracted from maize plant residues comparing the results with those of HAs extracted previously from other organic matrices. Moreover interesting environmental application of HAs from organic wastes such as the solubilisation of tetrachloroethene (PCE) was done. PCE is one of the more diffuse pollutants in groundwater [3], and it was reported as causing both acute and chronic human health problems.

2. Materials and Method

HA-like substances were extracted from the maize plant residue harvested at the senescence [1]. Chemical characterisation (elemental analysis, CP MAS ^{13}C NMR) and surface tension measurement of HA-like substance were done.

PCE solubility trials were carried out in triplicate using one millilitre of pure PCE (99% pure, density 1.67 kg L^{-1}). PCE was equilibrated for 72 h in vials with solutions at concentrations in the range of 0.2–10 g L^{-1} of HA-like substance surfactants. Then, the supernatant was sampled and characterized by GC-MS.

3. Results and Discussion

HA-like were extracted from maize plant residue at a yield of 81.1 g kg dm^{-1} ± 4.9 of dry matter and from organic wastes at the rate of 79.8 ± 6.3 g kg dm^{-1}.

The surface tension of HA aqueous solutions and the CMC values were reported in Table 1.

Table 1: CMC and surface tension values and solubilzation data of PCE determined for humic acid (HA) extracted from maize and others surfactant substances

	γ_{cmc} (mN/m)	**CMC (mg L^{-1})**	**Log K_{dom}** (mL g^{-1})	**Slope (K_{dom} C_w^{sat})** (g PCE g^{-1} aqueous surfactants)
HA_L[a]	42.9	4,039	3.08	0.1789
HA_{LC}[b]	50.4	999	3.17	0.2230
HA_{LF}[c]	40.8	471	2.97	0.1410
HA_{LFC}[d]	47.6	665	3.50	0.4786
cHA_L[e]	36.1	400	-	-
cHAL2[f]	37.8	970	-	-
$HA_{commercial}$[g]	50	7400	2.10	0.0263
HA-like $_{maize}$[h]	43	1,986	2.56	0.0550
Tween 80[i]	-	-	3.62	0.6895
Triton X 100[j]	-	-	3.86	1.0766

[a] humic acid from lignocelluloses wastes.
[b] humic acid from lignocelluloses wastes after 15 d of composting.
[c] humic acid from lignocelluloses wastes plus organic fraction of municipal solid waste.
[d] humic acid from lignocelluloses wastes plus organic fraction of municipal solid waste after 15 d of composting.
[e] humic acid from lignocelluloses wastes plus organic fraction of municipal solid waste after 15 d of composting [1].3
[f] humic acid from lignocelluloses wastes [6].
[g] commercial humic acid from leonardite [7].
[h] humic acid from maize residue.
[i] Tween 80
[j] Triton X- 100

The CMC is an important parameter to describe a surfactant as it defines the surfactant concentration at which solubility increases because of the micelle formation. It became very important to assess the effect of the characteristics of HA on the CMC as like for synthetic surfactant, CMC is found to be influenced by molecular characteristics, with particular attention to the hydrophilic versus hydrophobic molecule contents [6]. However more than one parameter affects CMC. When molecular weight (MW), CP MAS ^{13}C-NMR and elemental analyses data were considered, a regression with a high determination coefficient was obtained: CMC = 12,919 – 59.48 alkyl-C – 51.71 N – 0.71 MW; in which CMC is expressed as mg L^{-1}, alkyl-C as %, N as g kg^{-1} and MW in Da (R^2= 0.94; P< 0.05, n = 7).

When PCE was equilibrated maize-HA-like solution, the apparent PCE solubility increased linearly (r = 0.97, P < 0.05, n = 6) with increasing concentration of HA. The ability of HA to solubilise PCE is represented by the partitioning coefficient (K_{dom}) or its logarithmic form (Log K_{dom}) (Table 1), which represents the ratio of the equilibrium concentrations of a hydrophobic organic compound within an HA complex and in the free aqueous phase [7].

Solubilization of PCE by maize-HA was from 3.6 to 9.6 times lower than data calculated using HA extracted from organic wastes (Table 1). The maize-HA was more hydrophilic than HAs from organic wastes such as suggested by the hydrophilic/hydrophobic ratio of 0.58 (calculated from NMR data) that was much lower than that calculated as average for HA from organic wastes of 1.49 ± 0.09. Greater hydrophilicity was the consequence of a higher content of non-ionic hydrophilic molecules, such as polysaccharide derived from both cellulose and hemicellulose contained in the parent material and above all, to the low content of alkyl-C (26.78 %) with respect to an average value calculated for previously extracted-HA of 44 ± 3.8 % (mean ± s.d., n = 4). PCE solubilisation has been described by the two-site model of solubilisation: (i) solute partitioning into the micellar hydrophobic core and (ii) dissolution and/or binding into the hydrophilic shell [8]. A higher O-alkyl content, forming a hydrophilic shell, of HA from wastes than that of commercial-HA was advocated previously to explain the higher performance of HA studied with respect to commercial-HA [7]. Now as maize-HA-like contains more O-alkyl-C than HAs from wastes, more capacity to solubilise PCE was expected and not lower, as shown in Table 2. On the other hand PCE solubilisation by non-ionic surfactants (dodecyl alcohol ethoxylates) [8] has been reported to be driven by two opposite forces [10]: (i) loss of solubilisation capacity with increasing hydrophilicity and (ii) enhancement of solubility capacity by specific interaction of PCE with polyethoxylene shell (non-ionic hydrophilic group).

Based on this information we have tried to give a mechanistic understanding of the results obtained in this work in comparison with literature data [7]: (i) HA from organic wastes showed higher ability to solubilise PCE, with respect to commercial-HA, although they have similar lipophilicity, because they contain a high O-alkyl fraction that acts as non-ionic group and increases PCE solubilisation in the hydrophilic shell; (ii) maize-HA-like showed lower solubility capacity than HA from organic wastes, although it contained an higher *O*-alkyl-C, because it is less lipophilic; (iii) maize-HA-like solubilised more PCE than commercial-HA because it contains more *O*-alkyl-C. Therefore, alkyl-C and *O*-alkyl-C contents acted in opposite ways in determining PCE solubilisation. These results found confirmation in the good multiple linear regression found between Log K_{doc} and alkyl-C and *O*-alkyl-C: Log K_{doc} = -1.37 + 0.062 alkyl-C + 0.055 *O*-alkyl, in which alkyl-C and *O*-alkyl were expressed as % ($R^2 = 0.93$, $P < 0.05$, $n = 6$).

4. Conclusions

This work shows that plant residue can be a valuable source of raw material to obtain bio-based surfactants. Different chemical compositions HA acids lead to both different surfactant properties and ability to solubilise PCE. The available data allowed a mechanistic understanding of how chemical composition affected both CMC and PCE solubility and development of linear multiple regression models.

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Some Strategies How to Suppress Interference of Humic Substances in HPLC Analysis of Pesticides in Environmental Samples

Milan Hutta*, Mária Chalányová, Róbert Góra, Radoslav Halko, Ivan Rybár
Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University,
Mlynská dolina CH-2, 842 15 Bratislava, Slovakia
E-mail: hutta@fns.uniba.sk

1. Introduction

Methods for analysis of both xenobiotics (e.g. pesticides) from soil, sediment and water, respectively, are often plagued by a lack of recovery and reproducibility. One source of this variability is the presence of soil and sediments constitutional substances or water contaminants, which usually vary in quality (type) and quantity, even in samples taken at the same time from the same source locality. The investigation of their individual and combined effects on analyte recovery is necessary for a better understanding of the concentration processes and the factors which may interfere with their recovery.

Chemical components naturally present in solid samples or dissolved in natural waters can reduce analyte recovery in numerous sample treatment methods. Constitutional soil organic matter or soluble organic materials found in water have been implicated as major constituents responsible for interference with various trace analytes (e.g. pesticide residue) in environmental analysis. A large fraction of the soluble organic content of soil, sediment and natural waters consists of humic and fulvic acids, which are complex, amorphous, highly colored hydrophilic acids. [1,2]. The fulvic acids are generally more abundant in water and have more oxygen-containing functional groups and a smaller size distribution than the humic acids [3].The major functional groups in both types of acids are similar, although their relative abundance may vary and so their interfering effect can be also variable depending on actual conditions.

In HPLC procedures, especially in solid sample analysis procedures (e.g. soil, sediment) general schema is widely accepted which comprises following steps: sampling - release of analyte(s) from the solid matrix by more/less selective leaching or extraction – liquid extract purification and pretreatment – liquid sample volume reduction – pretreated sample reconstitution in proper mobile phase – HPLC end analysis – data evaluation – final result and its interpretation.

With the slight modification this scheme is applicable also in water analysis by HPLC.

For instance, change-over to enantiomer enriched or pure agrochemicals requires improvement of already existing methods of stereo-selective analysis and also design of novel methods and mastering of new non-traditional approaches in analytical procedures, especially in the case of their analysis in environmental samples (water, soil, sediment). In contrary to the great produced amounts the effective doses per application area are very small—reaching values from 100 g/ha down to 10 g/ha due to their high biocide activity. Thus, mass proportion of both inorganic and organic constituents of the matrix to the analyte enormously increases, posing harder demands on the overall analytical procedure.

2. Materials and Methods

High-performance liquid chromatographs, column-switching (CSW)-HPLC, Large-volume injection (LVI)-HPLC, LVI-SP(by sample pumping)-HPLC, “solid sample injection” HPLC.

3. Results and Discussion

Trace and/or ultra-trace analysis of pesticides residues is needed after their application in the environmental compartments, however, results of majority analytical methods are often harmed by co-extracted substances. The analysis is usually complicated by general presence of constitutional organic matter (e.g. soil organic matter SOM or dissolved organic matter DOM in water). From point-of-view of analytical chemist detection their presence in environmental sample is very important. If not under control, they can cause, beside soil microorganisms such effects as are irreversible and/or selective adsorption of analytes to humic substances colloids or particles, irreversible and selective adsorption of humic substances to the surfaces of analytical or preparative separation system components (stationary phase surface coverage, separation selectivity change, column clogging and damage, chelation of metallic surfaces of e.g. HPLC instrument hydraulic lines), catalyzed decomposition, selective decomposition of pesticides in soil, sediments and aqueous environments, enantiomerization of chiral substances?, interference with analytical signals of majority detection techniques etc. From the point-of-view of R&D analytical chemist it complicates effectiveness of analytical procedure design and development by bringing uncertainty to the interpretation of results e.g. got from recovery studies.

Our team developed various approaches focused to environmental trace HPLC analysis of various pesticides aiming suppression of humic substances adverse interfering effects. The approaches are based on utilization of cloud point extraction and micellar liquid chromatography [4–7], LVI achiral–chiral CSW-HPLC [8], LVI-SP-HPLC [9,10], off-line flow-through extraction of soil micro-samples LVI HPLC [11,12], on-line flow-through extraction of soil micro-samples CSW-LVI-HPLC [13]. In many cases we take benefit from crude extract dilution by a proper solution (in contrary to e.g. Soxhlet extraction and concentration approach) and direct large volume injection (milliliters) into HPLC column. This approach enables decrease of adverse effects of normal injection technique caused by frequent over-saturation and precipitation of interferents within a small top column space in a short time period, when only small dilution of substances occurs due to desired high efficiency of analytical column. Humic substances are probably the main interfering component of soil and natural water organic matter that are operationally defined and must be analyzed and characterized [14,15] for various reasons. The enemy of many chemical analyses must be known better just to make it an acceptable friend, because humic substances are exceptionally interesting also as an analyte. Present state of HPLC application to solution of so-called „pesticide problems“ and /or problems of environmental analysis of pesticides involves optimization of HPLC separation systems for efficient resolution of pesticides and/or their mixtures with humic substances in model systems with focus of application to the analysis of technical and formulated samples; preparative applications of e.g. (enantio-)selective separation as a part od new pesticides testing and production; (enantio-)selective pesticide residue analysis that plays the key role in the assessment of the environmental fate of (chiral) pesticides; trace and ultra-trace (enantio-) specific HPLC analysis in environmental samples (air particulates, soil, water, sediment) where humic substances are frequently acting as serious interferents. The last mentioned item is especially interesting and studied by the staff of the Laboratory of Liquid Chromatography, Department of Analytical Chemistry, FNS CU in Bratislava.

4. Conclusions

Future trends in analysis of complex multi-component mixtures of residual pesticides at trace and ultra-trace concentration levels in complex matrices (soil, biota etc.) will benefit not only from development of combined techniques (e.g. column-switching HPLC–DLLS-NMR-MS, combination of chromatographic and electro-separation methods with MS, enantio-selective and sensitive detection etc.), but also from much deeper knowledge about humic substances and their behavior under various conditions.

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On-site and Off-site Cleaning of Heavy Metal Polluted Soils by Soluble Humic Substances

O.K. Borggaard[a*], J.K. Jensen[a], M. Soleimani[b], S.B. Rasmussen[a], P.E. Holm[a]

[a]Department of Basic Sciences and Environment, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg, Denmark; [b]Department of Soil Science, Isfahan University of Technology, Isfahan, 84156-83111, Iran
E-mail: okb@life.ku.dk

1. Introduction

Thousands of soils in Denmark and other countries need cleaning because they are polluted with toxic heavy metals [1-4]. Since heavy metals are non-degradable, they must be removed. For moderately polluted soils, this can be done on-site by phytoextraction, i.e. uptake by plants that are harvested, further treated or disposed. Strongly polluted soils must be excavated and cleaned off-site in a soil deposit by soil washing [2-6]. To speed up on-site as well as off-site cleaning, a strong heavy metal complexing agent (chelant), often EDTA, is used with both methods. However, although EDTA effectively promotes the cleaning, it is persistent, xenobiotic and toxic and should be replaced by natural, nontoxic compounds such as soluble humic substances (HS) [1-6]. The possibility of replacing EDTA by HS as phytoextraction stimulating and soil washing promoting chelant is tested in this paper.

2. Materials and Methods

The capacities of HS and EDTA to mobilize heavy metals (Cd, Cu, Ni, Pb, Zn) from more or less polluted calcareous and non-calcareous soils were compared using multi-step and single-step batch extractions [1-3]. HS from various sources (forest litter, compost percolate and processed cow slurry) was tested and the concentrations of HS and EDTA expressed as dissolved organic carbon (DOC) ranged from12.5 to 100 mM DOC. The influence of HS on heavy metal uptake by willow (*Salix viminalis*) is under investigation by means of big field lysimeters containing 5.5 Mg (tonnes) polluted soils.

3. Results and Discussion

Even though increased heavy metal solubility is the aim of using a chelant both to enhance phytoextraction and to improve soil washing, the desired increases are different. In the off-site soil washing as high as possible solubility (without destroying the soil) is the goal, whereas an increased solubility that stimulates the plant uptake without increasing leaching is the aim of using a chelant in the on-site enhanced phytoextraction. As heavy metal solubility increases at increasing chelant concentration (Table 1), a high chelant concentration will therefore be optimal for soil washing, whereas enhanced phytoextraction requires a moderate, balanced concentration that increases plant uptake but not leaching.

Table 1: Influence of chelant concentration as mM DOC on amounts of Cd, Cu and Pb in percent of total metal contents that are extracted by EDTA and HS in 10 extractions from calcareous and non-calcareous soils

Extractant (conc.)	**% Cd**	**% Cu**	**% Pb**
Calc. soil, pH 8.0; totally 15.6 mg Cd/kg, 1000 mg Cu/kg, 500 mg Pb/kg			
EDTA (25)	72	53	25
EDTA (50)	81	65	27
EDTA (100)	87	77	32
[a]HS (25)	24	41	3
[a]HS (50)	38	54	4
[a]HS (100)	44	53	4
Non-calc. soil, pH 5.8; totally 73 mg Cu/kg, 12 mg Pb/kg			
[b]HS (25)	-	34-39	34-37

[a] From processed cow slurry.
[b] From beech litter, spruce litter and processed cow slurry.

Apart from the concentration, extracted heavy metals depend on the chelant and the soil characteristics (Table 1). The substantial efficiency of EDTA to extract heavy metals is in agreement with many previous investigations [2-6]. However, although HS is less efficient than EDTA, this natural chelant extracts substantial amounts of Cd and Cu but also of Pb from the non-calcareous soil. In contrast, HS seems unsuited for extraction of Pb from calcareous soil (Table 1). The origin of HS appears unimportant as similar percentages of Cu and Pb are seen to be extracted by HS from forest litter (beech and spruce) and processed cow slurry (Table 1). Furthermore, similar amounts of Cd, Cu and Pb were extracted after one extraction with a solution to soil ratio of 100 L/kg as by 10 successive extractions with a solution:soil ratio = 10 L/kg [3]. Accordingly, single-step extraction seems as effective as multi-step extraction, which is of substantial practical (labour-saving) importance in soil washing but disagrees with previous findings [4].

These results indicate that HS may replace EDTA as soil washing agent when cleaning non-calcareous soils polluted with Cd, Cu, Pb and probably other heavy metals. HS can also be used for cleaning calcareous soils polluted with Cd, Cu and maybe other heavy metals, whereas Pb requires stronger chelants than HS [3].

The suitability of using HS instead of EDTA to enhance phytoextraction was tested on two calcareous soils; Soil 1 was from a field strongly polluted with heavy metal and Soil 2 from a moderately polluted field [1]. Growth performance of willow has been determined on these soils showing severely reduced growth on Soil 1 but unrestricted growth on Soil 2 [7]. The releases of Cu and Zn were determined by multi-step batch extraction, which was considered to simulate repeated additions of the stimulating (accelerating) chelant in enhanced phytoextraction. The accumulated releases of Cu from Soil 2 by addition of 25 mM DOC as EDTA or HS from compost percolate are shown in Fig. 1. EDTA extracts considerably more

Cu than HS and the shapes of the extraction curves are different. The EDTA curve is convex and the first step extracted nearly 50 % of the amount of Cu extracted during the 10 extractions, whereas the HS curve is straight-lined. Extraction curves with similar shapes were found for Zn.

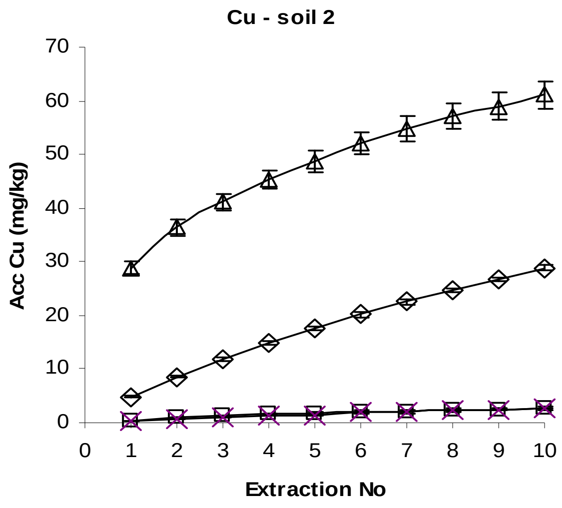

Figure 1: Accumulated Cu extracted from Soil 2 by 25 mM DOC as EDTA (Δ), HS from compost percolate (◊) and control, 0.05 M KNO_3 (□). Solution:soil ratio 10 L/kg. Vertical bars denote SDs.

The release of Cu in a concentration of 3 mg/L (solution to soil ratio = 10 L/kg) as in the first extraction is toxic for many plants [1]. Furthermore, leaching of Cu in such a high concentration will cause groundwater pollution, which is especially problematic in Denmark and other countries using untreated groundwater as drinking water. Thus, the Danish quality criterion for drinking water is 0.1 mg Cu/L [7]. In addition, EDTA is toxic in itself and persistent [1,4,6]. Therefore, the use of EDTA may very much be considered 'a replacement of one pollutant with another pollutant' [7].

The mobilization of Cu by HS is more modest compared to EDTA. Each step, releases about 0.29 mg Cu/L (Fig. 1) and 0.19 mg Zn/L [1]. Such low concentrations are harmless to the plants and soil fauna, and will not threaten groundwater quality if leached [1]. Furthermore, in contrast to EDTA, HS improves seed germination and plant growth [1,6] indicating that replacement of EDTA by HS is environmentally friendly. On the other hand, although HS is considered to enhance phytorextraction due to these moderate increases of the heavy metal solution concentrations [1], this has not yet been confirmed. In fact, a pot experiment with a salt tolerant plant (*Paspalum vaginatum*) showed no significant increased uptake of Cu and Zn from polluted marine sediment by application of HS from vermicompost [6]. In order to get reliable knowledge about the effect of HS on phytoextraction, we have initiated a long-term experiment with large field lysimeters, each containing 5.5 Mg (tonnes) of Soil 1 or Soil 2, where the effect of compost HS on heavy metal leaching and uptake in willow is investigated (monitored). So far, willow has been shown to decrease percolating water and heavy metal leaching as expected [7] but no significantly increased uptake of heavy metals in plant leaves and twigs has been found. However, HS has only been applied during one growth season (May-October) and the amount added corresponds to a solution to soil ratio of only 0.2 L/kg, which is much lower than the ratio of 10 L/kg used in the laboratory test (Fig. 1).

4. Conclusions

The comparison of EDTA and HS has indicated that HS may replace EDTA as soil washing agent in off-site cleaning of non-calcareous soils contaminated with heavy metals such as Cd, Cu, Ni, Pb and Zn, whereas the efficiency of HS to clean calcareous Pb-polluted soils is very limited. The possibility of using HS instead of EDTA to enhance on-site phytoextraction of Cu and Zn from polluted calcareous soils is likely according to a laboratory test but the result needs be tested in the field. Replacement of the synthetic, persistent and toxic EDTA with natural HS in off-site and on-site cleaning of heavy metal polluted soils would be an important environmental achievement.

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SESSION 5: INDUSTRIAL PRODUCTION AND COMMERCIAL APPLICATIONS

Humic Nanoparticles versus Manufactured Nanoparticles in Soil Remediation

Nicola Senesi
Department of Agroforestal and Environmental Biology and Chemistry,
University of Bari, Via Amendola, 165/A, 70126-Bari, Italy
E-mail: senesi@agr.uniba.it

In the first part of this presentation specific properties of humic substances (HS) which support their nanoparticle (NP) nature will be highlighted. In the second part, some comparative results on the adsorption efficiency for selected OPs of various types of soils added with either a compost rich in HS or with a selected NP will be discussed in detail.

HS are recognized to be the most widespread and ubiquitous components of natural nonliving organic matter (NOM) in the global environment. The estimated level of soil organic carbon (SOC) on the earth surface occurring as HS is 30 x 10^{14} Kg. In particular, approximately 60-70 % of the total SOC has been estimated to occur in HS. These substances consist of a physically and chemically heterogeneous mixture of relatively high-molecular weight, yellow to black organic compounds of mixed aliphatic and aromatic nature, formed by secondary synthesis reactions (humification) of products of the microbial and chemical decay and transformations of biomolecules released from organisms into the surrounding environment both during life and after death. The HS are universally recognized as the most relevant and chemically, biologically and physically active components of NOM thanks to their typical composition, macromolecular structure, polyfunctionality, surface properties, presence of multiple reactive sites, variable sizes and shapes, and intrinsic porosity. Further, several studies have demonstrated that HS are able to interact efficiently with various organic pollutants (OPs) in soil through various mechanisms and processes, among which the most important is adsorption, and that this action is increased markedly upon addition to soil of organic amendments rich in HS, such as composts. Nowadays, manufactured (or synthetic) nanoscale materials, or NPs, are increasingly used or proposed to be used for soil decontamination by adsorption/trapping of various OPs. This, thanks to their appropriate average dimensions (ranging from 1 to 100 nm), high porosity and hydrophobic surfaces. However, the ascertained toxicity to organisms of these engineered NPs is posing increasing serious concerns for human and environmental exposure. Based on the intrinsic chemical and physical properties of HS, including the average size (from about 1 nm to 1000 nm) and the typical cross-linked spatial networks containing highly-branched polymer chains exhibiting fractal features, HS can be qualified as natural NPs in the environment.

Four different soils with various content of SOC and different chemical properties have been considered, and adsorption of the polycyclic aromatic hydrocarbons (PAHs) pyrene and fenantrene, two very common OPs, has been measured comparatively on each original soil, and on the soil added with either 1% of a compost rich in HS or 5% of the NP fullerene. Results show that: (a) in any case, the adsorption data best fit into a Langmuir-type isotherm, which means that a maximum of adsorption, i.e., saturation, is reached by filling all available vacant sites; (b) as expected, the adsorption capacity for the two PAHs of the three soils, either in the absence or the presence of compost or fullerene, is a function of their original content in SOC; (c) for any soil, the values of adsorption distribution coefficients for pyrene and fenantrene, which are an indication of the adsorption capacity of the substrate for the PAHs, increase in the order: original soil<soil+fullerene<soil+compost. These results indicate that the content of native SOC is the most important factor influencing the adsorption capacity of soil for PAHs, even if additional powerful sorbents, such as compost or fullerene, are added. However, the addition of either a source of HS, such as compost, or an engineered NP, such as fullerene, is able to enhance at various extent the adsorption capacity of soil for PAHs. More important, the HS-rich compost appears more efficient than fullerene in enhancing the adsorption capacity of soil for pyrene. Thus, the compost amendment practice could be preferred to the application of engineered NPs to soil for OP remediation purposes.

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Effects of Brine Fulvic Acid on Plants Growth

Tadatomi Sato[a], Tatsuo Kaiho[a], Masahiro Kato[b]

[a]Kanto Natural Gas Development Co., Ltd., 661 Mobara, Mobara, Chiba 297-8550, Japan;
[b]Chiba Prefectural Agriculture and Forestry Research Center, 808 Daizen-no-cho, Midori-ku, Chiba 266-0006, Japan
E-mail: Tatsuo.Kaiho@gasukai.co.jp

1. Introduction

Brine Fulvic Acid (BFA) is obtained from fossil seawater called "Kan-Sui (KS)" in Chiba, Japan. The KS is drawn from the stratum to produce biogenic natural gas (NG) and iodine. KS contains approximately 50 mg/L of total organic carbon (TOC). The main component of TOC is allotted to BFA. Brine Fulvic Acid is structurally different from other fulvic acids (FA) such as weathered coal FA or peat FA. Brine Fulvic Acid has relatively a low content of aromatic moieties and a high content of aliphatic moieties. Characteristics differences of them may reflect the origin of resources [1]. As we reported at IHSS 2006, BFA has positive effects on the growth of plants (e.g. rice seedlings, peanuts, and tomatoes) [2]..We will report about bio stimulating activity of BFA on turfgrass, a new extraction method of BFA from KS and also their three-dimensional excitation emission matrix (3DEEM) spectra in this paper .

2. Materials and Methods

Preparation of BFA. The KS was pumped up from the wells, at depths from 200m to 2000m in Kujukuri, Chiba, Japan. After separation of NG and sand, KS was applied on the activated carbon column (AC) for desalination. BFA retained on AC was eluted with an aqueous alkaline solution and hot water. The mixed eluent was neutralized with phosphoric acid and then further concentration was conducted by using membrane filter to obtain 30,000 mg/L BFA solution. The 3DEEM spectra of BFA before and after extraction were measured.

Bio stimulating activity of BFA. Three seedlings of cool-season turfgrass "*Penncross*" (Shoot length: 15–20cm, root length: 5–9 cm) were transplanted to the individual plastic pot filled with sand. The grass was watered at 2 to 4 day intervals by hand throughout the experiment. Otsuka liquid fertilizer (NPK15-8-17%) was applied to all of the treatments over the duration of the experiment at one quarter the recommended rate (10g/L/m^{-2}) every two weeks. The twelve pots were divided into three groups of four pots represented a different treatment. The BFA was applied at weekly intervals at the rates of 25 ppm and 50 ppm. Control pots were also used which only received Otsuka fertilizer throughout the experiment. All of the products used were in liquid form.

3. Results and Discussion

Eighty days after the first treatment, the turfgrass samples were collected. Significantly higher dry weights were recorded with BFA treatment on the turfgrass in comparison of control. Leaf chlorophyll content (SPAD value) and numbers of shoots were also greater than control. The 3DEEM spectra of BFA and KS were essentially identical.

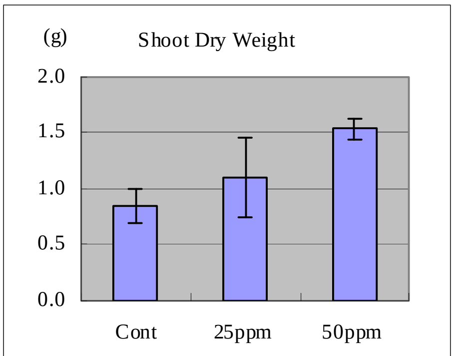

Fig 1

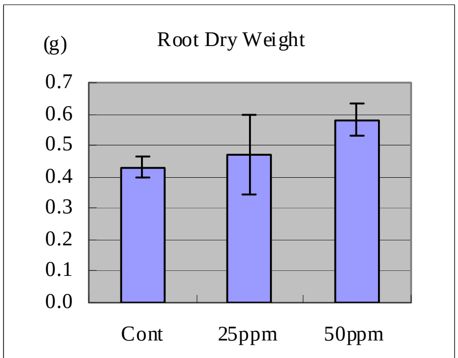

Fig 2

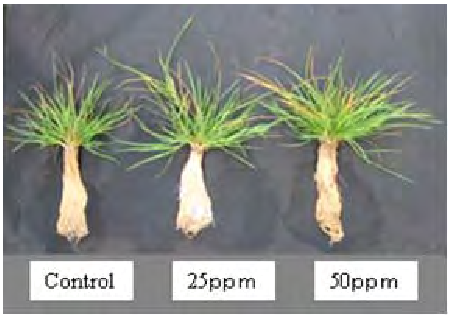

Fig 3

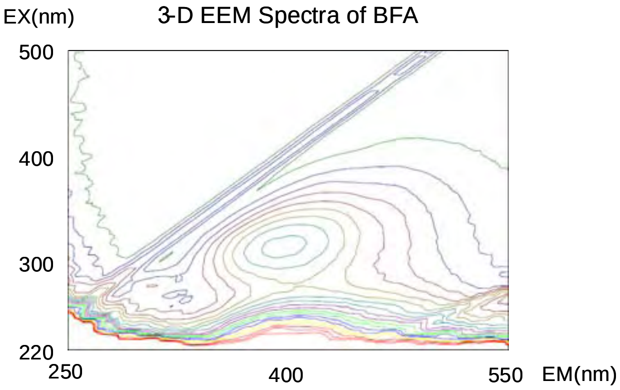

Fig 4

4. Conclusions

BFA has bio stimulating activity on turfgrass in the same manner with rice seedlings. BFA namely Kan-Sui FA is now on the market in Japan.

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Thermolysis of Lignin for Value-added Products

Paul de Wild[*], Ron van der Laan, Ruud Wilberink
Energy research Centre of the Netherlands (ECN), Westerduinweg 3, 1755 LE Petten,
The Netherlands
E-mail: dewild@ecn.nl

1. Introduction

Utilisation of lignin is growing due to an increasing interest in renewable raw materials. The development of second generation bio-fuels like bio-ethanol from ligno-cellulosic biomass will lead to another source of lignin and lignin containing residues (Fig. 1).

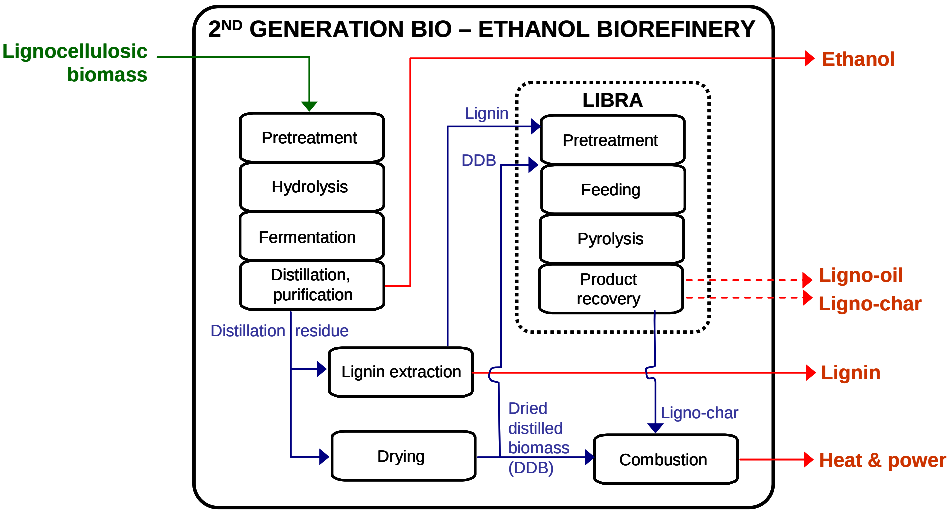

Figure 1: Lignin valorisation in a 2nd generation bio-ethanol biorefinery

Industrial lignin pyrolysis processes are rare, despite lignins potential as a valuable petrochemical substitution option for fuel, performance products (polymers) and individual chemicals. In literature on the pyrolysis of lignin for the production of chemicals, yields of mono-phenolic compounds are reported that rarely exceed 5–6 %, based on lignin [1–3]. Consequently, the main practised option to date for lignin is the use as a low-cost solid fuel for generating heat. To exploit the potential of lignin as a renewable feedstock for valuable materials, new conversion technologies are clearly needed. Currently, LIBRA, a Lignin Biorefinery Approach, is under development at ECN to valorise lignin into bio-phenols and bio-char. LIBRA aims to provide added-value to the whole lignocellulosic biorefinery. LIBRA is based upon a specific thermolysis procedure including the use of catalysts and dedicated product recovery. Primary target products are phenolic bio-oil and bio-char. Biochar might find applications as soil improver, growth enhancer and filtration material. The phenolic-oil is an interesting for e.g. bio-bitumen, phenol subtitutes in wood-resins and for several high-value phenolic compounds like guaiacols, syringols and alkylated phenols for pharmaceutical, food, and/or transportation fuel applications.

2. Materials and Methods

The deciduous lignin was prepared via the Alcell organosolv process from a mixture of hardwoods. The herbaceous lignin is produced by Asian Lignin Manufacturing (ALM) from soda-pulping of annual non-woody plants like wheat straw and Sarkanda grass. It is marketed by Granit SA, Switzerland. The third lignin is a lignin-rich residue from the production of bioethanol in a biorefinery. The original lignins were thermally characterised under N_2 by TGA with a Mettler Toledo TGA 850. For the pyrolysis experiments, the lignins were used as 1-3 mm particles. Continuous experiments were conducted using an atmospheric pressure, 1 kg/hr (maximum solid feed rate) bubbling fluidised bed test facility at 400 °C (Figure 2) featuring automated operation and data acquisition. Liquid and gaseous products were analysed using standard chemical analysis methods. Most organics were measured off-line with a TRACE-GC-ULTRA GC/MS with DSQ-II mass spectrometer. NDIR spectrometry (ABB-Advance Optima) was used for on-line monitoring of CO, CO_2, CH_4. Karl-Fischer titration was applied to analyse the water content in the liquid organic samples. The specific (BET) surface area and pore size distribution of the chars are measured by gas adsorption / desorption. To elucidate the pyrolysis-induced structural aspects of the lignin chars, the three lignins and their corresponding chars are going to be studied using ^{13}C-cross-polarization, magic-angle-spinning (CP/MAS) NMR measurements and scanning electron microscopy (SEM). All CP/MAS spectra are recorded on a Chemagnetics CMX-300. Electron microscopy is conducted with a JEOL spectrometer.

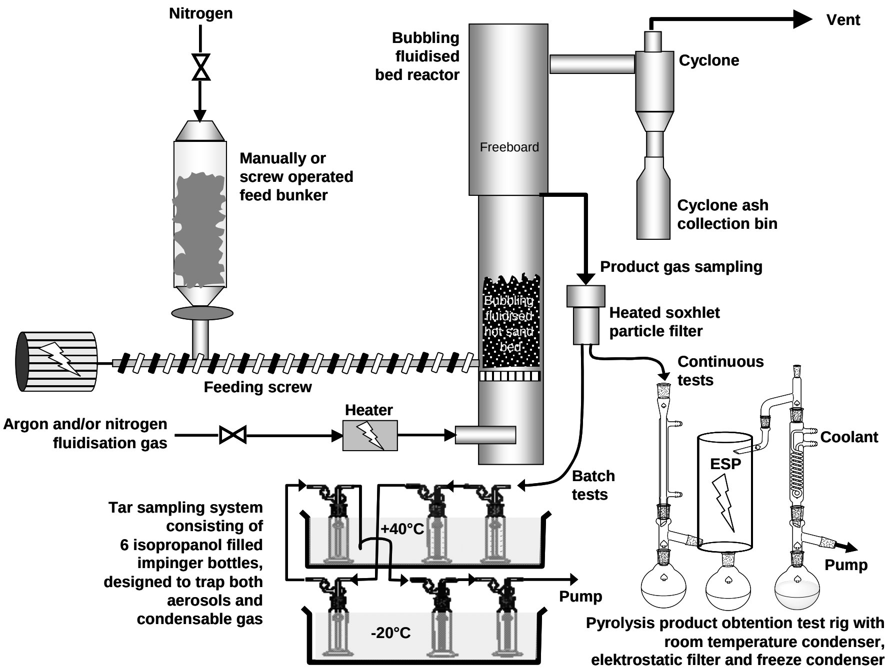

Figure 2: Experimental set-up for the lignin pyrolysis tests

3. Results and Discussion

Chemical analysis of the three tested lignins revealed that the major difference between the three lignins is the ash content that is highest for the biorefinery material. Consequently, its C, H and O content and the heating value are lower when compared to the pure technical lignins. From TGA results it is deduced that the main weight loss starts at 200 °C for the Alcell lignin, at 150 °C for the GRANIT lignin and at 170 °C for the biorefinery lignin. It levels out at 500–600 °C leaving behind char with a residual weight of 40%–50% of the original amount. The temperature at which the thermal degradation under isothermal conditions is maximal is around 350–360 °C. Contrary to the technical lignins Alcell and Granit, the biorefinery lignin shows a rather narrow thermal stability window. From the thermal characterisation results a temperature of 400 °C was considered to be suitable for the subsequent pyrolysis trials. After some batch trials, successful continuous pyrolysis experiments were carried out with the three lignins at a solid feed rate of approximately 100 –200 g/h. The experiments were deliberately stopped after 2-3 hours because of char build-up in the reactor bed. The Table below presents the main results. The identified phenols constitute half of the total amount of GC-detectable phenols. For Alcell they consist of syringols with lesser amounts of guaiacols, alkylated phenols and catechols. For Granit and the biorefinery residue the main phenols are guaiacols and alkylated phenols with minor contributions from syringols and catechols.

Products (wt% of dry feedstock)	Alcell	Granit	Biorefinery residue
Gases, total	13	18	9
CO_2	6	8	7
CO	4	6	2
CH_4	3	4	0.3
Bio-oil, total	37	42	40
Monomeric phenols	6	10	7
Oligomeric phenols	10	11	13
Other components ("light ends")	1	0.3	1
Water	13	21	9
Char + ash	44	30	53
Balance (%)	94	90	102

Gas-adsorption measurements with the chars revealed BET areas of 20–200 m^2/g. Determination of pore size distribution is underway. Structural characterisation experiments by NMR and SEM are underway and first results will be presented at the congress.

The phenolic fraction is under evaluation for conversion to transportation fuels [4], for separation of individual phenols, for use in wood-adhesives and for use as additive in petrochemical bitumen. The bio-char is studied as a possible soil improver and as a feedstock material to produce activated carbon for gas- and water-filtration applications.

4. Conclusions

Three lignins derived from hardwoods by the Alcell organosolv process, from grass and straw by soda pulping and from annual plants as a raw residue from the production of bio-ethanol have been characterised by various physico-chemical characterisation methods whereafter they were processed according to LIBRA, a pyrolysis-based Lignin Biorefinery Approach to investigate their potential as renewable feedstocks for valuable products. Lignin can be valorised in bio-char (40%) and bio-oil (40%). The bio-oil contains 16–21 wt% (d.b.) of a phenolic fraction constituting of monomeric (6–10%) and oligomeric (10–13%) compounds. LIBRA clearly indicates the potential for pyrolysis to valorise lignin.

Acknowledgements

Alcell and Granit lignin were respectively provided by Dr. R. Gosselink from A&F – Wageningen University Research and Prof. A.V. Bridgwater of Aston University, UK. A global biorefinery developer is gratefully acknowledged for their provision of a test sample of a typical bioethanol-based biorefinery lignin residue.

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Field Evaluations of Humic Products in Crop Production: a Partial Review

Daniel C. Olk[a], Yasser Dergham[b], David J. Dunn[c], Saad A. El Shall[d], Michael M. Kenty[e], Shaban A. Kotob[f], Stuart Lebo[g], Craig Muma[h], Michael Raske[i], Brett Rhodes[j], J. Rene Scoresby[k], Daman S. Walia[f], and Gibbs Wilson[h]

[a]USDA-ARS, Ames, IA, USA; [b]Humintech Company, Düsseldorf, Germany; [c]Univ. of Missouri, Portageville, MO, USA; [d]Agricultural Research Center, Giza, Egypt; [e]Helena Chemical Co., Collierville, TN, USA; [f]ARCTECH, Inc, Chantilly, VA, USA; [g]LignoTech USA, Rothschild, WI, USA; [h]Horizon Ag Products, Modesto, CA, USA; [i]Innovative Crop Solutions, Radcliffe, IA, USA; [j]Ag Logic, Iowa Falls, IA, USA; [k]Green Light /Valent USA, Mosinee, WI, USA
E-mail: dan.olk@ars.usda.gov

1. Introduction

Humic products in liquid or solid forms are made by extraction of a range of natural materials, including lignite and composted plant residues. They have been used as a plant growth supplement for many decades. Yet their field efficacy is hotly debated within the research and farming communities. One reason for this uncertainty is that virtually no documentation of their field efficacy exists in the peer-reviewed literature. Manufacturers of humic products have conducted extensive field evaluations of their products; however their findings have not been widely disseminated.

To address this paucity of information, a session entitled, “Microbial and Humic Amendments: Advances in Understanding their Effects on Soils and Plants” was held at the 2009 annual meeting of the Soil Science Society of America / American Society of Agronomy in November 2009 in Pittsburgh, PA, USA. Presentations by industry representatives and university researchers reported the effects of humic products on crop growth and economic yield for a wide range of crops in five continents. This presentation is a partial summary of that session.

2. Results and Discussion

Nearly all field evaluations of humic products have been single experiments that were not repeated at multiple locations with exactly the same treatments and management practices. To some degree this trend results from the desire to evaluate different humic products in different environments and sometimes at different application rates. An additional factor in the U.S. is the frequent need to obtain state-by-state certification of a humic product: many states require one successful field evaluation to be conducted within their boundaries. The number of field replicates has varied among the field studies, but in most cases there were four or fewer. A large number of studies were statistically evaluated, but many were not.

We have conducted single site field studies in all regions of the U.S. Overseas, we conducted studies in Canada, Egypt, France, Germany, India, Iraq, Italy, and the United Kingdom. Crops included apricot, broccoli, celery, clover, kiwi, maize (corn), mango, melon, orange peach, pear, pepper, potato, rice, sorghum, soybean, strawberry, sugar beet, sugar cane, tomato, and wheat. Economic yield was numerically greater in the majority of cases. For example, LignoTech USA found an increase in harvestable yield in 33 of 38 cases for 12 crops grown in eight U.S. states and five other nations. Mean yield increase was 7%. Similarly, Helena Chemical together with Horizon Ag also found positive responses in the vast majority of many field trials throughout the U.S. for a range of crops. Several companies determined the benefits of their humic product to plant traits in addition to economic yield, especially plant biomass and nutrient uptake. Further, ArcTech measured fruit number, shoot length, leaf number, leaf area, leaf chlorophyll content, and root growth for apricot trees. Humintech measured fruit size, leaf greenness, and plant height. Lignotech measured tuber number, stem number, and size class distribution for potato, ear number for maize, and size class distribution for celery.

Although soil type affects efficacy of fertilizers and pesticides and hence also seems a likely factor of humic product efficacy, only a few studies investigated soil type systemically. In one example, a Horizon Ag product was shown to significantly (P<0.0001) improve rice grain yield in Missouri, and this benefit did not differ between two nearby fields that differed substantially in their availability of soil P and K.

Second, in a 2.5-hectare maize field in Iowa, the grain yield response to a humic product of Innovative Crop Solutions varied with soil type in 2009. The yield response was positive in well-drained fertile soils covering half of the field, but in a yet more fertile, low-lying soil that covered most of the remaining field, yield responses were muted or in cases negative. This product was also applied to maize in about 120 farmers' fields at points scattered across Iowa. For a subset of 30 farms where plants were hand-sampled shortly before harvest, 25 farms reported a positive yield response, with a mean response of about 650 kg ha^{-1} (Fig. 1), which was significant (P<0.001). Grain yield as measured by combine was determined for most of the 120 farms. About 70% had a numerically positive increase in grain yield with product application, with a mean increase of about 450 kg ha^{-1}. Several of the fields lacking a positive response were located in one locale having particularly fertile soils, and the product was applied there at a crop growth stage that is not recommended by the manufacturer. The few no-tilled farms in this survey had yet higher grain yields and grain yield responses to product application than did tilled farms. These and other initial trends with crop management practices and soil type will be further evaluated in future years.

Only a few of our studies attempted to identify the mechanism for the humic product benefit to crop growth. In arid soils of Egypt, application of an ArcTech product resulted in increased resistance of deciduous fruit trees to soil salinity, as measured through decreased Na uptake even with greater uptake of other nutrients and larger crop biomass. In Italy, application of an Fe-humate product by Humintech corrected an Fe deficiency in kiwi nearly as well as did application of an Fe chelator. LignoTech USA noted greater diversity of soil microbial species with application of their product. A selected number of the Iowa maize fields that received the product of Innovative Crop Solutions were monitored for duration of grain-filling. It was found to be lengthened through (i) an earlier onset at pollination, and (ii) a delayed end. Together with a transient increase in stem internodal length at mid-season, these trends suggest a hormonal-like stimulation of crop growth.

3. Conclusions

In the vast majority of field trials, humic products improved crop growth. At times the yield boost was substantial, although more often it was 10% or less. Even such modest returns provided a viable economic return, because application rates of the humic products are low. Modest yield boosts likely contribute to the mixed results of previous field evaluations reported previously in conference proceedings and university extension bulletins. Many of our field studies were conducted in low-carbon soils, yet a positive effect was also found in the high-carbon soils of Iowa.

The statistical significance of the yield boost would be more easily proven if the number of field replications were greater than has been the case. Yet this step would increase the cost of field evaluations, with no direct benefit to the manufacturer. Mean yield boost could also be strengthened if unfavorable testing sites were identified and excluded from evaluation, much as these products should be marketed. However, our knowledge of humic product efficacy does not yet provide an adequate basis for predicting the magnitude of product response for specific crops, soil types, and land use (e.g., previous crops, fallow), and for weather patterns unique to each growing season.

Currently, there is no published information on the mechanisms through which humic products promote plant growth in field conditions. This information is viewed by many humic product manufacturers as having limited value to the marketing of their products and not worth the substantial research costs necessary to determine mechanisms. Yet a mechanistic understanding would likely enhance our capability to predict crop yield response under specific conditions, and it might facilitate product certification.

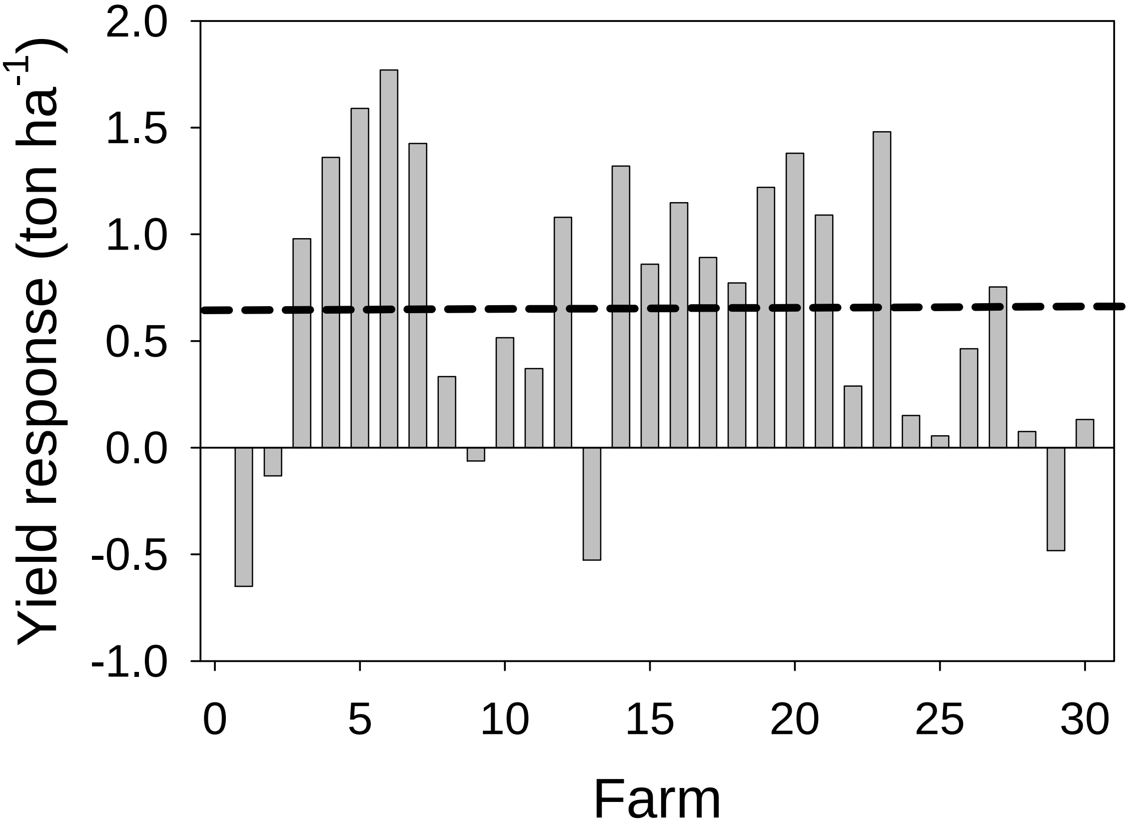

Figure 1: Response of maize grain yield to humic product application for 30 fields in Iowa. Data were based on grain mass of eight plant samples per plot. Grain weight was extrapolated to a hectare basis by assuming a population of 74,000 plants ha^{-1} for all fields. Mean yield response is indicated as a dotted line

Evaluation of Microbioreactors in the Stabilization of Different Kinds of Organic Residues for Organic Fertilizers Production

Paulo R. Dores-Silva*, Maria D. Landgraf, Maria O. O. Rezende
Instituto de Química de São Carlos. Av. Trabalhador Sãocarlense, 400, São Carlos, SP, Brazil, CEP 13566-590
E-mail: tortuga@iqsc.usp.br

1. Introduction

The transformation of the organic matter (OM), a result of the combined action of earthworms and the microflora which lives in its digestion treat, is defined as vermicomposting [1,4].

The earthworms ingest and digest the organic residues dejecting the excrement in a special form, made of ground aggregates and organic matter, named as coprolite. The coprolites have nutrients in high concentration due to the earthworm metabolism. The slurry material is in a more advanced decomposition form, ie, of humification, and easy assimilation by the roots. The production of vermicompost is accelerated due to the digestion of cellulose and semi-decomposition caused by worms that work symbiotically with microorganisms, acting as a kind of microbioreator. This strengthens their ability to reduce organic matter and production of raw material humified. [5,6] .

Due to the necessity of providing a suitable target to organic residues, this study aims to verify the change in physical and chemical parameters promoted by the use of worms (microbioreators) to stabilize the various organic residues.

2. Materials and Methods

For the production of vermicompost three boxes of plywood, enclosed but not sealed, were used. For each experiment it was used: cattle manure (CM); cattle manure + rice straw (RS) in a 1:1 ratio by volume (RS); sugar cane bagasse + cattle manure in a 1:1 ratio by volume (SC) and vegetable waste from hydroponics + cattle manure in a 1:1 ratio by volume (VW), for a total of 12 boxes, with dimensions of 0.70 m width, 0.70 m long and 0.70 m in height each. The residues for the study were put inside these boxes, keeping the humidity between 40 to 60% and temperature between 20 and 30°C.

The first collection was made after careful homogenization of the mixtures prepared in the boxes. The inoculation of the earthworm *Eisenia foetida* was made with the implementation of 1000 worms per m^3 [5]. After an interval of 90 days after the inoculation, the vermicompost collection was done to perform the physicochemical analyses, in accordance to the procedures described in Kiehl (1985) [5].

The physicochemical characterization of the initial samples (blank) and vermicompost produced after 3 months of vermicomposting was done, according to the following parameters: pH in $CaCl_2$, phosphorus and total Kjeldahl nitrogen (TKN) by spectrophotometer Hach, method 480 and 399, respectively , total organic carbon (TOC) via TOC-V COH Shimadzu, cation exchange capacity (CEC), by occupying the active sites to exchange with hydrogen ions in 1 mol L^{-1} glacial acetic acid and OM content, gravimetrically.

3. Results and Discussion

Table 1 shows the physicochemical characterization results of the initial sample (blank) and of the so described produced vermicomposts.

Table 1: Physicochemical characterization of the initial sample (blank) and of the produced vermicomposts (standard deviations for *n* = 3)

Parameters	**OM mg kg^{-1}**	**P mg kg^{-1}**	**TKN mg kg^{-1}**	**TOC mg kg^{-1}**	**C/N**	**CEC**	**pH**
CMI	329800 (28900)	887 (43.87)	9884 (284)	184300 (4076)	22.09 (1.02)	19.45 (0.87)	8.98 (0.03)
CMV	274600 (1.09)	890 (64.80)	11200 (842)	113100 (2340)	11.69 (0.54)	24.64 (0.22)	8.43 (0.03)
RSI	635700 (79800)	519 (19.80)	11490 (254.56)	403400 (47200)	41.06 (5.59)	29.92 (0.26)	8.93 (0.04)
RSV	541000 (17500)	541 (34.63)	10550 (106.07)	318300 (13300)	25.55 (1.37)	32.16 (0.57)	8.29 (0.01)
SCI	513300 (32900)	544 (14.89)	6450 (318.24)	301900 (19400)	55.62 (6.19)	23.53 (0.79)	7.73 (0.07)
SCV	455600 (12000)	592 (44.23)	9330 (84.85)	268000 (11500)	33.55 (2.10)	29.55 (0.74)	7.13 (0.06)
VWI	540200 (31200)	931 (41.35)	10320 (84.94)	317700 (18400)	35.92 (2.05)	32.65 (0.40)	8.97 (0.02)
VWV	451200 (41600)	1106 (74.28)	14880 (96.34)	265400 (24500)	20.81 (1.60)	35.55 (0.25)	8.55 (0.03)

CMI = Cattle manure initial
CMV = Cattle manure vermicomposted
RSI = Rice straw + cattle manure initial
RSV = Rice straw + cattle manure vermicomposted
SCI = sugar cane bagasse + cattle manure initial
SCV = sugar cane bagasse + cattle manure vermicomposted
VWI = vegetable waste from hydroponics + cattle manure initial
VWV = vegetable waste from hydroponics + cattle manure vermicomposted

As it is observed, there was a decrease in the OM content in the experiments when they are compared to the original matrix, this occurs because, concomitantly to the degradation process promoted by the existent bacterial population in the initial matrix, the earthworms feed on the organic matter and, besides the carbon incorporation promoted by the earthworms, the mineralization process of the organic carbon to CO_2 is occurring simultaneously.

Analyzing the Table 1 it is possible to notice a slightly decrease on pH, then a decrease in the basicity of the matrices. From the earthworms esophagus some small amounts of calcareous are released, the calcifer glands that secrete calcium carbonate, which controls the content of this material in the body of the animal. The CO_2 produced by breathing is eliminated along with the excess of absorbed calcium from the soil to form $CaCO_3$, which is released to the outside along with undigested particles in the form of excrement. This explains the lower pH of the vermicomposting when it is compared to the initial pH of the matrices [3].

The TKN and P quantities increased during the vermicomposting process, all of them are larger than those found in the initial matrices. The increase in TKN and P enhances the fertilizer capacity of the final vermicompost, as nitrogen and phosphorus are two essential nutrients for growth and development of plants. According to Atiyeh et al., [2], the increase of nutrients such as nitrogen and phosphorus is directly related to the mineralization of organic matter by earthworms.

Looking at Table 1, the C content decreases after the vermicomposting process, as a behavior observed for all studied matrices, which means that the process of vermicomposting is effective in C removal.

C shows a decrease more intense in the CMV with a removal percentage of 38.63%, which is related to the lowest C/N ratio in the CMI, the other residues have a higher C/N ratio due to the presence of organic matter poor in nitrogen and rich in carbon (lignine), what explains the lowest reduction percentage obtained from the produced vermicomposts from these residues.

The CEC showed an increase trend after the vermicomposting process. This occurs because of the organic colloids from the earthworms humus have a greater ability to adsorb (bind) cations in soil solution, being able to release then or to make changes, if there is a concentration of different ions or a pH change.

The values for the C/N ratio showed a decrease during the vermicomposting process, this occurs due to the observed reduction of C and to the increase of TKN quantity. The speed-up in the humification process, promoted by the earthworms during the vermicomposting, result in a reduction of C/N ratio [2].

Counting the fact of the excellent adaptation of the earthworms in the matrices, the vermicomposting process is presented as an ideal process to be used in the stabilization of the studied residues, because, in the end of the process, besides obtaining an organic fertilizer with a high quantity of nutrients, it provides another product which presents a commercial value: - the earthworms.

4. Conclusions

The obtained results show that the vermicomposting process is effective in the stabilization of organic matter, being able to be used for the organic fertilizers production, recycling the generated wastes from farming activities and ranching, increasing the TKN and P contents, the CEC when it is compared to the initial matrix, reducing the C/N ratio to acceptable values for organic fertilizers and making the pH values near to the neutrality, providing a sustainable alternative and environmentally adequate final disposal of the studied organic residues.

Acknowledgements

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Extraction of Humic Substances from Composts Using Different Alkaline Solvents

M. Elisabete F. Silva[a], Víctor Guitán[a], Luís Lemos[a], Olga Nunes[b],
Ana Cristina Cunha-Queda[c*]

[a] Departamento de Ambiente, Escola Superior de Tecnologia e Gestão de Viseu, Campus Politécnico de Repeses, 3504-510 Viseu, Portugal; [b]LEPAE-Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Porto, Portugal; [c]Instituto Superior de Agronomia, UIQA, TULisbon, Tapada da Ajuda 1349-017 Lisboa, Portugal
E-mail: crisqueda@isa.utl.pt

1. Introduction

Composting is a useful method to produce stabilized and mature organic material, rich in humic substances, which may contributes to the essential functions of global soil fertility and health. In particular, the role of humic acids is emphasized, for example, their addition to a soil has a positive effect on the assimilation of nutrients by plants and microorganisms. So, there is a growing tendency to use humic substances as liquid organic amendment. Depending on the extraction method, these organic amendments can be applied to the soil as sodium or potassium humate. Usually the extraction of humic substances is carried out with sodium pyrophosphate, however the resulting humate shows high concentration of sodium, which can cause problems for plant growth. In this work a comparative study of the humic substances extracted using two different alkaline solvents as sodium pyrophosphate and potassium pyrophosphate was carried out.

2. Materials and Methods

Compost samples produced from organic materials after 240 days (C1) and 270 days (C2) of composting process were used to extract humic substances (HS). HS were evaluated according to methodology describe in the Spanish official method for the analysis of fertilizing organic products [R.D. 1110/12 July 1991]. HS were extracted simultaneously with 0.1 M sodium pyrophosphate and 0.1 M potassium pyrophosphate. This methodology allowed the separation of several fractions: the humic substances (HS), the humic acids (HA) and fulvic acids (FA). In order to remove interfering compounds the HA and FA fractions were further a purified by dialysis [1]. Both HA and FA were reported as % of carbon. The E4/E6 ratio was obtained by spectrophotometry (Jenway 6300 Spectrophotometer) using extracts of 3.0 mg of HA or FA in 10 mL of 0.05 mol L^{-1} $NaHCO_3$. The ratio of absorvance at 465 nm and 665 nm gave the E4/E6 ratio. FTIR spectra were recorded on a Bruker Tensor 27 spectrophotometer using KBr pellets (2 mg sample:200 mg KBr).

3. Results and Discussion

On table 1 are showed the results obtained for the characterization of humic substances extracted from the different composts.

Table 1: Data from chemical analysis of humic substances from compost in day 240 (C1) and day 270 (C2)

Compost	**Alkaline solvent 0.1 M**	**HA (%)**	**FA (%)**	**E4/E6 ratio**	
				HA	FA
C1	$Na_4P_2O_7$	3.0	6.8	nd	nd
	$K_4P_2O_7$	2.5	7.2	nd	nd
C2	$Na_4P_2O_7$	3.4	5.4	5.0	7.2
	$K_4P_2O_7$	3.4	6.2	6.0	7.3

nd – not determined

Values of carbon content for FA extracted with potassium pyrophosphate were higher than those obtained using sodium pyrophosphate. However, the carbon content for the HA was similar for both solvents. The total amount of HA increased with time of composting, but the amount of FA decresead. The absorbance ratio E4/E6 of the humic substances is a tradicional parameter used to estimate the degree of humification and/or the molecular size [2]. Results of E4/E6 ratio are similar for both HA and FA obtained.

FTIR spectra (figure 1) of FA or HA were similar irrespectively of the solvent used for extraction, indicating they present the same functional groups. The broadband at around 3400 cm^{-1} can be attributed to the stretching vibration of bandes OH groups, while peaks at 2927 and 2854 cm^{-1} are attributed to aliphatic methylene groups. The band at 1640 cm^{-1} can be related with aromatic rings, while the bands at 1510-1550 cm^{-1} are attributed to amides. Phosphate absorbs between 600 and 500 cm^{-1}. Peak at 1384 cm^{-1} is assigned to nitrate [3].

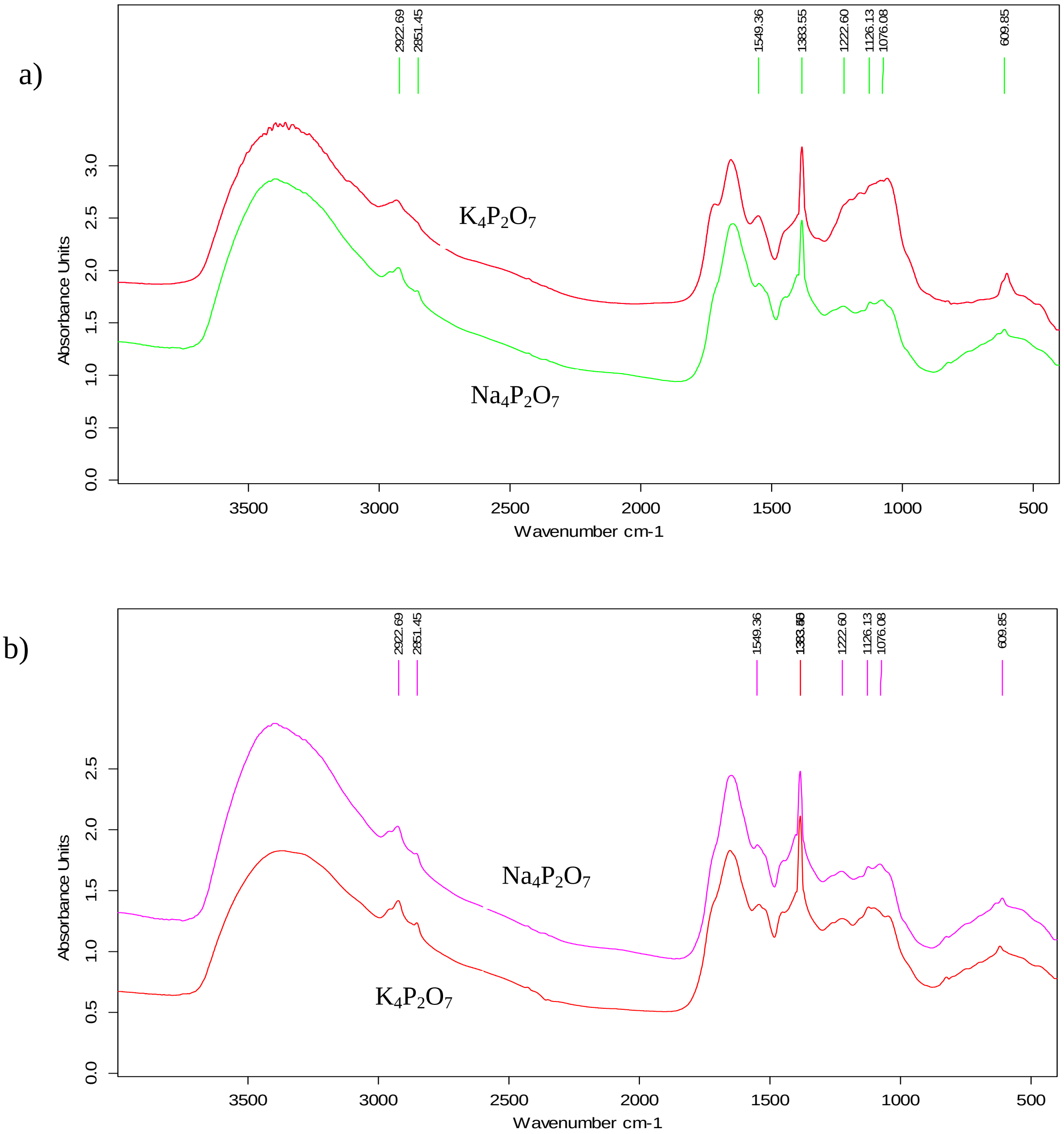

Figure 1: FTIR spectra of fulvic acids (a) and humic acids (b) extract with 0.1 M sodium pyrophosphate ($Na_4P_2O_7$) and 0.1 M potassium pyrophosphate ($K_4P_2O_7$)

4. Conclusions

In this study it has been shown that humic substances extraction was similar for both alkaline solvents. This is an interesting result, as using potassium pyrophosphate the humates produced will present potassium, which will be more favorable in terms of crop fertilization than sodium humates.

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Fulvic Acid Production From Turkish Leonardites

Bekir Zühtü Uysal [a*], Yusuf Mert Sönmez[a], Duygu Uysal[a], Selahaddin Anaç[b], Duygu Öztan[a]

[a]Department of Chemical Engineering, Faculty of Engineering and Clean Energy Research and Application Center (CERAC), Gazi University, Maltepe, 06570 Ankara, Turkey; [b]Turkish Coal Enterprises, Hipodrom Cad., No:12, 06330 Ankara, Turkey
E-mail: bzuysal@gazi.edu.tr

Abstract

Fulvic acid was produced from three Turkish leonardites by employing a method of precipitation by ethyl alcohol solution. Yields were determined to be in the range between 4.1 and 7.8 g/kg dry leonardite. FT-IR analyses of the samples produced were made. Effect of ethyl alcohol concentration in the precipitating solutions was also investigated experimentally.

1. Introduction

Fulvic acid is a macromolecular polymer with a structure and characteristics that change along with its origins and humification processes [1]. Fulvic acids, like humic acid, occur naturally in water, soil and peat. They are produced by soil micro-organisms to result in the decomposition of plants, also known as humification. Fulvic acids and humic acids have been thought of as two distinct entities and their characteristics have been described accordingly. The molecular weights of fulvic acids are less than those of humic acids. Fulvic acids are generally known to be more oxygen-rich and carbon-poor than humic acids. Similarly to humic acids, fulvic acid contains many reactive functional groups, including carboxyls, hydroxyls, carbonyls, phenols, quinones and semiquinones. These reactive groups make fulvic acid an effective chelators of both mineral ions and heavy metals and provide antioxidant activity. Fulvic acids make also nutrients in the soil more accessible for assimilation by plants. They have been found to favorably affect seed germination and plant growth, as well as increase the number and length of roots of plants [2]. Fulvic acid from peat has also been used for the clinical treatment of diseases induced by damage of oxygenated free radicals, such as arthritis, cancer, ulcers and rheumatism disease [3]. Fulvic acid is regarded as an organic natural electrolyte that can balance, activate and energize the biological properties with organic materials it comes into contact [4].

Fulvic acid is rapidly being recognized as one of the key elements in many outstanding health and scientific breakthroughs. This research involves the experimental studies to produce fulvic acid from three Turkish Leonardites as a part of the efforts for industrial scale production of fulvic acid.

The method of purification of fulvic acid by XAD-8 resin adsorption and desorption followed by freeze-drying works very well in laboratory scale work [5, 6]. However, due to long times required by this method it may not be very practical in plant scale production. Moreover, our experience has shown that the overall efficiency may drop to about 35% for this method. Faster methods giving better efficiencies are preferred in industrial applications. Therefore, the method consisting of precipitation of fulvic acid by ethanol is employed in this research [7].

2. Materials and Methods

Leonardite samples from three different reserves, i.e. Husamlar, Soke and Mugla were used in the experiments. Following the usual method of extracting humic content by alkaline solution, acidification was performed in two steps for precipitating out heavy metals at pH between 5-8 and humic acid at a pH about 2. Ethyl alcohol solution was then added to the filtrate to make fulvic acid precipitate. Fulvic acid thus obtained was dried at 80^{o}C. The product was analyzed with FT-IR Spectroscopy and compared with IHSS 2S103F fulvic acid. Experiments were also performed to see the effect of the strength of ethyl alcohol solutions at the precipitation step of fulvic acid.

3. Results and Discussion

Amounts of fulvic acids produced from 100 g of Husamlar, Soke, and Mugla leonardites were 633 mg, 330 mg, and 369 mg, respectively. When the calculations were done regarding the moistures of samples, dry coal based contents of fulvic acids were 0.78% for Husamlar sample, 0.41% for Soke sample, 0.58% for Mugla sample. Figure 1 shows FT-IR spectra for fulvic acid obtained from Husamlar leonardite and pure sample obtained from the International Humic Substances Society. The agreement between them is quite satisfactory. These results also agree well with the FT-IR spectra reported by Kwon et al.[8] and Khanna et al.[9].

Five different concentrations of ethyl alcohol obtained with Hüsamlar leonardite in the aqueous solution at the fulvic acid precipitation step were also tested. The results are given in Figure 2. Considering the natural scatter of experimental data and the cost of ethyl alcohol one may conclude that it would be assuring and safe to employ a concentration in the range between 65 and 75%.

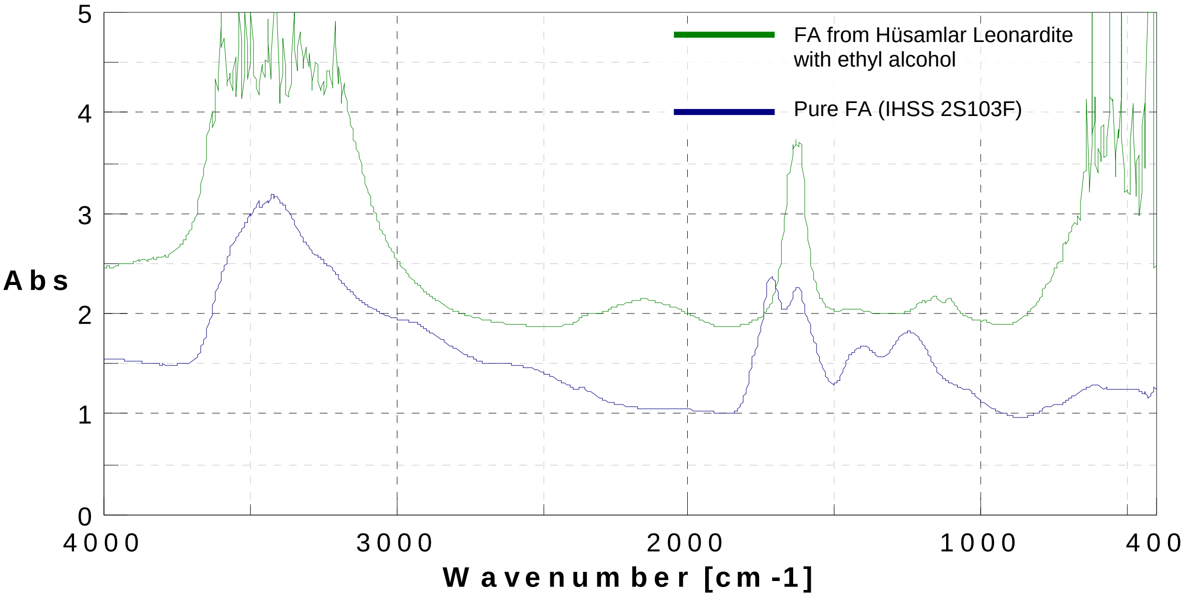

Figure.1: FT-IR comparison results for fulvic acid from Hüsamlar Leonardite and IHSS 2S103F pure fulvic acid

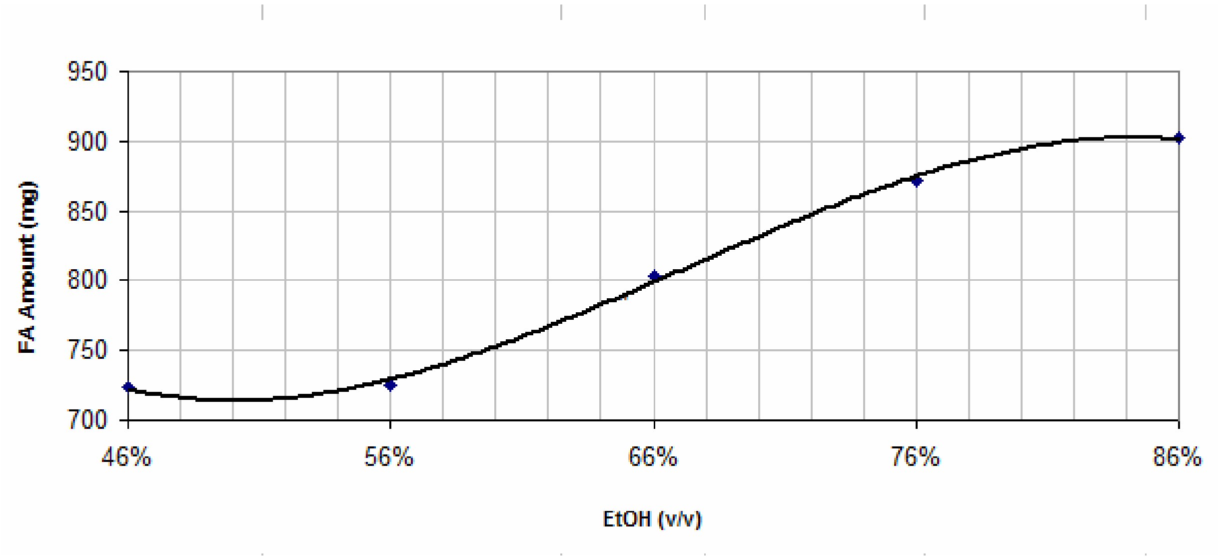

Figure.2: Effect of ethyl alcohol concentration in 100 ml of precipitating solutions

4. Conclusions

Three different Turkish leonardites were used to produce fulvic acid by the method of precipitation by ethyl alcohol. The yields for fulvic acid were found to be in the range of 4.1-7.8 g/kg dry leonardite. It is determined that it is suitable to employ an alcohol content between 65 and 75% in the solution precipitating fulvic acid.

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Ammonoxidation of Ligneous Materials and the Question of Nitrogen Binding

F. Liebner[a*], G. Pour[a], E. Brendler[b], A. Potthast[b], T. Rosenau[a]

[a]University of Natural Resources and Applied Life Sciences, Department of Organic Chemistry, Muthgasse 18, A-1190 Vienna, Austria; [b]TU Bergakademie Freiberg, Institute of Analytical Chemistry, Leipziger Straße 29, D-9599 Freiberg, Germany
E-mail: falk.liebner@boku.ac.at

Complementary to conversion of polysaccharides, utilization of lignin is becoming an integral part of modern biorefinery concepts, following the notion that simple burning for energy production – as widely practiced today – is not at all a sustainable solution. One of the promising application fields of lignins pertains to their use as soil-improving materials, which, as an example, can greatly contribute to combat the progressive desertification being one of the most alarming processes of environmental degradation.[1]

Ammonoxidation or oxidative ammonolysis, has been established as suitable chemical process for enriching lignin with nitrogen and for converting lignin into soil-improving materials, which were proven to have properties similar to natural humic substances.[2] The ammonoxidation of lignin thus represents an artificial humification procedure.

However, only little is known about the nitrogen binding forms of ammonoxidized lignins, and even nitrogen binding in natural humic substances is still a controversial issue in soil science and geochemistry. Contradictory to former conclusions from wet-chemical analyses, amid-type nitrogen is nowadays thought to comprise the majority of organically bound nitrogen.[3] There is strong evidence from the "older" literature that low molecular phenolic intermediates could play a decisive role in the humification process.[4]

The present paper reports on ammonoxidation of phenolic and quinonid key compounds in humification, namely 2,5-dihydroxy-[1,4]benzoquinone and methoxy-[1,4]benzoquinone, using ammonia, methylamine and dimethylamin as nitrogeneous nucleophilic agents. The results of GC/MS, Curiepoint-pyrolysis GC/MS, X-ray photoelectron spectroscopy, NMR spectroscopy, and quantum mechanical calculations support the polyphenol theory of natural humification and advance the current understanding of nitrogen binding along the course of lignin ammonoxidation.

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SESSION 6: IHSS TRAVEL AWADS. JOUNG SCIENTISTS IN HS AND NOM

Electrochemical Quantification of the Redox States of Humic Substances

Michael Aeschbacher[a], René P. Schwarzenbach[a], and Michael Sander[a*],

[a] Institute of Biogeochemistry and Pollutant Dynamics (IBP), ETH Zurich, Zurich, Switzerland

E-mail: michael.sander@env.ethz.ch

1. Introduction

Humic substances (HS) can act as electron transfer mediators in biogeochemical redox reactions and as redox buffers in temporarily anoxic environments where they accept electrons under anoxic conditions and donate electrons to O_2 under oxic conditions. This regenerable electron acceptor capacity (EAC) of HS is believed to significantly decrease methanogenesis in bogs and peatlands (1). Assessment of such processes requires accurate quantification of the EAC and electron donor capacities (EDCs) of HS, the extent of electron transfer reversibility to and from HS, and the kinetics of re-oxidation of reduced HS by molecular oxygen. Previous methods have quantified HS redox states indirectly by the use of chemical probes (e.g. Fe^{3+}) (2). These methods have limitations including low accuracy due to indirect quantification of transferred electrons, pH-dependent reduction potentials E_h of the chemical probes such that results obtained at different pH could not be compared, slow electron transfer kinetics between chemical probes and HS samples. We developed a new electrochemical approach to quantify the redox state of humic substances that overcomes these limitations (3). In this approach EACs and EDCs were determined by mediated electrochemical reduction (MER) and oxidation (MEO), which relied on organic radicals with pH-independent E_h to facilitate electron transfer between HS samples and a working electrode (WE). The amount of transferred electrons was quantified by chronocoulometry. For method validation, direct electrochemical reduction was used to generate HS standards with clearly defined redox states. The versatility of the novel method is demonstrated by cyclic reduction O_2-reoxidation experiments using Leonardite Humic Acid Standard (LHA) and by determining the EACs of a diverse set of IHSS HS samples and correlating them to HS structural properties.

2. Materials and Methods

Direct electrochemical reduction (DER) was carried out in an electrochemical cell with a glassy carbon WE, an Ag/AgCl reference electrode, and a platinum wire counter electrode. Reduction of HS was carried out at a WE potential of E_h=-0.59V (pH 7; 0.1 M KCl).

MER and MEO were run in a cell of similar geometry to the one described above for DER. Reticulated vitreous carbon was used as WE material, which, due to its high surface area, achieved faster electron transfer kinetics than glassy carbon. Applied potentials were E_h= -0.49 V in MER and +0.61 V in MEO, and the cells contained the redox mediators diquat (DQ) and 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), respectively. Spiking of small volumes of HS samples to MER and MEO cells resulted in reductive and oxidative current peaks.

Cyclic reduction and O_2-reoxidation of LHA. LHA was reduced by DER and reoxidized by O_2 in excess, in a total of three cycles. After a certain time unreacted O_2 was removed by Ar-purging. After each reduction and re-oxidation step, small HS aliquots were withdrawn for redox-state quantification using MER and MEO.

3. Results and Discussion

DER (E_h = -0.59V, pH 7) of LHA resulted in high initial reduction currents of several 100 μA, which decreased over 60 hours to <50 μA. Reductive currents in the absence of LHA were negligible (< 3 μA), showing that LHA was selectively reduced without the production of significant amounts of H_2 at the WE. DER therefore allows transferring defined amounts of electrons to HS. DER was used to generate LHA samples S0-S10 (red trace in Figure 1a) with known redox states (Q_{DER} from 0 to 966 $\mu mol_{e-} g_{LHA}^{-1}$ transferred). These samples were spiked to MER and MEO, resulting in reductive and oxidative current peaks (Figure 1b). Peak currents and peak areas increased in MEO and decreased in MER with increasing pre-reduction of LHA from sample S0 to S10. Peak integration yielded the amounts of electrons transferred, Q_{MER} and Q_{MEO}. Balancing of Q_{DER}, Q_{MER}, and Q_{MEO} yielded the following results (Figure 1a): (i) to each HS samples the same total amount ($Q_{DER} + Q_{MER}$) of electrons was transferred and (ii) for each HS sample Q_{MEO} was equal to $Q_{DER} + Q_{MEO}(S0)$. MER and MEO therefore allowed for accurate quantification of changes in HS redox states (here DER-transferred electrons): $Q_{DER} = Q_{MER}(S0) – Q_{MER}(SX) = Q_{MEO}(SX) – Q_{MEO}(S0)$. Regression analysis showed that the recovery of DER-transferred electrons was quantitative in MER (104±3%) and close to quantitative in MEO (90±3%).

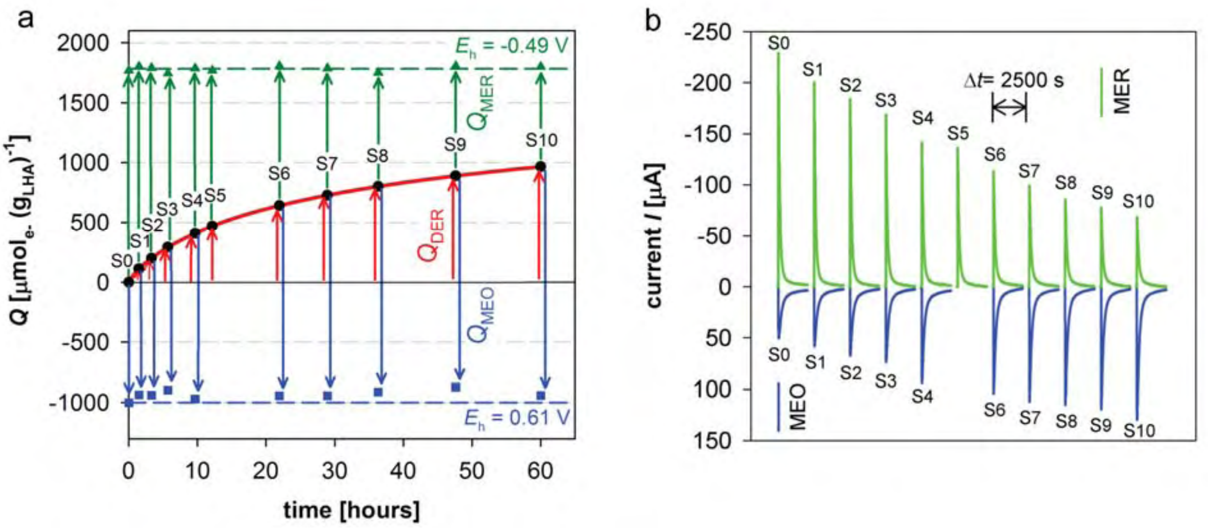

Figure 1: **a.** Direct electrochemical reduction of Leonardite Humic Acid Standard (LHA, red curve). S0 to S10 represent LHA samples to which known amounts of electrons Q_{DER} (red arrows) were transferred by DER. Green and blue arrows represent the amounts of electrons transferred in mediated electrochemical reduction (MER) and oxidation (MEO). **b.** Reductive (green) and oxidative (blue) current peaks obtained for LHA samples S0 to S10 in MER and MEO, respectively. Figures are from ref. 3.

The novel electrochemical approach was used to determine the electron transfer reversibility to and from LHA in cyclic reduction O_2-reoxidation experiments (Figure 2a). Q is the excess electron content of the LHA sample relative to an unreduced sample and was quantified by DER (red curves), MER (green triangles), and MEO (blue squares). The results show that aeration of reduced LHA resulted in extensive re-oxidation and hence regeneration of much of the EAC over the three cycles. The kinetics of LHA re-oxidation by O_2 were biphasic with a fast initial re-oxidation (seconds to minutes), followed by a slower component (hours to days) (data not shown). These results demonstrate that HS may act as largely reversible redox buffer in temporarily anoxic systems, supporting that HS reduction reduces microbial methanogenesis by providing an additional EAC. Quantitative detection of electrons in LHA samples after incomplete re-oxidation by O_2 using MEO suggested that the O_2-reoxidation recalcitrant fraction originated from redox active moieties with slow reoxidation kinetics, most likely slow first electron transfer from reduced benzoquinones to O_2.

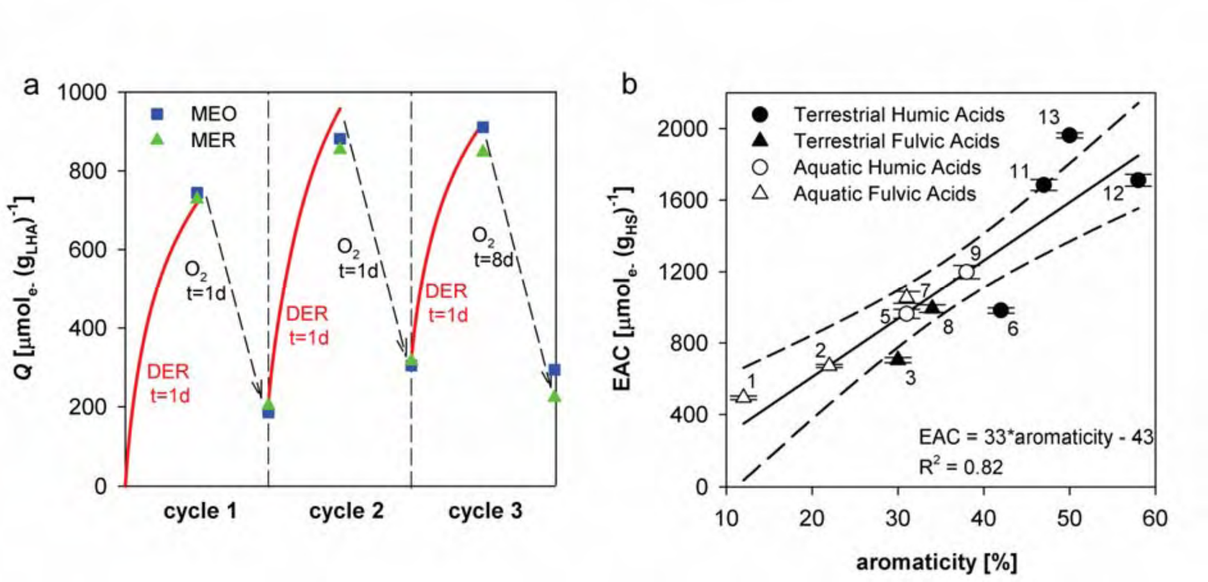

Figure 2: **a.** Cyclic direct electrochemical reduction (DER; red curve) and O_2 reoxidation (dashed line) of Leonardite Humic Acid Standard (LHA). After each step, the excess amount of electrons on LHA samples relative to the unreduced sample, Q, was quantified by mediated electrochemical reduction and oxidation (MER and MEO). **b.** Linear correlation of MER-quantified electron accepting capacities (EACs) (μmol_{e-} g_{HS}^{-1}) for a set of IHSS humic substances with ^{13}C-determined aromaticities (%) (IHSS; http://ihss.gatech.edu/ihss2/). The humic substances are: #1: Pony Lake Fulvic Acid Reference; #2 Suwannee River Fulvic Acid Standard; #3: Elliott Soil Fulvic Acid Standard; #5 Suwannee River Humic Acid Standard; #6: Waskish Peat Humic Acid Reference; #7: Nordic Lake Fulvic Acid Reference; #8: Pahokee Peat Fulvic Acid Standard; #9: Nordic Lake Humic Acid Reference; #11: Pahokee Peat Humic Acid Standard; #12: Leonardite Humic Acid Standard; #13: Elliot Soil Humic Acid Standard. Dashed lines represent 95% confidence intervals. Figures are from ref. 3.

MER was used to quantify the EACs of a large set of IHSS humic and fulvic acid standards and references (Figure 2b). The obtained EACs were between 490 $\mu mol_{e-} g_{HS}^{-1}$ (Pony Lake Fulvic Acid Reference) and 1960 $\mu mol_{e-} g_{HS}^{-1}$ (Elliott Soil Humic Acid Standard). In general humic acids had higher EACs than fulvic acids from the same source material, and terrestrial humic acids had higher EACs than aquatic humic acids. The linear correlation of the EACs of the HS samples with their aromaticities (Figure 2b) and C/H ratios (not shown) suggests aromatic redox active moieties in HS, most likely quinones. The EACs determined by MER correlated linearly (R^2 =0.94) with EAC quantified indirectly by Fe^{3+} oxidation of H_2-Palladium reduced humic substances (4). However EACs quantified by MER were about a factor of three higher, most likely due to slow reoxidation of HS with Fe^{3+} and hence underestimation of EACs with this method (2).

4. Conclusions

The developed electrochemical approach allows for an accurate balancing of transferred electrons to and from HS samples and therefore an exact quantification of HS redox states.

Applied to cyclic reduction and O_2 reoxidation experiments, the method showed electron transfer to HS is largely reversible, but that a small pool of O_2-reoxidation recalcitrant moieties is present in HS, likely benzohydroquinones. The EACs of a large set of IHSS HS samples correlated linearly with aromaticity, consistent with quinones as major redox active moieties in HS.

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Exploration of Fulvic Acid as a Tool for Developing Mucodhesive, Sustained Release and Acid Buffering Vaginal Tablets for Fungal Infection

Aamir Mirza*, Sushma Talegaonkar, S. P. Agarwal and Zeenat Iqbal

Dept of Pharmaceutics, Jamia Hamdard, New Delhi, India
E-mail: aamir_pharma@yahoo.com

1. Introduction

Aqueous environment, the anatomy and its physiology render vagina conducive to the growth of bacteria, fungi, yeast and other microorganisms. Candida albicans is a common cause of vulvovaginitis and its infection is frequent in pregnant women and diabetics. Vaginal milieu has an acidic pH 4-5, which offers a natural protection from various infections. Continued infections in the vaginal cavity accentuate the pH into an alkaline range which could be brought down into physiological range (4-5) by using acid buffering agents. Itraconazole is a broad spectrum antifungal but marred with problem of high hydrophobicity. Itraconazole has also been described as “practically impossible to apply to the body” [1]. Thus it could be explored for the development of suitable vaginal drug delivery systems (VDDS).

To obviate the aforementioned caveat here fulvic acid explored in various capacities likes buffering agent, imparting sustained property and providing mucoadhesion. In pharmaceutical research fulvic acid is reportedly used as solubility enhancer with drugs like Carbamzepine, furosemide and Ketoconazole [2]. In the present investigation it was used first as a complexing agent to provide appreciable water solubility along with sustained release property. The compositions comprising more than 40 % (w/w) fulvic acid also exhibited mucoadhsion properties, which could be potentially used for prolonged adhesion to the vaginal mucosa. Amount of fulvic acid optimized also exhibited advantageous buffering potential in an acidic range.

2. Experimental and methods

Phase solubility study. Phase solubility study was carried out at room temperature (25°C) according to the method reported by Higuchi and Conners [3], analyzed by UV spectrophotometer at 260 nm

Preparation of complex by Solvent evaporation. Complex was developed by solubilizing Itraconazole with HCl in the presence of propylene glycol and poured it into aqueous solution of Fulvic acid. Molar ratio1: 1 and 1:2 (Drug: Fulvic acid) were determined by phase solubility studies. The solution was then sonicated for an hour. The solution thus obtained was dried in a rotary evaporator.

Characterization of the solid complexes. Complex was characterized by DSC, XRD and FT IR as per the methods described in earlier reports [2].

Preparation of mucoadhesive, sustained release and acid buffering tablets. Different batches of tablets (500 mg each) were developed according to Table 1. Tablets T 1 to T 4 had 1:1 complex while T 5 to T 8 had 1:2 complex.

Optimization of tablet. The acid buffering bioadhesive tablets were optimized on the basis of hardness, mucoadhesion and release profile. The release profile of the tablets were studied in 900 ml of simulated vaginal fluid containing 1% sodium lauryl sulphate using USP dissolution II apparatus at 50 rpm and 37°C. Analysis was done on UV spectrophotometer at 260 nm. *In vitro* mucoadhesion study was carried out by the slightly modifying reported method [4].

Evaluation of optimized tablets. Following studies were carried out to evaluate the optimized tablets (T 4 & T 7).

Determination of pH of tablet and acid buffering capacity: first the tablets were kept in 2 ml distilled water and pH of the swelled tablet was measured by touching the tip of the electrode to the wet mass of the tablet. For buffering capacity, the tablet was crushed in 10 ml of 0.9% Nacl (normal saline) solution. Sodium hydroxide (1.0 N) was added in 20 µl increments under constant stirring. The pH was measured with a standard combination electrode 30 sec after each addition. Stirring was stopped during pH measurements. This procedure was repeated until the pH rose above 7.0. The titrations were performed in triplicate for each gel. Curves were used to calculate the amount of NaOH required bringing the pH of each solution to 5.0 (a measure of the buffering capacity of the tablet).

Swelling and spreadability studies of tablets: swelling characteristics of the bioadhesive tablets were evaluated by dynamic swelling studies. Each sample was weighed and then placed in 10 ml citrate buffer of pH 4 in a glass vial at 37 °C ± °C. The samples were periodically weighed after removing excess water on the surface with a filter paper. The swelling was calculated and then plotted as a function of time. For spreadability study, the tablets were allowed to swell in 2 ml of distilled water; the swollen mass was gently transferred to the center of a glass plate and compressed under several glass plates (100g each every minute). The spread diameters recorded each time and compared with (candid-V).

Ex vivo Bioadhesion studies: adhesion property on buffalo mucosa was assessed using texture analyzer (pretest and test speed 0.5 and 0.1 mm/sec, contact 3.0 minutes, contact force 1.0 N and load cell 500 N). Tablet was applied on the upper probe and the force required to detach the tablet from moistened tissue surface was determined as peak value in resultant force – time plot.

In vitro antifungal studies: in vitro anti fungal studies were performed against clinical isolates of candida albicans in Sabouraud's agar medium by cup plate method. The marketed formulation (Sporanax 100 mg Capsule) was dissolved in 2 ml of sterilized water and transferred into the well of agar plate. Samples for tablets and pure drug were similarly prepared. In control, 2 ml of distilled water was taken. Covered petriplates were incubated at 32°C in the BOD incubator for 40 hrs. The zone of inhibition was measured at the end of 40 hrs.

3. Results and discussions

According to phase solubility study 1:1 and 1:2 molar ratio were selected, as the graph was AL type.

Characterization of complex. All the analysis (DSC, FT IR and XRD) confirmed the formation of complex. Thermograms of pure drug and FA showed sharp peaks at 169 °C and 150°C-160°C respectively, which were absent in the complex. FT IR of the complex was showing the stretching of the fingerprint region and all the characteristic peaks either overlapped/diminished in inclusion complex. XRD of pure drug showed a characteristic crystalline pattern while that of FA elicited an amorphous nature. XRD of inclusion complex also exhibited amorphous nature thus indicating an entrapment of drug.

Optimization of tablet. On the basis of hardness, mucoadhesion and release profile, T 4 and T 7 were found to be good candidates for further studies. T 7 was showing more sustained action than T 4.

Determination of acid buffering capacity. The pH of the optimized tablets was found to be acidic (4.8±0.068, n=2), almost similar to the required pH of the vaginal tract. The pH of the marketed formulation was also acidic (Infa-V= 5.19 ± 0.26, n=2). Amount of NaOH required to bring 1g equivalent of tablet to pH 5.0 was found to be 0.052 mEq. This data was comparable an earlier study literature, reporting the amount of NaOH required to bring 1 g equivalent of Advantage 24 to a pH 5 was 0.080 mEq.

Swelling and Spreadability studies of optimized tablets. With increasing the amount of FA, tablets provided higher swelling rates. The equilibrium swelling of T 4 after 15 min was 66.2% while T7 swelled up to 48.6%. After maximum swelling disintegration started. Being gel, candid-V spread smoothly and covered largest area in any point of time. T4 spreads little more than T 7 but were comparable to Candid-V.

Ex vivo Bioadhesion studies. Tablets (T4 and T 7) showed very good retention (> 0.3 N) in the *ex vivo* retention studies which was also comparable to marketed preparation.

In vitro antifungal studies. All the wells except containing control and powdered Itraconazole produced appreciable zone of inhibition.

Table 1: Composition of different formula of the tablets

Ingredients	**T-1**	**T-2**	**T-3**	**T-4**	**T-5**	**T-6**	**T-7**	**T-8**
Itraconazole	100	100	100	100	100	100	100	100
Fulvic acid	320	340	360	380	320	340	360	380
Effervescent	-	5	10	15	-	5	10	15
MCC PH 102	75	50	25	-	75	50	25	-
Silica gel	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mg stearate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5

4. Conclusion

The study reports novel pharmaceutical roles of Fulvic acid wherein it is proposed to be used for its capacities in designing and developing a newer VDDS.

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Application of Mini-electrodialysis to Small Volume Saline Sample Preparation for FTICR-MS Analysis

Hongmei Chen, Aron P. Stubbins, Patrick G. Hatcher

Department of Chemistry and Biochemistry, Old Dominion University,
Norfolk, VA 23529, USA
E-mail: hxchen@odu.edu

1. Introduction

The molecular level characterization of dissolved organic matter (DOM) strongly requires advanced techniques for its isolation and identification. Electrospray ionization combined with ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (ESI FTICR-MS) has shown great promise in characterizing DOM and evaluating its nature as a function of source and reactivity (Sleighter and Hatcher 2007, and references therein). The most promising improvement in DOM isolation to date appears to be reverse osmosis coupled with electrodialysis (RO/ED), which can isolate up to 95% of marine DOM, compared to ~10-40% for previous techniques (Koprivnjak et al 2009). However, the RO/ED system demands large sample volumes as its dead volume is about 3 L. It is attractive to find a method which can take advantage of the higher recovery from ED but requiring as small sample volume as it for C_{18} and other traditional techniques, thus it can be applied to high frequency sampling with small volumes such as pore water, industrial monitor samples, et al. We have developed a way by coupling ED with rotary evaporation and report here the details and efficacy of this approach. Our study involves both artificial and natural (saline) water systems where we tested the recoveries and molecular information retrievable with theis system.

2. Material and Methods

The ElectroPrep system, which we call a mini-ED, from Harvard Apparatus is a versatile patented sample preparation technology based on (2D) electrophoresis/dialysis. It has a sample chamber made of Teflon, a completely inert material specifically suited for high sample recovery and low blanks. The membranes of the ElectroPrep system are made of cellulose acetate material. The commercially available membranes with molecular weight cut-offs (MWCO) of 500 Daltons are chosen for use. A water sample (1.5 mL) is placed in the chamber enclosed on two sides by membranes. Ultra quality MilliQ water (~1 L) is placed in the container surrounding the chamber. The system is conducted under the voltage of 200 Volts. All the samples are pre-filtered with 0.2 μm filter before analysis to remove particulates, including bacteria. Samples were analyzed on a Bruker Daltonics 12 Tesla Apex Qe FTICR-MS using similar parameters as previous study in our group (Sleighter and Hatcher 2008).

3. Results and Discussion

Great Dismal Swamp fresh water was diluted with artificial seawater to yield a DOC concentration of ~10 mgC/L. DOC recovery was 55% from a starting salinity of 35 and 57% from a salinity of 20.

Generally, anytime one attempt to process and analyze low DOC waters, extreme care must be taken to avoid contamination. A system blank was tested by loading MilliQ water as the surrounding water and salinity ~31.5 artificial seawater inside the chamber, and operating the system as one would for a sample. Although the artificial seawater DOC increases by 2 mgC/L after desalting the ESI FT-ICR MS for the blank contains mainly salt-derived molecules with mass defects between 0.7 and 0.8, with no significant peaks where normal DOM molecules are generally observed (mass defects 0.00 to 0.30, Fig. 1). When diluted Dismal Swamp (DS) DOM is mixed with the desalted seawater blank, the number of peaks with mass defects of 0.7~0.8 increases; however the mass spectrum in which these salt-derived peaks are removed is nearly identical with DS DOM diluted with MilliQ water (Fig. 1). The blank FT-ICR MS salt-derived peaks are clearly separated from those of the intended analyte, DOM, and as such they are readily discarded and are not considered a major concern. Given that the ED membranes used are cellulose acetate, it is likely the C blank from the mini-ED is also cellulosic and, as such, would have very low ionization efficiency in negative ion mode ESI.

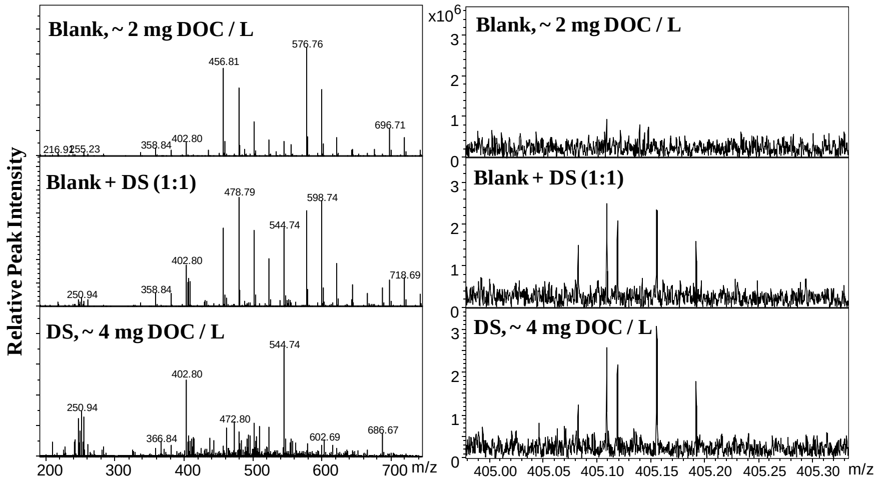

Figure 1: Negative ESI FTICR mass spectra of blank (desalted artificial seawater), diluted Dismal Swamp (DS) DOM, and the mixture. To the right are the expanded spectra in the region of 405.00 – 405.35 as an example for better comparison.

The DOM recovered by ED is compared with C_{18} extracted DOM and whole water DOM for a Congo estuary sample, with salinity ~ 0.2. Salt-derived peaks are almost completely removed by ED as compared with the original salty water (Area A in Fig. 2). The suppressed peaks with mass defect between 0.00-0.06 in the original salty water show up after ED desalting but are not observed with C_{18} extraction (Area B, in Fig. 2). These peaks are not artificial as they are also observed in the whole original water in a sample from a nearby station with no salt (Area B, in Fig. 2). These peaks have relatively lower mass defect, representing compounds either with lower H/C ratio such as lipid-like compounds or with higher O/C ratio such as tannin-like compounds (Fig.3). Some of them are observed in the original salty water DOM but more are observed in the ED-desalted DOM (Fig. 3).

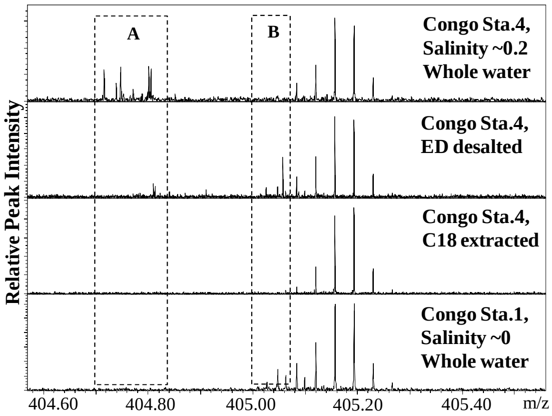

Figure 2: Negative ESI FTICR mass spectra expanded in the region of 404.50 – 405.50 for better comparison. Area A represents salt-derived peaks with mass defects between 0.7 – 0.8, and Area B represents peaks with mass defects between 0.00 – 0.06 which are missed in salty whole water and C_{18} extracted DOM.

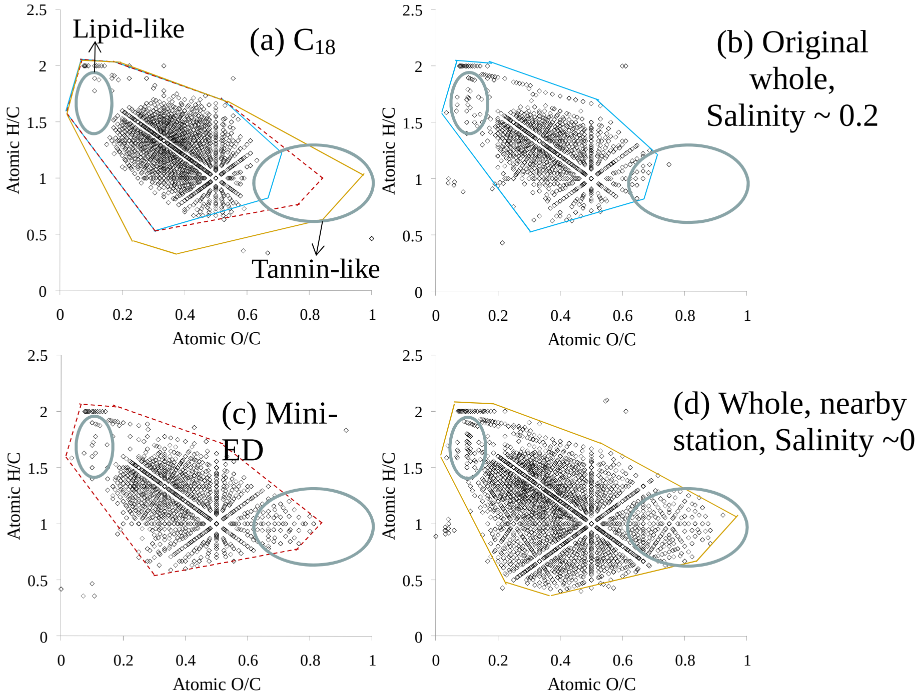

Figure 3: van Krevelen diagrams of the DOM, from the assigned formulae (C, H, O) in each sample

Another comparison between ED and C_{18} as desalting methods was conducted with open ocean seawater from North Atlantic Surface Water, with salinity ~ 35. As shown in Fig. 4, the results are quite comparable and promising considering that C_{18} extraction used ~500 mL initial seawater and concentrated to ~5 mL but ED only used ~15 mL initial seawater and concentrated to ~1.5 mL. This comparison indicates that C_{18} extracts similar material as is retained by ED, in part due to the fact that open ocean DOM is not enriched with large amounts of polar water-soluble substances like tannins. It is also possible that the most polar materials that would be excluded by C_{18} are also excluded by ED because they are low molecular weight and pass through the ED membrane.

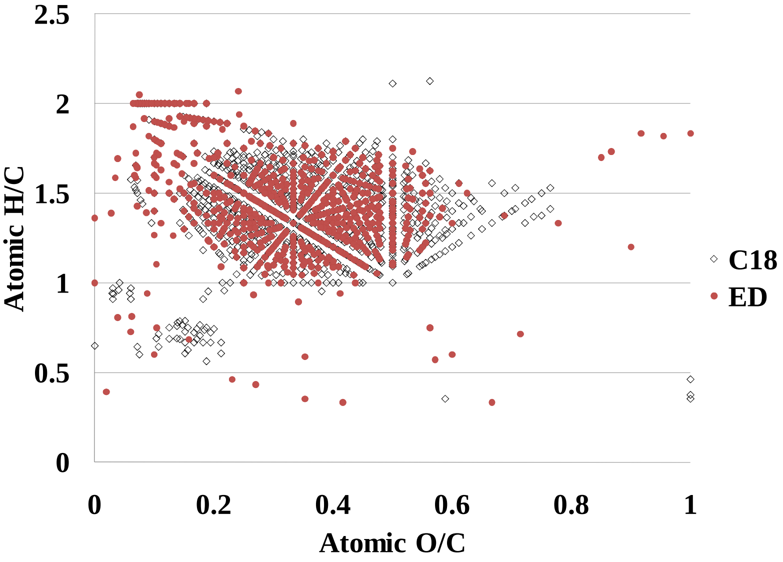

Figure 4: The van Krevelen diagrams of the DOM, from the assigned formulae (C, H, O) in North Atlantic Surface DOM by C_{18} extraction (black open dots) and ED desalting (red full dots)

4. Conclusions

An efficient desalting method, mini-electrodialysis (mini-ED) for preparing small volume samples of salty water prior to analysis by ESI FTICR-MS has been developed and tested. Salty samples with DOC concentrations higher than 6 mgC/L can use this method. A recovery of ~55% DOM can be expected which is higher than the recovery of 30~40% from traditional C_{18} extraction. There is no significant contamination from the ED system that affects FTICR-mass spectra. More representative molecular information can be expected from this method when compared with C_{18} extraction.

Acknowledgements

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Gold- and Germanium-bearing Brown Coals of Primorskiy Region of Russia: Interactions of Humic Acids with Rare and Precious Metals

A. Volk[a*], S. Bratskaya[a], V. Ivanov[b], N. Barinov[b], A. Ustinov[a], N. Polyakova[a], V. Avramenko[a]

[a]Institute of Chemistry, Far Eastern Branch, Russian Academy of Sciences,159, ave 100-letiya Vladivostoka, Vladivostok 690022, Russia; [b]Far East Geological Institute, Far Eastern Branch, Russian Academy of Sciences159, ave 100-letiya Vladivostoka, Vladivostok 690022, Russia
E-mail: avolk@mail.ru

1. Introduction

Humic acids (HA) are highly functionalized biopolymers, which were generated as a result of decomposition of plants and animals residues. They represent the major part of organic carbon of environment. HA have wide variety of functionalized groups, which determine HA active participation in processes of accumulation and distribution of rare and precious metals. The mechanisms of interactions of various metals with HA can be very different. For some metals, for example gold, opinions of scientists significantly vary: some of them report on reduction of gold by HA [1], while the other support idea of Au(III) compexation [2]. These contradictions promote interest to further studies of precious metal ions interactions with HA.

The most enriched natural source of HA is brown coals, contents of HA in some of them reach 80-90%. Brown coal resources of Primorskiy region amount to few billion of ton and include several gold- and germanium-bearing deposits. Understanding mechanisms of accumulation rare and precious metals in brown coals is of great importance for the development of rational methods of these metals recovery with a special focus on separation technologies allowing recovery of metals without decomposition of organic matrix, which can be further used as valuable by-product.

Here we discuss characteristics of HA extracted from gold- and germanium-bearing brown coals of Primorskiy region of Russia and mechanisms of these HA interactions with rare and precious metals.

2. Materials and Methods

The brown coals of five deposits of Primorskiy region of Russia were studied; one part of samples was from gold-bearing, and the other - from germanium-bearing coals. Isolation and purification of HA from the extracts were performed according to the protocol of the International Humic Substances Society (IHSS). Analysis of gold, platinum and palladium content was performed by atom adsorption spectroscopy (Solaar M6, Thermo Electron, USA).

Analysis of germanium was performed by total reflection X-ray fluorescence (TXRF 8030C FET Company, Germany). Sorption of gold (III), platinum (IV), palladium (II) and germanium(IV) on brown coals HA was studied at pH 1.5, equilibration time was 72h. Alkaline extracts of HA containing ions of gold (III), platinum (IV), palladium(II) or germanium(IV) were fractionated using a chromatographic column packed with Toyopearl 65 and distilled water as an eluent. In each 5 ml of the eluent contents of metals and total organic carbon were determined. Size-distribution analysis of colloidal gold was performed employing Zeta Sizer Nano ZS (Malvern, UK). X-ray photoelectron spectroscopy (XPS) studies were carried out by means of the high vacuum photoelectron spectrometer (Specs, Germany) equipped with a monochromatic MgKα X-ray source. EDS - Electron Microprobe Spectroscopy was performed employing the Carl Zeiss scanning electron microscope (EVO-50XVP) with an Oxford Energy Dispersive X-Ray spectrometer (INCA-350 System).

3. Results and Discussion

The contents of HA in the studied gold- and germanium-bearing brown coals were rather different: Au-bearing coals had up to 50% of extractable HA, while HA recovery from Ge-bearing coals was about 12-20% only. Analysis of HA extracted from some samples of brown coals showed that they have similar chemical composition: the average contents of the elements in the brown coals HA were found as C - 55±2 mass%, H – 4.5±0.5 mass%, O - 36±2 mass%, and N - 2.8±0.7 mass% that reflects high aromaticity (H/C atomic ratio 0.94±0.04) and high oxidation degree (O/C atomic ratio 0.49±0.05). Potentiometric titration data indicated that extracted HA had higher content of strong carboxylic groups that was also corroborated by ^{13}C NMR spectra. The high content of carboxylic groups determines good complexing properties and high colloidal stability of this type of HA.

Analysis of recovery of gold and germanium from brown coals to alkaline extracts showed that these metals can be efficiently extracted, for both metals recovery can reach 90% in some samples. This suggests that substantial part of these metals in brown coals is bound to HA. Evaluation of recovery degree for palladium and platinum was complicated by low bulk content of these metals in most studied samples of brown coals.

To study mechanisms of HA interactions with rare and precious metals the sorption isotherms of gold(III), platinum(IV), palladium(II) and germanium(IV) on HA were obtained. Fig. 1 shows that sorption capacity of HA changes in series Au>Pd>Pt>Ge. High values of affinity of gold to HA, which remarkably exceeded values previously reported for gold binding to HA by complexation mechanism [3], suggest possibility of different mechanisms of sorption of gold and other metals by HA.

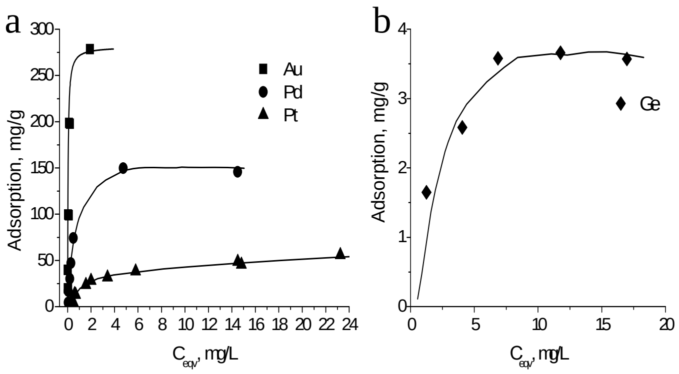

Fig.1: Sorption isotherms of precious (a) and rare (b) metals on brown coal HA, pH=1.5

Fig. 2 illustrates distribution of gold, platinum, palladium and germanium between humic fractions. The data shows difference in distribution metals: platinum and palladium bound uniformly to the high and low molecular weight fractions, gold was eluted with high molecular weight fraction, and germanium with low molecular weight fraction. Such behavior of germanium can explain very low values of its sorption on non-fractionated HA, which can result from very modest content of the low molecular weight fraction in HA under study.

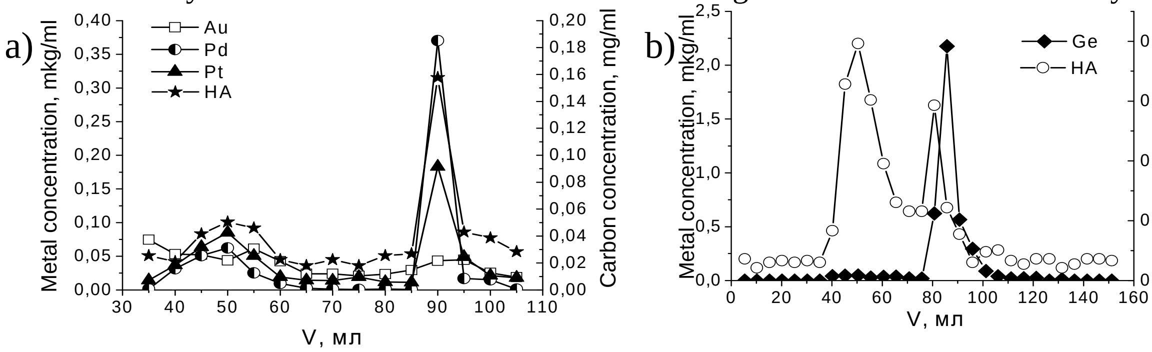

Figure 2: Size exclusion chromatography of brown coal extracts with: a) Au, Pt, Pd; b) Ge, pH=7

Interaction of gold (III) with HA was investigated by UV-vis spectroscopy (Fig. 3). After addition of gold (III) in absorption spectrums of HA we detected peaks characteristic for colloidal gold. When the concentration of HA decreased the maximum of absorption shifted to the long-wave region due to increase in gold particle size. The presence of colloid gold nanoparticles and dependence of their size on HA concentration in solutions were also confirmed by photon correlation spectroscopy (Fig. 3).

XPS spectra of HA after sorption of gold(III) and palladium(II). The high resolution spectrum of palladium have 2 peaks, which attribute to Pd(+2) state. The high resolution spectrum of gold included of two peaks both represented Au(0). Results of XPS indicate that gold is reduced in presence of HA, whereas palladium form complexes [4]. The sample of HA brown coals with the largest content of gold was analyzed by EDS. SEM-imaging (Fig. 4) confirmed the presence of gold particles with dimension below 1 μm, that corroborate with mechanism of gold reduction and stabilization in brown coals by HA.

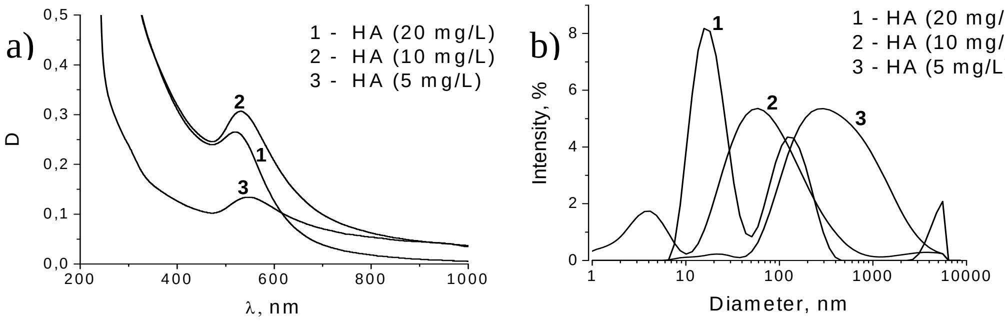

Figure 3. a) Absorption spectrum of colloidal gold (20 mg/L), formed in HA solutions, pH=7 and b) size distribution of colloidal gold in these solutions

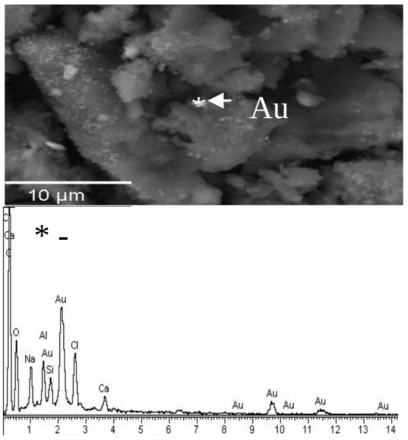

Figure 4: BSE-SEM images of natural gold particles associated with HA brown coals.

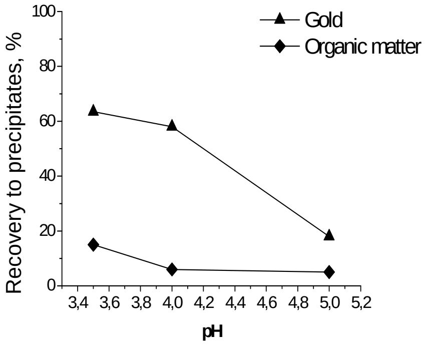

Figure 5: Efficacy of gold recovery by centrifugation of brown coal alkaline extracts with native gold content

Existence of nano particles of gold in brown coal alkaline extracts suggests possibility to separate gold-bearing HA fractions from the main bulk of organic matter. Taking into account different colloidal stability of gold and HA, it was suggested that suitable method for such separation may be centrifugation. Fig. 5 shows efficacy of gold recovery by centrifugation of brown coal alkaline extracts of the gold-bearing brown coals with the highest gold content (0.5-0.76 g/t). At pH = 3,5-4 and rotation speed 5000 rpm the gold recovery reaches 58–63% when only 5–15% of HA is simultaneously precipitated. The recovery of germanium without decomposition of organic matter apparently is possible due to irregular distribution of metal in HA fractions.

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The Role of Different Natural Organic Fractions in the Phytoremediation of the Endocrine Disruptor Compound 4-Nonylphenol

C. Eliana Gattullo*, Elisabetta Loffredo, Nicola Senesi
Dipartimento di Biologia e Chimica Agro-forestale ed Ambientale, University of Bari,
Via Amendola 165/A 70126 Bari, Italy
E-mail: e.gattullo@agr.uniba.it

1. Introduction

4-Nonylphenol (NP) is a well known endocrine disruptor able to interfere with the hormonal system of animals and humans by imitating the female hormone 17β-estradiol. NP derives principally from the anaerobic degradation of nonylphenol ethoxylates, industrial surfactants commonly used in cleaning products and pesticides. It enters the environment mostly by the disposal, application and discharge of effluents and sludges produced by sewage treatment plants. NP was found in the river Po (Italy) waters up to 158 ppb [1], much more than the limit concentration of 6.6 ppb recommended by EPA in freshwaters [2], and up to 4.6 ppm in US soils [3]. The removal of NP from waters and soils through conventional physicochemical methods or by using microorganisms is expensive. Phytoremediation is a new sustainable technology which uses plants to remove or transform contaminants present in sediments, soils and waters. It is largely accepted that humic substances (HS), particularly humic acids (HA), and natural organic matter (NOM) play an important role in the bioavailability and environmental fate of xenobiotic compounds. To the best of our knowledge, no information are reported in the literature about the influence of these organic fractions on the phytoremediation of NP and other endocrine disruptors. Therefore, in this work the authors investigated the effects of one HA and one NOM fraction at two concentrations on the ability of two herbaceous species to remove NP from water during germination.

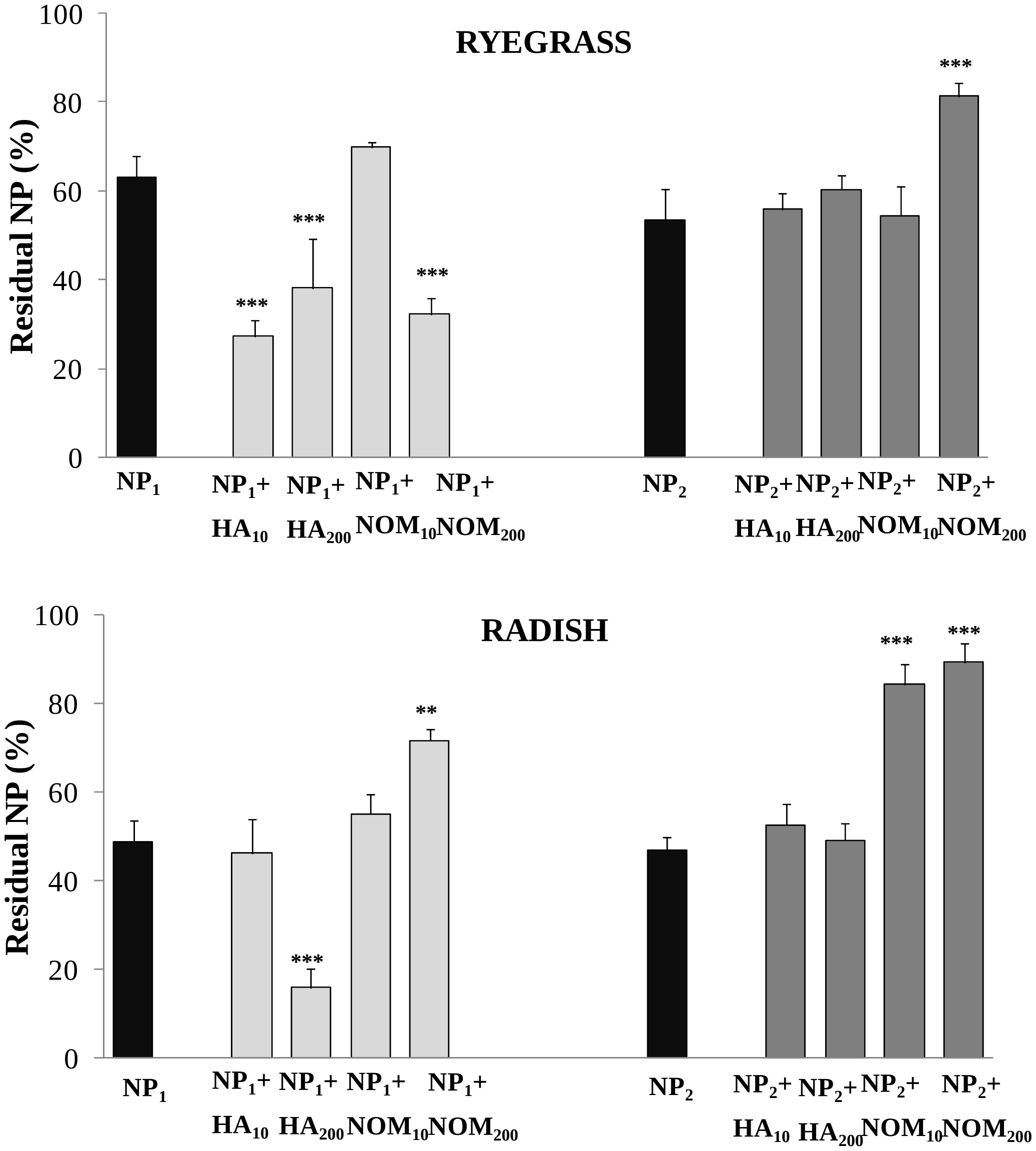
2. Materials and Methods

The Elliot soil HA standard (HA) and the Suwannee river NOM used in this study were obtained from the International Humic Substances Society (IHSS). Ten seeds of ryegrass (RY, *Lolium perenne*) and five seeds of radish (RA, *Raphanus sativus*) were placed separately on filter paper in Petri dishes and added with 3 mL of the following media: bidistilled water (control), NP at concentrations of 1 (NP_1) and 2 (NP_2) ppm, the interaction products between NP at the two concentrations and HA or NOM at concentrations of 10 and 200 ppm (HA_{10}, HA_{200}, NOM_{10} and NOM_{200}). All germination media were added with methanol at 2% in order to increase NP solubility, and pH was adjusted at 6.3. The germination was performed in a Phytotron growth chamber at 23°C in the dark for 5 days for RA and 7 days for RY. In order to test the seedling response to the various treatments, the germination percentage, root and shoot length and fresh weight were measured at the end of germination. The residual NP in the germination medium was determined by using high performance liquid chromatography (HPLC) analysis and a fluorescence detector (λex = 230 nm, λem = 310 nm). All treatments were replicated 5 times and data were statistically analyzed by one-way analysis of variance (ANOVA) and the least significant difference test (LSD).

3. Results and Discussion

The NP alone at both concentrations was not toxic to RY and RA, whereas most of NP interaction products with the two organic fractions inhibited seedlings growth. In previous studies, a synergistic negative effect on germination and seedling growth was observed when HS of different origin and nature were present simultaneously with some organic xenobiotics [4]. In particular, RA was negatively affected by all combinations of NP and HA, which determined a slight reduction of shoot length or fresh weight or both of them, and all combinations of NP and NOM, which strongly reduced shoot length and fresh weight. A significant inhibition of germinating seeds of RY was also produced by the interaction products between NP and NOM at the different concentrations. In particular, NP_1+NOM_{10} and NP_1+NOM_{200} reduced seedling fresh weight and, along with NP_2+NOM_{200}, shoot length. Differently, the interaction products of NP and HA at any concentration did not result toxic for RY.

No significant variation of NP concentration was measured after 7 days in all germination media in the absence of plants. The residual NP measured at the end of germination of both plants in any media and expressed as percentage of the initial amount added is reported in Fig. 1. In the case of RY, the residual NP at the lower dose was 63% when present alone and significantly less, between 27 and 38%, in the presence of HA_{10}, HA_{200} and NOM_{200}. At the higher dose, the residual product varied from 54 to 60% in the different treatments, with the exception of NP_2+NOM_{200} where a much higher (81%) residual NP was measured. In the case of RA, the residual NP at the lower dose was about 50% when present alone and significantly lower (16%) or higher (72%) in the treatments NP_1+HA_{200} and NP_1+NOM_{200}, respectively. At the higher dose, NP removal was about 50% when NP was present alone or in combination with HA at both doses, whereas it resulted significantly lower (84 and 89% still present) in the combinations with NOM at the two concentrations.



*** $P \leq 0.001$; ** $P \leq 0.01$; according to LSD test

Figure 1: Residual NP in the different media at the end of germination

As far as we know no similar studies are reported in the literature, therefore it is not possible to comparatively discuss the results obtained. However, in a previous study on the phytoremediation of the endocrine disruptor bisphenol A in waters, the authors measured a markedly higher removal efficiency by RY and RA, probably because of the higher water solubility of bisphenol A with respect to NP [5]. The effects of the two natural organic fractions on NP removal during seeds germination varied on dependence of their nature and concentration, plant type and NP dose. At the lower dose of NP, HA generally enhanced the product removal by seedlings, whereas NOM exerted a general opposite effect. The different role of HA and NOM in influencing NP removal by germinating seeds might be ascribed to the different chemical, structural and functional characteristics of HA and NOM which may interact differently with NP. Further, it can be hypothesized that simple molecules present in the NOM fraction may represent an easily available C source for microorganisms which are present in the germination medium with a consequent reduced NP microbial degradation. Deeper studies are in course to better elucidate this matter.

4. Conclusions

This study demonstrated that NP can be efficiently removed, at least partly, during germination of the two herbaceous species RY and RA which showed a moderate stress to NP treatments at the doses adopted in this study. The capacity of both plants to remove the product resulted appreciable when NP was present alone. This capacity increased or was not modified when HA at 10 and 200 ppm was combined, respectively, with NP at the lower and the higher dose. Differently, the combinations of NP and NOM increased product toxicity for seedlings and generally decreased their efficiency of NP removal with respect to NP alone, with the only exception of NP_1+NOM_{200} for RA.

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Characterization of the Interactions Between Aquatic Humic Substances from Tropical Rivers and Endocrine Disruptors, Using an Ultrafiltration Procedure and Gas Chromatography – Mass Spectrometry

Wander Gustavo Botero[a], Luciana Camargo de Oliveira[a], Bruno Barbosa Cunha[a,b], Daniele Goveia[a,b], Julio Cesar Rocha[a], Newton Luis Dias Filho[c], Leonardo Fernandes Fraceto[b], Andre Henrique Rosa*[b]

[a] Institute of Chemistry of Araraquara – UNESP, Araraquara-SP, Brazil; [b] Department of Environmental Engineering – UNESP, Sorocaba-SP, Brazil; [c] Department of Physics and Chemistry – UNESP, Ilha Solteira-SP, Brazil
E-mail: ahrosa@sorocaba.unesp.br

1. Introduction

Population increase, the development of new products, and intensified use of terrestrial and aquatic resources in industry and agriculture have resulted in the introduction into the environment of new compounds having varying toxicities and health effects in human and animals. Particular attention has recently been given to a class of substances, present in surface waters and effluents, known as endocrine disruptors (ED), due to the risks that they pose to life, even at the low concentrations found in the environment.

Since ED are an emerging class of pollutants, the literature is still sparse concerning their behavior and interactions in hydric systems. Due to their structural characteristics, aquatic humic substances (AHS), the most important class of complexation agents present in the natural environment, can perform an important role in the transport and behavior of ED [1]. Characterization of the interactions of ED with AHS, exploring the formation and stability of possible ED-AHS complexes, can furnish important information concerning the availability of ED to aquatic biota.

This work presents the use of a procedure based on ultrafiltration to study interactions between aquatic humic substances, extracted from two different tropical rivers, and endocrine disruptors (17α-ethynylestradiol and bisphenol A), with determination of equilibrium time, complexation capacity and competition between the ED for AHS complexation sites.

2. Materials and Methods

Isolation of aquatic humic substances using XAD-8 resin. The AHS were extracted from water samples collected in Itapanhaú river, São Paulo State, Brazil and Ribeira do Iguape river, São Paulo State, Brazil, concentrated by filtration of the water samples through 0.45 μm cellulose-based membranes, acidification to pH <2 with 0.5 mol L^{-1} HCl, and isolation on the XAD-8 collector, following the recommendations of Aiken [2].

Interaction of aquatic humic substances and endocrine disruptors. The analytical procedure proposed by Burba [3], based on ultrafiltration, was used for studies of the interactions between AHS and the ED. This technique utilizes a tangential ultrafiltration system (Sartorius Ultrasart X), fitted with a 1 kDa membrane (Gelman Pall-Filtron OMEGA), that permits separation (from the free ED) of AHS-ED complexes having molecular size greater than 1 kDa. In this study were determined of the time to equilibrium of complexation between AHS and ED, the complexation capacity of AHS for ED and competition between ED and AHS.

Chromatography analysis. Optimization of SPE extraction of analytes using Nexus cartridges (Varian) was performed according to the Nexus applications recommendations for endocrine disruptors. The derivatization of standard solutions and samples was performed in a test tube, according to the procedure proposed by Jeannot [4] and the Varian GC-MS system was employed for analysis.

3. Results and Discussion

According to Oliveira [5], complexes with organic matter tend to stabilize as a function of time, due to inter- and/or intramolecular rearrangements and transfer of complexing species to complexation sites deeper within the molecules.

Complexation equilibrium between the ED and AHS extracted from the different rivers was achieved after about 20 minutes (for both ED), with the results being comparable to those previously obtained in studies of complexation of HS, extracted from different matrices, with potentially toxic inorganic elements, where the kinetics of complexation was around 30 minutes [6].

The complexation capacity (CC) was determined by plotting the free ED concentration (mg L^{-1}) as a function of the total ED concentration (mg L^{-1})[6].

No comparative results have been reported in the literature concerning the complexation capacities of AHS for endocrine disruptors. Table 1 presents complexation capacities of AHS for organic and inorganic substances, including the results of the present study.

Table 1: Complexation capacities of aquatic humic substances for endocrine disruptors and other organic and inorganic substances

Species	CC (mg g^{-1} TOC)	AHS sample	Reference
Cu	74.90	Water from the Iguape River-SP	[7]
Cu	91.51	Water from the Rio Negro- AM	[7]
Al	55.90 – 67.45	New Zealand rivers	[8]
4-Nitrocatecol	25.80	Peat	[9]
1-Naphthol	30.40	HA	[10]
Bisphenol A	3.68	Ribeira de Iguape river	*This work*
Bisphenol A	3.24	Itapanhaú river	*This work*
17α-ethynylestradiol	32.80	Ribeira de Iguape river	*This work*
17α-ethynylestradiol	28.60	Itapanhaú river	*This work*

From the CC obtained, it can be seen that the AHS from both sources showed greater affinity for 17α-ethynylestradiol than for bisphenol A. This can be explained by the behavior of the ED molecules at the AHS complexation sites, which is largely governed by the quantity of –OH groups present, with formation of hydrogen bond type links.

Pacheco [9] suggested that HS form micelles having specific sizes, from which it can be inferred that even though 17α-ethynylestradiol has a higher molecular mass than bisphenol A, greater Van der Waals type interaction occurs within the HS micelles, due to the greater number of hydrogens present in the molecule. Another important characteristic of HS is related to the presence of homologous groups that can form a coating around the AHS complexation sites, directly influencing the stability of the complexes formed [6].

Comparing the CC results for the different chemical species listed in Table 1, it can be seen that the ED show a lower affinity than metal species for AHS, which could be related to the size and steric hindrance of these compounds. However, they show affinities comparable to those of organic compounds such as 4-nitrocatecol and 1-naphthol [9].

Competition studies are used to evaluate the affinity, for a substrate, of any given compound in relation to others. Figures 1A and 1B illustrate the results obtained for the competition between the ED and AHS extracted from the Ribeira de Iguape and Itapanhaú rivers, respectively.

For both samples there was release of bisphenol A and complexation of 17α-ethynylestradiol, in agreement with the complexation capacity data. The results demonstrate that 17α-ethynylestradiol has a greater affinity than bisphenol A for the AHS. Physico-chemical data suggest that the humic molecule possesses a flexible structure as a result of intra/intermolecular interactions and hydrogen bonding. According to Swift [11], this flexible structure results in molecules that approximate spheres (micelles), having a Gaussian molar mass distribution with a high mass density in the center, decreasing towards the extremities of the sphere. Hence, for both of the ED studied, it can be inferred that their main interaction with the HS arises from hydrogen bonding of the oxygenated groups and –OH groupings present in these molecules. Due to the presence of a greater number of hydrogens available for Van der Waals type interactions, 17α-ethynylestradiol succeeds in occupying complexation sites present in the humic substance micelles that were previously occupied by bisphenol A.

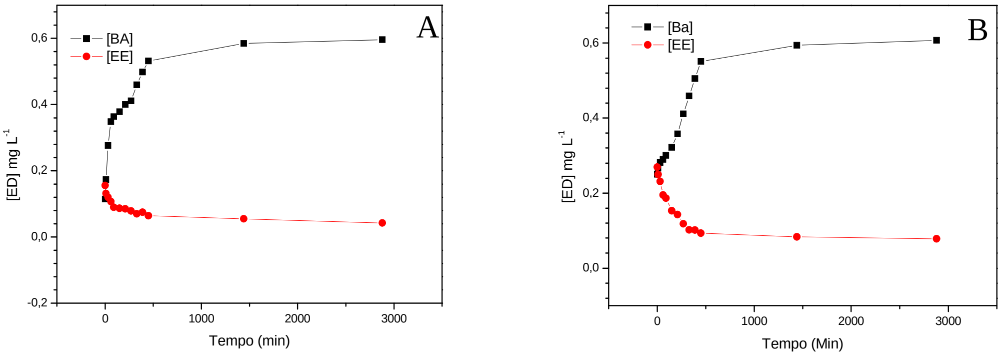

Figure 1: Competition between 17α-ethynylestradiol and bisphenol A for AHS extracted from (A) Ribeira de Iguape river samples, and (B) Itapanhaú river samples

4. Conclusions

These results demonstrate that the availability of endocrine disruptors could be directly related to the presence of humic substances in aquatic systems. Consequently, studies of the interactions between AHS and endocrine disruptors are vital for a better understanding of the transport and/or reactivity of this type of contaminant in the environment.Summary of the work performed, and the main conclusions.

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Removal of Humic Substances From Water by Granulated Activated Carbon and by a Special Bacterium Mixture

Szilvia Joó, Rita Földényi[*]
University of Pannonia, Department of Earth and Environmental Sciences, 8200 Veszprém, Egyetem u. 10., Hungary
E-mail: jooszilvi@gmail.com; foldenyi@almos.uni-pannon.hu

1. Introduction

The presence of humic substances (HS) in row water causes one of the main problems in drinking water purification. If disinfection is carried out by chlorine, then HS may become dangerous due to formation of carcinogen materials, like trihalomethanes (THMs) and chlorinated phenol derivatives. The most typical disinfection by-products are trichloromethane, dichloroacetic acid and trichloroacetic acid [1]. Removing HS from drinking water and analysing their solubility properties have an obvious importance in the prevention of cancer. Different technologies are available, like membrane filtration, activated carbon adsorption, ozonization etc. Concentration of THMs depends on the amount and quality of natural organic matter in the water. The possibility of reacting HS with chlorine and formation potential of disinfection by-products are shown by the ratio of UV absorbance measured at 253 and 203 nm wavelength (A_{253}/A_{203}) [2]. Higher A_{253}/A_{203} ratio means higher concentration of THMs after disinfection. Activated carbon (AC) adsorption is a widely used method in drinking water plants for removing organic materials from row water [3] [4] [5].

“Effective microorganisms” (EM) consist of 18 species of bacteria like lactic acid bacteria, photosynthetic bacteria, nitrogen fixing bacteria. Aerobic and anaerobic species live together in an anaerobic environment helping each other. The bacteria were isolated from the nature and they are not harmful, and their use was found efficient as well as safe in different areas. The EM mixture is applied for instance in agriculture, waste management, waste water treatment and remediation, protecting natural waters. They degrade and transform organic materials into easily available nutrients for plants. EMs roll back pathogen microbes and produce numerous bioactive compounds like organic acids, antioxidants, enzymes and vitamins [6] [7] [8]. EM enabled composts to provide more rapidly crop-available nutrition than its natural compost.

Our aim was to determine the efficiency of HS removal from water by granulated AC, predict the possibility of forming THMs from different humic substances and investigate the effect of EM on degradation of HS.

2. Materials and Methods

The efficiency of AC adsorption was analysed in removal of HS (sodium humate: HANa (Roth+Co, Karlsruhe); fulvic acid: FA (Organit Ltd.); humic materials from Hungarian sandy soil, brown forest soil and chernozem). The presence of HS was followed by UV-VIS spectroscopy at λ=254 nm, and the A_{253}/A_{203} ratio was also determined. Granulated activated carbon (FILTRASORB 400, Chemviron Carbon, specific surface area: 1050 m^2/g) was evaluated in removing different kind of HS and was used in static as well as in dynamic (column) equilibrium experiments at different pH values (pH=5; 6; 7; 8). All samples were in triplicate, and adsorption isotherms were calculated according to ref. [9]. Soil suspensions were made of sandy soil, chernozem and brown forest soil by pure water. 5 g of soils were weighted exactly into flasks and 50 cm^3 of water was added. The flasks were shaken for 1 hour and left to stand overnight for equilibration at 25°C. After filtration suspensions were analysed.

Degradation of humic materials was analysed by EM bacteria community (Greenman Ltd.) after 1 and 7 days. Solutions of fulvic acid, sodium humate and soil suspensions of sandy soil were investigated in 0.01 mol/dm^3 phosphate-buffer at pH=7. Experiments were carried out with and without EM. The bacteria suspension was applied in 1:100 dilution. 10 g of sandy soil was shaken with 100 ml of solution for one hour and left to stand overnight for equilibration at 25°C. Equilibrium phases were separated by filtration – paper or membrane (pore size: 0.45 μm) – and were analysed for HS. All samples were in triplicate. HS were measured by Jasco V530 UV-VIS spectrophotometer (λ=254 nm) and determination of total organic carbon (TOC). Carbon/Nitrogen range was also determined by means of using Thermo Euroglas TOC 1200 equipment which let us conclude the nutrient supply.

3. Results and Discussion

According to A_{253}/A_{203} ratio in case of the same amount of soils, humic materials from brown forest soil are expected to form THMs in the highest concentration, than chernozem and sandy soil rank. During static equilibrium experiments the efficiency of removing HS from soil suspensions changed between 60-84 %. In case of chernozem the highest performance was found (84 %) than sandy soil (82 %) and brown forest soil (60 %) rank.

HANa has shown a two-step L-type of isotherm (Fig. 1) which means high affinity during adsorption [10]. As HANa has a big hydrophobic moiety there is no competition between this and the water molecules. Due to interaction with the activated carbon as well as with the former adsorbed molecules the humate can accumulate in more layers. FA gives a two-step S-type of isotherm (Fig. 1) meaning strong competition with solvent molecules for the active sites of the adsorbent. It adsorbs with lower affinity than HANa because FA is smaller and more hydrophilic. The two-step isotherm of FA, however, refers to multilayer adsorption and over 70 mg/dm^3 equilibrium concentration more FA is adsorbed on AC than HANa.

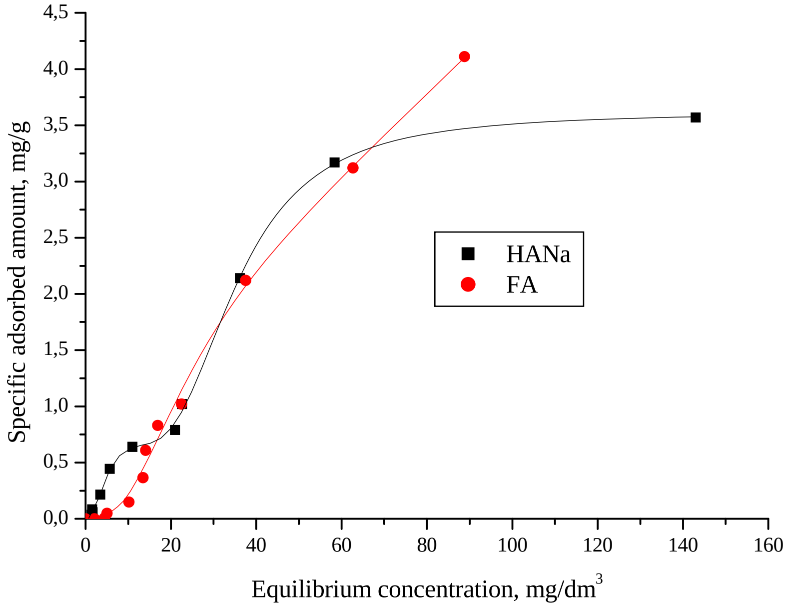

Figure 1: Isotherm of HANa and FA on activated carbon

The removal of the investigated HS was found between 51 and 92 % during the dynamic equilibrium experiments. The binding of FA by the AC column was more efficient than that of HANa. The maximum efficiency of removal was 92.83 % at pH=6 for FA, and 84.36 % at pH=5 for HANa. This value was found at pH=8 - being around the real pH of Hungarian surface waters - 76.50% for HANa and 82.46% for FA, and it is still quite a good result.

Degradation of HS by the applied bacteria community was quite efficient. The most significant affect was found in FA solution and in one of the suspensions of sandy soil with membrane filtration. The efficiency is much lower in case of HANa when scarcity of nutrients was found because C/N range was raised. At the most efficient degradation high concentration of nitrogen could be measured. The smaller FA is more accessible for the microorganisms then the bigger HANa. Some differences between UV absorbance and TOC values can be explained by various processes having different rate, and appearing of various degradation by-products. Non-aromatic groups stared decreasing first, and later aromatic groups degraded.

4. Conclusions

According to A_{253}/A_{203} ratio humic substances of brown forest soil are the most dangerous from THM forming aspect; however, more HS can be solved from sandy soil than from the others. The HANa and the FA have shown two-step isotherms and they adsorbed on the applied activated carbon with high affinity. HS removal by AC was found efficient, however, it depended slightly on pH value, the type and form of HS. This method leads to decrease the amount of forming THMs in water plants.

Degradation of HS by the applied bacteria community was also efficient, especially in FA solution and in one of the suspensions of sandy soil with membrane filtration.

The application of activated carbon is important in general combined processes (e.g. ozonization and AC; coagulation and AC) [2], [11-14], which is supported by these results.

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Enhanced Infiltration Regime for Treated Wastewater Purification in Soil Aquifer Treatment (SAT)

Itamar Nadav[a] , Arye Gilboa[b], Jorge Tarchitzky[a] and Yona Chen[a*]

[a]The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100 .Israel; [b]Weizmann Institute of Science, Rehovot, Israel
E-mail: yonachen@agri.huji.ac.il

1. Introduction

Fresh water (FW) shortage is common in many countries in the Middle East and the Mediterranean region due to long drought periods, increasing population and the concomitant rise in water demand. The Dan Region Reclamation Project (Shafdan) is the largest WWTP in Israel. The Shafdan treats about 130 Mm^3 annually of raw wastewater from the city of Tel-Aviv and several municipals surrounding it. The secondary TWW are then further treated to tertiary TWW degree by slow sand filtration using soil aquifer treatment (SAT). The tertiary TWW are then pumped out from the groundwater and then is transferred to the southern region of Israel for unrestricted agriculture irrigation. The hydraulic loading is divided into a flooding period of nearly 24 h, and drainage period of 48-72 h to allow aerobic conditions in the soil, this flooding regime will be referred as "Shafdan regime". As a result of long term of TWW infiltration, a reduction in the infiltration rate of the basins gas been observed. The main factor in infiltration reduction of the soil is related to OM originating from the TWW accumulation at the top soil layer and therefore induction of water repellency.

The objective of this research is to establish an effective infiltration regime for the Shafdan WWTP infiltration basins that could reduce OM accumulation and reduce the degree of water repellency in the top soil layer. Elevated infiltration rates will increase the basins capacity to treat larger quantities of TWW.

2. Methods

In order to achieve enhanced infiltration rates four infiltration model ponds (5x3 m and 1 m high) were built at the Shafdan WWTP site; those ponds were designed to simulate the large infiltration basins.

The control pond (p.1) was filled with FW through a single source in a flow rate of 1 m^3 h^{-1}, according to the current regime used at the Shafdan wastewater treatment plant ("Sahfdan regime"). The first TWW treatment (p.2) was subjected to the same regime as the control but using TWW instead of FW. The objective of that treatment was to examine the influence of organic matter on the infiltration rate in the Shafdan regime. The next treatment pond (p.3) was filled through 8 garden micro-sprinklers which were placed on the pond surface and its overall flow rate was the same as p.1 and p.2 (1 m^3 h^{-1}). That filling method was tested in order to achieve a faster wetting time of the all pond surface. The last treatment, (p.4) was a rapid filling regime. The pond was filled with TWW in high flow rate of about 10 m^3 h^{-1} (one magnitude faster than the p.1, p.2 and p.3 treatments). The objective of this pond was to achieve full surface wetting and a high and rapid pressure head on the soil surface.

On each infiltration cycle, all ponds were simultaneously filled with 24 m^3 of TWW/water according to the methods mentioned above followed by drainage period of 72 h. OM content, hydraulic conductivity (HC) and the degree of repellency as water drop penetration time (WDPT) were measured throughout the experiment in each pond.

3. Results

At the beginning of the experiment before any TWW infiltration the OM content in the top soil was about 0.1%. The original OM content results from natural vegetation in that sandy region. In the first period of the experiment (Mar. - Sep. 07) there was a gradually increase in OM content in all model ponds. During the fall and the winter (Oct. 07-Feb. 08) there was a severe increase in OM content in all model ponds, whereas in the last period of the experiment during the spring and early summer (Mar.- Jun 08) there was a decrease in OM content in all TWW infiltration model ponds. Along the entire experiment no changes in OM content where observed in the FW control pond (p.1). The OM content in that pond remained relatively constant at about 0.1% (w/w) as the original OM content at the beginning of the experiment.

From the beginning of the experiment differences in OM accumulation were observed between the different ponds. Although, the observed trends of increased OM content in the winter and decreasing in the summer where common to all the TWW ponds. The highest OM accumulation was observed in p.3 (micro-sprinkler filling) along the experiment which reached a peak of 6% in the mid winter. Some of that organic matter was contributed from algae growth on the top soil layer. The lowest OM accumulation along the entire experiment was found in p.4 (rapid filling). The highest OM content in that treatment was about 3% at the mid winter, about half of the OM content of p.3. WDPT values measured in the different model ponds are shown in. At the beginning of the experiment WDPT values for all ponds were 0 s as the original soil repellency. Along the experiment, a gradual increase in water repellency was observed in all TWW ponds which reached a peak in the mid winter followed by a decrease in water repellency in the summer. The highest repellency degree was found for p.3 in the mid winter with WDPT values of up to 2 min (120 s).

The lowest repellency degree was found in the mid winter for p.4 with peak WDPT values of 60 s. The order of repellency degree between the ponds remained constant throughout the experiment with the highest degree of repellency for p.3 and the lowest degree of repellency for p.4. No repellency characteristics where observed in p.1 (FW) pond throughout the entire experiment as expected.

At the beginning of the experiment the HC values were high as the original HC of the sandy soil around the ponds and as in p.1 (control). Along the experiment a HC decrease was observed in all ponds, including p.1. The peak in HC values reduction was observed in the mid winter and was followed by increasing HC values towards the summer months. Similar to the trend presented in Figs. 1 and 2, the lowest HC values found was for p.3 pond at mid winter.

In contrast with the constant OM content and WDPT values found in p.1, the HC values had changed during the winter and had lower values than in the summer. That reduction in HC values can be explained by the reduction in air temperatures which leads to changes in water flow through the soil (Lin et al., 2003). Similar trend of HC values decrease in the winter was observed in the infiltration basins as well.

In all parameters measured during the experiment (OM%, WDPT and HC), the lowest infiltration characteristics were found for p.3 and the highest infiltration characteristics were found for p.4. The control pond (p. 1) showed almost no changes in infiltration characteristics along the experiment excluding the HC reduction in the winter as explained before, and thus p.1 had the highest water permeability soil surface from all model ponds.

4. Conclusions

Treated wastewater infiltration rates into the soil can be lower of those of FW infiltration. Lower HC and higher WDPT values were found in the winter period in the TWW infiltration model ponds in comparison with the FW pond. The main factor affecting these values appears to be OM accumulation at the soil surface which induced water repellency characteristics. Higher OM accumulation during the winter exhibits lower HC and higher WDPT values and thus reduced infiltration rates. The TWW filling regime used determines the rate of OM accumulation in each pond. The micro-sprinkler filling (p.3) caused higher OM accumulation, higher water repellent soil surface conditions, and as a result the lowest infiltration rates. The

Shafdan regime (p.2) caused mid OM accumulation and infiltration rates. The most efficient filling regime was found to be the fast filling (p.4) which induced the lowest water repellent soil surface. In that filling method the OM accumulation rate was the lower, the HC values were higher, and the WDPT values were lower. No water repellent characteristics were found in p.1 (FW) as expected.

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Humic Substances Bound Polycyclic Aromatic Hydrocarbons (PAHs) in Rhizosphere of Crops from Contaminated Field

Bin Ma, Huai-Hai Chen, Yan He, Jian-ming Xu*

Zhejiang Provincial Key Laboratory of Subtropical Soil and Plant Nutrition, College of Environmental and Natural Resource Sci., Zhejiang University, Hangzhou 310029, China
E-mail: jmxu@zju.edu.cn

Abstract

Formation of humic substances bound PAHs in soil would bring about the bias of rhizosphere effect on PAHs degradation. The aim of this study was to evaluate the role of humic substances bound PAHs in rhizosphere of crops from long-term PAHs polluted soils by using sequential extraction approach. The scheme of sequential extraction includes organic solvent extractable PAHs, humic acid bound PAHs, crude humin bound PAHs, and organic-C enriched humin bound PAHs. Humic substances bound PAHs accounted for a large proportion of PAHs in both rhizosphere and bulk soils. The profiles of organic extractable and humic substances bound PAHs varied with plant species. The discrepancy of toxicity equivalency concentration between rhizosphere and bulk soils was much more significant than that of total PAHs concentrations. The novelty of this study is evaluation for concentration and toxicity equivalency concentration of humic substances bound PAHs fractionations in rhizosphere of crops sampled in a field polluted with PAHs for long term. This study has highlighted the large proportion of humic substances bound PAHs and vital role of rhizosphere in cleanup of cancerogenic toxicity of soil than amount of PAHs in polluted soils.

1. Introduction

Given that polycyclic aromatic hydrocarbons (PAHs) can partition strongly with soils via atmospheric deposition as well as anthropic activities [1-3], soils represented the major environmental repository for these contaminants[4]. It is commonly recognized that PAHs persisting in soil would exhibit declining extractability and bioavailability to organisms with increasing contact time[4-6]. The sorption of PAHs on soil sediments is an essential process controlling bioavailability and toxicity of PAHs [7-10]. Increasing amount of PAHs is retained in soil as humic substances bound residues with increasing soil contact time [6]. Mostly in previous studies, callous extraction methods were used to extract PAHs from soil in a single extraction step. In those studies the amount of humic substances bound PAHs that cannot be extracted would be assumed degraded, therefore, would overestimate the amount of degraded PAHs [6].

Phytoremediation is now considered as one of the most promising remediation approaches for PAHs in soil [11]. Rhizosphere effect, including promoting microbial activities and releasing secretions, dominates the phytoremediation through interactions with microorganisms and absorption of root directly [12]. The fate of PAHs in rhizosphere is expected distinct with bulk soil as the bioavailability of PAHs would be significantly different. Up to date, however, the discrepancy has been masked by the exhaustible extraction procedures which used single organic solvent as extractants [13]. Further, the contribution of rhizosphere to degradation of PAHs would obscure by the formation of humic substances bound PAHs in soils.

Toxic equivalency factor (TEF) method is a numerical procedure which can provide an interim approach until more data become available to assess the risk of mixtures containing structurally related compound [14]. The PAH TEFs are developed based on cancer effects of individual PAHs. The widely used PAHs TEFs were synthesized and adapted by Nisbet and LaGoy [15] into a set of referenced order of magnitude estimates. In order to evaluate the toxicity risk of soils polluted by PAHs, toxicity equivalence factors (TEFs) were employed to convert the PAHs concentrations to toxicity concentrations based on the relative carcinogenic potential of other PAHs to Benzo[a]pyrene (B[a]P).

Extractable PAHs in soils were extracted by organic solvent and then the humic substances bound PAHs bound to humic acid, crude humin, and organic C-enriched humin were extracted. Toxicity equivalence factors (TEFs) were used to convert PAHs concentrations into toxicity concentrations. A unique feature of this study is the simultaneous application of extractable and humic substances bound PAHs in soil for determining the contribution of rhizosphere effect to PAHs phytoremediation.

2. Materials and Methods

Soil sampling. Soil samples were collected from field closed to a smelting factory which generates PAHs pollution to the surrounding due to coal combustion for more than 40 years. A plot was selected with maize, oat, rape, and soybean, respectively. Triplicate rhizosphere soil samples were collected by removing the soil adhering to the plant roots. The falling soil samples collected by shaking were treated as bulk soils. Soils and plants tissues were lyophilized and passed through a 2-mm sieve.

Extracted with exhaust organic solvent. The soils were extracted with methanol, *n*-butanol, and DCM sequentially. The exhaust organic solvent extractable PAHs was the sum of three sequential extractions. The supernatants were cleaned up with 6ml C18 extraction cartridge separately. The solid-phase extraction cartridges were conditioned with 5 ml of DCM, followed by 5 ml of methanol. The PAHs were eluted from the sorbent with 8 ml of DCM. The eluted solution was dried under gently nitrogen, and then the solvent was exchanged into *n*-hexane. The determination was implemented by GC-MS (Agilent 6890N/5975B). Separation was achieved using an HP-5MS column (30m× 250um×0.25 um). The temperature program comprised 60 ºC for 2 min, then 30 ºC min^{-1} to 120 ºC and 5 ºC min^{-1} to 270 ºC, where it was held for 15 min. The MS transfer line was 280 ºC. The MS operated in selective ion monitoring (SIM) mode. The 10 USEPA PAHs were listed in Tab. 1.

Investigation into humic substances bound PAHs residues in soils. The humic substances bound PAHs residues in soil after sequential solvent extraction were extracted as described previously [16]. In briefly, the extracted soil was shaken with 10 mL of 0.1 M NaOH for 12 h and the supernatant was removed repeatedly. The combined supernatant was acidified to pH 1 using the solution of 6 M HCl, then extracted with DCM repeatedly until it was colorless in DCM. The collected DCM was cleaned up with SPE and determined with GC/MS. The residual soil samples were defined as crude humin [17]. Methanol (100 mL) was added into the residual soil, shaken for 30 min and loaded in an ultrasonic bath for 1 h with methanol. Then the successional extractions were carried out with 100 mL methanol: DCM (1:1 by volume), 100 mL DCM and 100 mL DCM step by step. Each time the mixture was centrifuged; the extract was collected and combined into a flask. The mixture was shaken and settled. The DCM was collected and repeated three times. Extraction was cleaned up and determined with GC/MS. The residual soil was first treated with 6 M HCl for 12 h and HF: HCl (1:1) for 12 h, then was rinsed with 2 M HCl three times. HCl solution was collected. The residual soil was shaken for 30 min and loaded in an ultrasonic bath for 1 h with 100 mL methanol. The mixture was then centrifuged at 2600*g* for 20 min, and the methanol was collected. Acid and organic solvent extract solution were combined and extracted with DCM for three times. DCM was cleaned up with SPE and determined with GC/MS.

Table 1: The toxicity equivalency factors of PAHs

PAHs	CAS number	Quantification ion/ confirmation ion for SIM	Toxicity equivalency factor
Naphthalene	91-20-3	128/127,129	0.001
Acenaphthylene	83-32-9	152/151, 153	0.001
Acenaphthene	208-96-8	154/153, 152	0.001
Fluorine	76-73-7	166/139, 165	0.001
Phenanthrene	85-01-8	178/165, 176	0.001
Anthracene	120-12-7	178/179, 176	0.01
Fluoranthene	206-44-0	202/200, 203	0.001
Pyrene	129-00-0	202/200, 203	0.001
Benzo[a]anthracene	56-55-3	228/226, 229	0.1
Chrysene	218-01-9	228/226, 230	0.01

Toxicity equivalency factors. The toxicity risk was evaluated by convert the PAHs concentrations to toxicity concentrations based on the relative carcinogenic potential of other PAHs to Benzo[a]pyrene (B[a]P) [15] (Nisbet and LaGoy 2002). The toxicity equivalency factors were presented in Tab. 1.

3. Results and discussion

The total humic substances bound PAHs concentrations accounted for 39.1% to 50.1% of total amount of PAHs in both rhizosphere and bulk soils sampled from the polluted site (Fig. 1a). In soils spiked with 32.5 μg pyrene kg^{-1}, the proportion of humic substances bound PAHs was increased from 3.7% to approximately 30.5% during 24 weeks in both pasture soils and woodland soils [18] (Macleod and Semple 2003). In the same soils amended with 100 mg pyrene kg^{-1}, only 8% humic substances bound PAHs were observed [19] (MacLeod and Semple 2000). In another study in soils spiked with 10 mg PAHs kg^{-1} and after 525-day aging time, humic substances bound PAHs proportion was 5.8%, 8.3%, and 17.0% for phenanthrene, pyrene, and benzo[a]pyrene, respectively [6] (Northcott and Jones 2001). In soils and sediments sampled in the pearl river of China, humic substances bound PAHs accounted for 33.7-57.4% of total PAHs [12] (He et al. 2008). Comparison of filed samples and spiked soils suggest that there may be a long time to reach balance in soils, and the proportion of humic substances bound fractionations could reached to approximately a half of total amount of PAHs in soils.

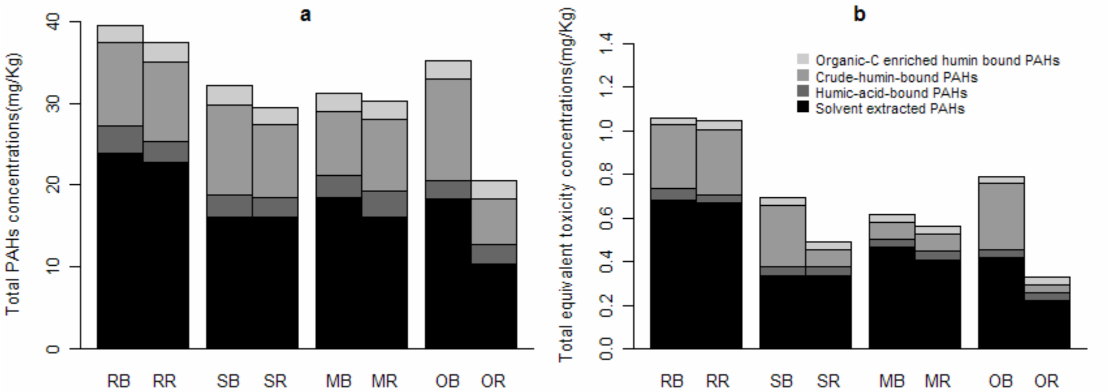

Figure 1: Total PAH concentrations (a) and toxicity equivalency concentrations (b) of of PAHs in rhizosphere and bulk soils based on sequential extraction. RB-bulk soil of rape, RR-rhizosphere soil of rape, SB-bulk soil of soybean, SR-rhizosphere soil of soybean, MB-bulk soil of maize, MR-rhizosphere soil of maize, OB-bulk soil of oat, OR-rhizosphere soil of oat

The humic substances bound PAHs in soils were considered to be bound with humic substance in soils. Based on the concepts of [17] Wang and Xing (2005), the PAHs bound with humic substance were sequentially extracted into humic acid bound PAHs, crude humin bound PAHs, and organic carbon enriched humin bound PAHs. Crude humin bound PAHs accounted for a large proportion of total humic substances bound PAHs fractionation in both rhizosphere and bulk soils, matching the content of the fractions of organic matter in most soils [16-19] (MacLeod and Semple 2000; MacLeod and Semple 2003; Wang and Xing 2005; He et al. 2008). The amount of crude humin bound PAHs in rhizosphere was lower than those in bulk soils under soybean and oat, and was higher in rhizosphere under maize (Fig. 1a). The formation of humic substances bound residuals was influenced by active microbial communities [5,18] (Eschenbach et al. 2000; Macleod and Semple 2003). The comparison of the amount of humic substances bound PAHs in sterile and non-sterile soils indicated that the soil biota is involved in the formation of humic substances bound PAHs in soils. In this study, the effect of rhizosphere effect on the formation of humic substances bound residues was either promotion or inhibition under different plant species. This contradiction might rise from the discrepancy of microbial communities between rhizosphere and bulk soils. Another possible explanation is that the organic matter fractions are different between rhizosphere and bulk soils. Currently, the knowledge of influence of microorganisms on the formation of humic substances bound PAHs is lacking [20-21] (Nieman et al. 2007; Tang et al. 2008). The key result from this comparison is that we observed the difference in the amount of humic substances bound PAHs residual between rhizosphere and bulk soils.

After substituting the equivalency toxicity concentration for the concentration of individual PAHs, the profiles of sequential extraction were remarkably distinct (Fig. 1b). The total toxicity level/concentration of all fractionations from soybean, maize, and oat was significantly lower than that under rape. In rhizosphere of soybean and oat, the sum of equivalency toxicity concentrations was significantly lower than that in corresponding bulk soils because the proportions of equivalency toxicity concentrations of crude-humin-bound PAHs were sharply decreased in rhizosphere. Furthermore, the proportions of equivalency toxicity concentrations of crude-humin-bound PAHs under maize were simultaneously decreased in both bulk and rhizosphere soil. The profiles of equivalency toxicity concentrations were distinct from the profiles of total PAHs concentrations, and varied with plant species.

4. Conclusions

The novelty of this study is the investigation of not only the concentration but also the toxicity equivalency concentration of humic substances bound PAHs fractionations in rhizosphere of crops sampled in a field polluted with PAHs for long term. Such investigation was rarely reported previously as that most of studies evaluated the effect of rhizosphere process with spiked soils and has not considered the toxicity equivalency factors of different PAHs. This study has highlighted more significant role of rhizosphere in cleanup of cancerogenic toxicity of soil than amount of humic substance bound PAHs in polluted soils. The sequential extraction experiment revealed the humic substances bound fractions of PAHs in field soils were significantly greater than in spiked soils. It is suggested that the results in spiked soils would overestimate the degradation of PAHs. The findings of this study suggested that the toxicity equivalency factors could be considered when evaluating the potential role of rhizosphere processes in PAHs cleanup in soils.

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Utilizing Visible-light TiO_2/Adsorbent Nanocomposite for Humic Acids Removal in Wastewater

Wei Zhang[a*], Linda Zou [a], Lianzhou Wang[b]

[a]SA Water Centre for Water Management and Reuse, University of South Australia, SA 5095, Australia; [b]ARC Centre of Excellence for Functional Nanomaterials, the University of Queensland, QLD 4072, Australia
E-mail: zhawy037@unisa.edu.au

1. Introduction

Recently, development of TiO_2/adsorbent nanocomposite (TNC) has become a strong candidate for dissolved organic compounds (DOC) removal in wastewater [1]. This could reportedly combine the advantages of both adsorption and photocatalytic techniques [2,-5]. On one hand, large surface-area adsorbents work as the support of nano-sized TiO_2 photocatalyst and concentrate the pollutants and intermediates around the TiO_2, of which photodecomposition rate is intrinsically low due to their limited surface area; on the other hand, the nanosized photocatalyst can decompose the pollutants thus regenerating the adsorbents in situ. However, it is well known that the band gap energy of TiO_2 is intrinsically wide between 3.0 and 3.2eV (3.0 for rutile and 3.2 for anatase). This means plain TiO_2 semiconductors only absorbs a small portion (5%) of solar spectrum in the UV region. Previous literature studies have demonstrated that wet chemical N-doping could enable the visible light activity of nano-sized TiO_2 photocatalysts [6]. Our preliminary results determined that the optimal wet chemical preparation conditions are obtained at equal N-to-Ti molecular ratio of precursors (triethylamine and titanium butoxide) and 350^{o}C calcinations. Consequently, coating visible-light sensitive TiO_2 photocatalysts on the surface of porous adsorbents within the same wet chemical procedure are carried out in current work. Zeolite adsorbent (F-9 from Tosoh, Japan) was used as the porous substrates. As-prepared TNC was assessed by aqueous humic acid (HA) removal, which exists widely as contaminants in Australian reservoir before proper treatments.

2. Materials and Methods

In a typical visible-light sensitive TNC preparation, titanium (IV) butoxide ($Ti(OC_4H_9)_4$) was first dissolved in ethanol by magnetic stirring at room temperature to obtain solution A. In addition, deionised water was mixed with HNO_3 by magnetic stirring at room temperature to obtain solution B. Solution B was then added drop-wise into solution A under vigorous stirring, and this was then followed by the hydrolysis of $Ti(OC_4H_9)_4$ under stirring conditions at room temperature until a transparent sol was formed. The N-doping was achieved by drop-wise addition of a certain amount of triethylamine to the formed TiO_2 sol under continuous stirring (overnight). In the last, adsorbent powder was added to N-doped TiO_2 sol with magnetic stirring. Prior to the addition, adsorbents were dried in oven at 150°C overnight to avoid any moisture content. The resulting mixture was subsequently dried and calcinated at 350°C for 2hr and the temperature ramp during the calcinations was 5°C /min. Different characterization of as-prepared TNC was also carried out. The photocatalytic and adsorption ability of as-prepared TNC resultants were studied using aqueous HA (Sigma Aldrich) as model pollutants. The HA content was expressed in terms of absorbance values measured at 254 nm (UV254) using Varian Cary 100 double-beam spectrophotometer. UV254 is widely accepted as a surrogate parameter to explain total organic compound (TOC) of HA.

3. Results and Discussion

X-ray photoelectron spectroscopy (XPS) in Fig. 1 suggested the successful N-doping into the TiO_2 lattice. Diffusion reflectance spectroscopy (DRS) in Fig. 2 further confirmed the resulting effect of N-doping as in enhanced visible-light absorbance (i.e. red shift). Typical scanning electron microscopy (SEM) images are shown in Fig. 3, where the surface alteration of adsorbents due to the TiO_2 modification could be observed. Synergistic interactions between adsorption and visible-light photocatalysis were observed, as under the assistance of visible-light irradiation, TNC achieved higher HA removal rates than those by adsorption process alone (see Fig. 4). Furthermore, easy separating ability was also obtained, as all TNC could be settled to the bottom of the reactor by gravity solely within few minutes.

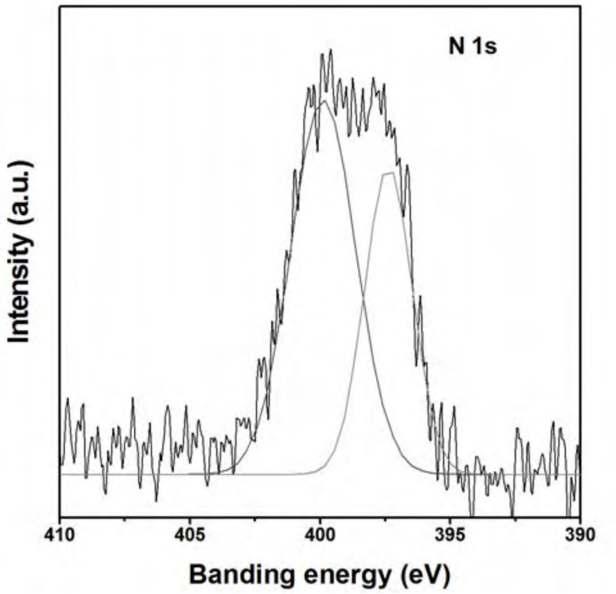


Figure 1: XPS spectra of N 1s in as-prepared TNC, where peaks at 396 and 401ev indicate the Ti-N and Ti-O-N bond formation, respectively

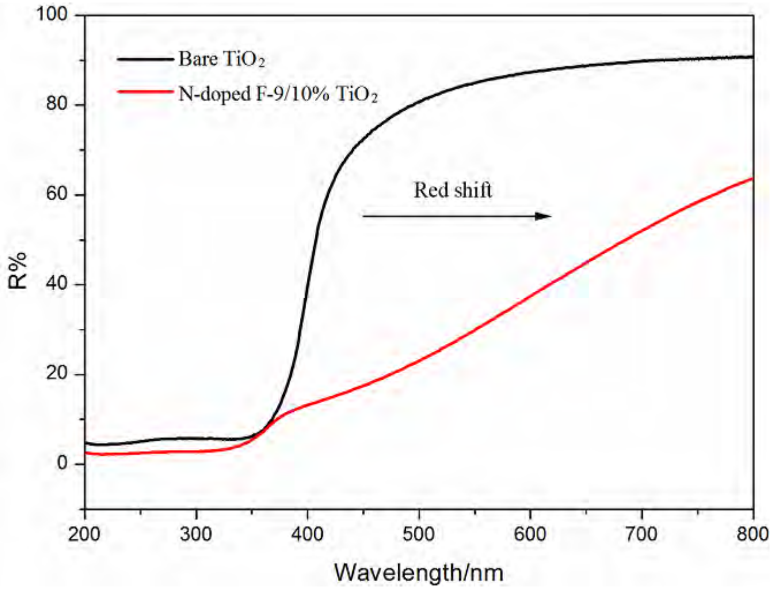
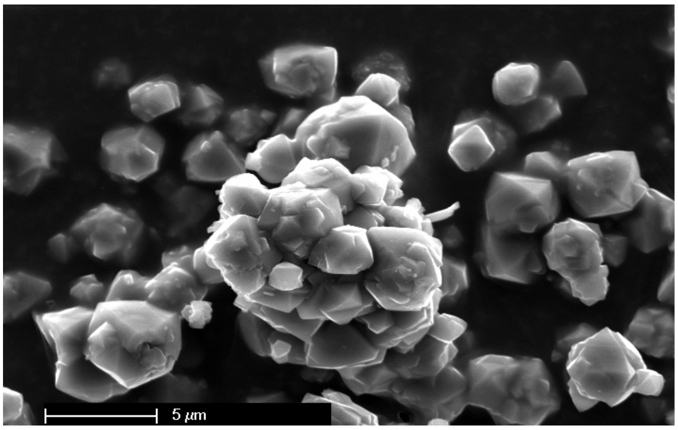
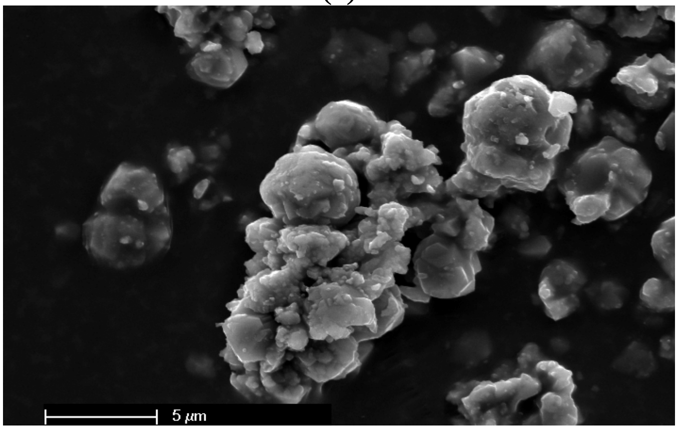


Figure 2: DRS spectra of as-prepared TNC, where a red shift of light absorbance due to N-doping is observed



(a)



(b)

Figure 3: SEM images of (a) F-9 and (b) F-9/10% TiO_2 nanocomposite

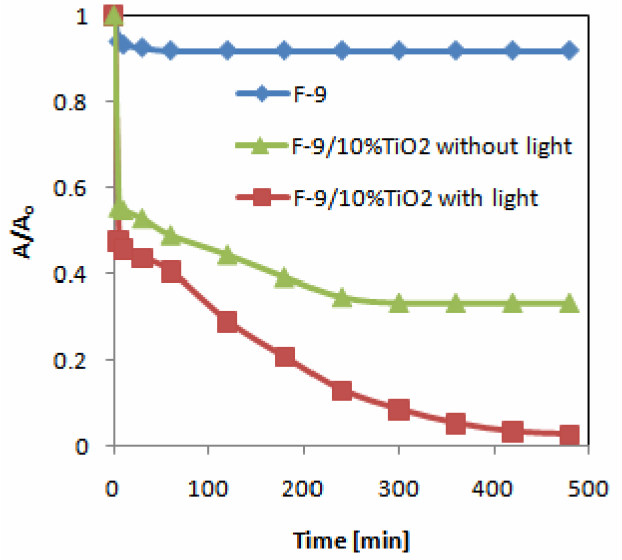


Figure 4: humic acid removal rate as the function of time, where A is HA absorbance at a given time, t; A_o is the initial HA absorbance before the experiment

4. Conclusions

The performance of the as-prepared N-doped TNC has demonstrated aqueous humic acid remediation ability, and also revealed its potential in the field of solar photocatalytic degradation of a wide range of dissolved organic compounds (DOC). Future efforts in this project will use natural HA contaminated water from a local reservoir for as-prepared TNC assessment.

Acknowledgements

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Abiotically controlled solubilization of soil organic matter, the hidden perspective

Ehsan Razavy Toosi[a*], Timothy A. Doane[b], William Horwath[b]

[a]School of Forestry, University of Canterbury, Private Bag: 4800, Christchurch, NewZealand
[b]Department of Land, Air and Water Resources, University of California, Davis,
1 Shields Avenue, Davis, CA 95616, USA
E-mail: ehsan.razavy@pg.canterbury.ac.nz

1. Introduction

Dissolved organic matter (DOM) is a small fraction of the total soil OM that contributes considerably to many soil biochemichal processes due to its mobility and highly reactive nature (Zsolnay, 1996). DOM is important in controlling the biogeochemistry of nutrient cycling, metal availability and C stabilization in soil (Neff and Asner, 2001). The flux of DOM is considered to be largely responsible for controlling microbial activity and turnover specially at greater soil depth where DOM is an important substrate (Park and Matzner, 2003).

Despite the growing body of information about the sources of DOM in soil, the processes involved in the production of DOM within the soil are poorly understood. Recently, Kemmitt et al (2008) challenged the well-established Winogrdsky's theory, proposing the "regulatory gate" hypothesis. Based on this hypothesis, soil OM is solubilized during a two-step process. In the first step, non-bioavailable compounds are transformed to bioavailable OM as a result of abiotic processes. During the second step, the altered (bioavailable) humified compounds become metabolites for the microbial community. This hypothesis has been questioned mainly because of its emphasis on the role of abiological factors in the controlling the bioavailability of OM (Kuzyakov et al., 2009, Paterson, 2009).

The difficulties in distinguishing biotic and abiotic sourced DOM have hindered our understanding of DOM processes in soil. We used ^{13}C isotope pool dilution method to evaluate the "regulatory gate" hypothesis. In this experiment, we monitored the production of the DOM from added sources of OM and extant soil OM in the presence or absence of the microbial community. The objective of this study was to evaluate the role of microbial activity on the net production of DOM from fresh OM source to test the assumption in the regulatory gate hypothesis.

2. Materials and Methods

Preparation of the soil and OM source. Soil samples were collected from topsoil (0-15 cm) from two sites, under Oak woodland (Sierra Field Station) and permanent pasture (Yolo Land) (both Mollic Haploxeralf). Soils were sieved and adjusted to the optimum water status (50% of the WHC) followed by a 10 day pre-incubation period at room temperature to equilibrate microbial activity.

Ryegrass was grown in $^{13}CO_2$ enriched conditions and used as the source of OM in incubation experiments (part A and B), after Bird et al., (2003). The plant material was oven dried at (50^{oC}), ground and sieved (mesh size 40). The plant material was added to soil as an OM source (part A) or was extracted to produce DOM (part B) with 0.01 M $CaCl_2$ (0.5:100 ratio) at 75^{oC} for 6 hours followed by shaking (15 min). The extract was subsequently filtered (0.45 µm) to obtain DOM solution. A new batch of DOM was prepared before each set of leaching (described below).

Incubation experiment.

Part A: incubated soils were mixed with sand (1:1 ratio, dry based) to provide uniform infiltration during leaching. The OM source was added to the mixture at the ratio of 1% (dry-based soil). 80 gr of the mixture was packed into leaching columns. Each leaching column was transferred to 1l Mason jar with septum to assess total and ^{13}C CO_2 production during the incubation. The containers were placed in the dark at 25^{o}C. Containers were opened and aerated regularly during the experiment to maintain aerobic conditions following the determination of total and ^{13}C-CO_2. A set of sterile subsample soils were autoclaved at 15 psi for 1 hr (121^{o}C) as a control. To check the microbial activity in the sterilized and unsterilized subsamples, CO_2 production was monitored at regular intervals. The incubation experiment was 90 days in length. Soil leaching experiment was done on days 1, 6, 18, 30, 42, 54, 66, 78 and 90. $CaCl_2$ solution (0.01M) was used as the leaching solution at the ratio of 1:1 (solution:soil, v:w). Mercury ($HgCl_2$, 0.7gr kg^{-1} soil) was added to the leaching solution to maintain the sterility of the autoclaved subsamples during the experiment. The collected leachate was filtered by 0.45 µm to obtain DOM (the solubilized OM).

Part B: the same procedure was followed for part B. However, instead of addition of OM to the soil mixture, the DOM extract solution was used as the leaching solution at the intervals mentioned above. To inhibit any form of biological activity in the sterilised subsamples, the same amount of $HgCl_2$ was added to the DOM solution along with each leaching set.

The DOM samples were analyzed for DOC, DO^{13}C, DON, UV absorbance (280 nm) and Humification Index (HI).

3. Results and Discussion

Microbial activity. Addition of OM and DOM caused a significant initial pulse of soil respiration. Part of the CO_2 originated from the added OM source, and the reminder came from the accelerated decomposition of the extant SOM. The observed increased mineralization of the extant SOM was due to “the priming effect” (Bingeman et al., 1953). We did not observe any CO_2 efflux in the sterilized soils, indicating the successful suppression of microbial activity in the sterilized soils.

DOM production. We observed that aside from a few exceptions, during the 90 days of the incubation, the contribution of the added OM was not more than 30% of the total produced DOM. Previous studies by ^{13}C (Hagedorn et al., 2002) and ^{14}C (Fröberg et al., 2007) have suggested that addition of OM has only a small contribution to total DOM production. Instead, the majority of the produced DOM in the mineral soil layers originated from the extant and humified SOM source.

Our results did not show a considerable difference in the proportion of DOM obtained from the added OM/DOM source either in the presence or absence of microorganisms. This is consistent with the results reported by Michelzik and Matzner (1999) and Neff et al (2001) that the process of the DOM export in soil is primarily controlled by abiotic factors. For example, diffusion has been suggested to regulate the solubilization of OM from immobile to mobile phase (potentially solubilizable pool) and provides the constant replenishing process of DOM in soil (Tipping, 1998; Stutter et al., 2007) that controls microbial activity.

4. Conclusions

DOM production in soil is believed to be regulated by biotic-abiotic interactions. The “Regulatory gate hypothesis” (Kemmitt et al., 2008), suggests the complete abiotic control over OM solubilization with the limited access of microorganisms to only the “abiotically solubilized OM”. We observed that the presence or absence of the microorganisms had no impact on the proportion of the DOM produced from freshly added OM during the 90 day incubation period. Under the limited microbial activity and likely suppressed extracellular enzymes activity under our experimental conditions, we can conclude that DOM production is largely controlled by abiotic factors. Monitoring the properties of the produced DOM, we observed that although the impact of the microbial activity on the properties of DOM was attenuated by the relatively large flux of DOM in our experiment, the impact of biological transformations on the properties of the abiotically solubilized OM (DOM) seems unquestionable.

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SESSION 7: NOM AND HS IN WATER

The role of humic NOM in advanced water technology

F. H. Frimmel*, G. Abbt-Braun, F. Saravia, L. A. Tercero Espinoza
Karlsruhe Institute of Technology (KIT), Engler-Bunte-Institute, Chair for Water Chemistry, Germany
E-mail: fritz.frimmel@kit.edu

1. Introduction

Natural organic matter (NOM) plays a major role in traditional water technology. The importance is based on the ubiquitous occurrence of NOM in all types of aquatic systems and in addition on the refractory character and the manifold functionalities of the organic matter concerned. Often adverse effects have been identified for humic NOM and its organic compounds in water use and hence water treatment is needed including separation methods like flocculation or filtration, oxidative reactions and biochemical degradation [1].

In many cases the classical technical solutions are not satisfactory. E. g. the reset NOM-concentrations remaining after treatment steps can lead to uncontrolled biological and chemical reactions with problematic product formation. Recent investigations therefore have focused on the basic advanced methods to lower NOM concentrations and/or to avoid or minimize the formation of adverse reaction by-products.

The paper deals with the NOM specific aspects of:

2. Membrane methods, (ultrafiltration, nanofiltration) and the advantage of powdered activated carbon (PAC) application or flocculation in hybrid membrane modules [2]. Flocculation, e. g. with $FeCl_3$ as flocculant, is highly efficient to remove NOM of humic structure (Fig. 1).

The results show that 87 % of the organic matter get eliminated and it is mostly the low molecular size substances which remain in solution. It is also interesting to note that there is even a higher elimination yield for UV absorbing matter. The elimination rate for AOX (adsorbable organic halogen) forming precursors which is deduced from the respective AOX-formation potentials (FP) is relatively poor. To tackle this problem, hybrid membrane systems are promising. We investigated the performance of an immersed ultrafiltration module with PAC for the elimination of NOM from a river water which was heavily contaminated with pharmaceuticals.

It became obvious that the method is well suited to eliminate NOM together with the organic contaminants (Table 1).

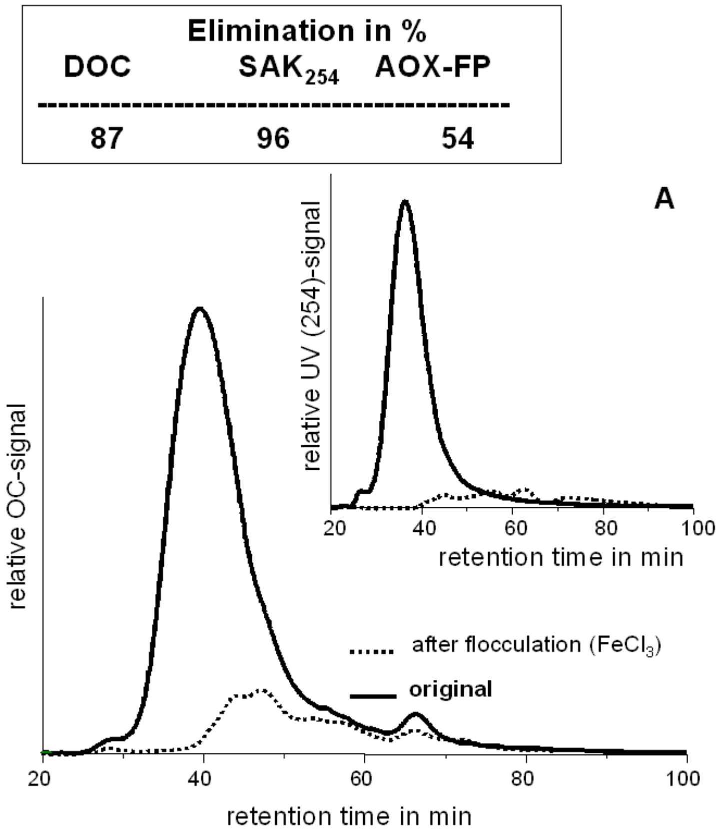

Figure 1: Elimination of brown water NOM by flocculation with $FeCl_3$ as revealed by OC (organic carbon) and UV detected SEC (size exclusion chromatography)

Table 1: Elimination of selected pharmaceuticals and NOM from river water using Norit SA-UF (PAC) and ultrafiltration

Filtration time h	NOM – DOC mg/L	PAC conc. mg/L	Carbamazepin µg/L	Clofibric acid µg/L	Diclofenac µg/L
0 raw water	20	0	3.9	6.6	5.8
0.5	0.8	18.4	0.07	1.3	0.2
25	0.4	27.6	0.03	0.1	0.02
50	<0.05	35.5	0.03	0.08	<LOD

3. TiO_2 as sorbent, photocatalyst and for disinfection in the presence of bromide (Br^-) in the raw water. Fig. 2 shows the fractions (F1, F2 and F3) of NOM as obtained by size exclusion chromatography (SEC) with UV (λ = 254 nm) detection [3]. The presence of 0.5 g/L TiO_2 in the NOM solution (ρ_0(DOC) = 11.5 mg/L) leads at irradiation with simulated sunlight to a rapid bleaching and degradation especially of the higher molecular substances.

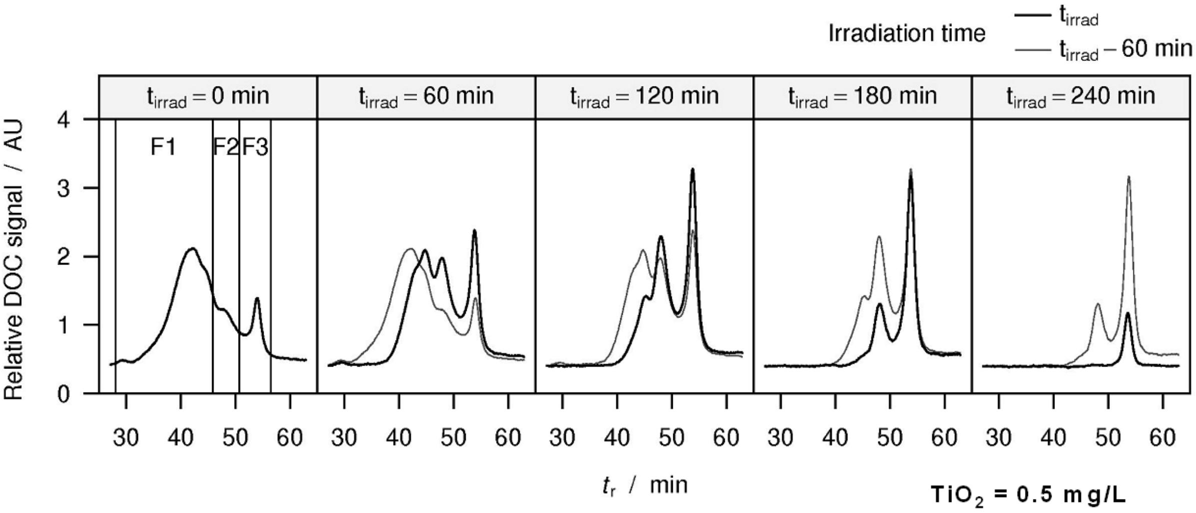

Figure 2: SEC-UV (λ = 254 nm) traces showing the time evolution of F1 – F3 under simulated sun light in the presence of TiO_2

The use of irradiated TiO_2 in aqueous suspension is also an alternative way for non chlorine based disinfection. Even though the attractive potential of the approach lies in minimized formation of disinfection by-products (DBPs), the formation of bromoform and/or high molecular organic bromine (AOX) has to be considered especially in raw waters with elevated bromide concentrations. Our investigations led to the suggestion of a reaction pathway and we could show that there is no significant formation of brominated DBPS. This underlines the attractivity of the alternative application of TiO_2 for disinfection.

4. Conclusions

Advanced technical methods have been developed for an effective treatment on NOM in raw water. Membrane filtration, hybrid systems and the application of photocatalysis turned out to be most promising. Even though we have reached the basis for a thorough understanding of the specific role of NOM in these water treatment relevant processes, the methods have not reached the level of large scale application, but they pave the way for a successful scale up of the elimination processes in the future.

Acknowledgements

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Rapid NOM Fractionation and Modified BDOC Analysis: Effective Tools for Water Treatment Performance Diagnosis and Holistic Optimization Efforts?

B. Eikebrokk[a*], G. Thorvaldsen[a] and T. Juhna[b]

[a]SINTEF, NO-7465 Trondheim, Norway; [b]Riga Technical University, LV-1048, Latvia
E-mail: bjornar.eikebrokk@sintef.no

1. Introduction

Natural organic matter (NOM) can control water treatment processes and affect corrosion, regrowth, and post precipitation processes within the distribution system. Knowledge of NOM properties and NOM behavior during treatment and distribution, and the linking of NOM characteristics to treatment performance and biostability are therefore very relevant issues in water supply. This paper demonstrates how relatively simple and easily applicable rapid NOM-fractionation and modified biodegradable dissolved organic carbon (BDOC) analyses can be applied as valuable tools in performance diagnosis and optimization efforts at existing water treatment facilities.

2. Materials and Methods

Diagnostic tools. Rapid NOM-fractionation (Fig. 1) was performed according to procedures developed in Australia [1] identifying four NOM fractions: 1) Very Hydrophobic Acids (VHA) adsorbed by DAX-8, 2) Slightly Hydophobic Acids (SHA) adsorbed by XAD-4, 3) Charged Hydrophilic Acids (CHA) sorbed by IRA958, and 4) Neutral Hydrophilic Acids (NEU) not adsorbed by any of the resins.

The BDOC analysis used here was a modification of the rapid single glass column BDOC procedure [2]. We used a novel set-up of six columns (Ø25 mm; H290 mm) coupled in series and filled with glass beads (Ø6 mm) as biofilm carriers. A three-way valve at the column effluent allowed water sampling for DOC analysis at certain time intervals. A variable speed pump allowed the water flow and the corresponding empty-bed contact times (EBCT) to be adjusted according to the biodegradability rates of the actual water/substrate. A scheme of the BDOC set-up is shown in Fig. 1. Measurements were performed in dark conditions at room temperature (22 °C). Between analyses of actual water samples, the columns were continuously fed with ozonated lake water. Prior to the analysis of a specific sample, the sample water was pumped through the columns for at least 18 hours for adaptation. An EBCT of 1.5 hours was found appropriate for typical Norwegian humic waters, thus yielding a total

EBCT of 9 hours in the six columns. The BDOC was then calculated as the difference between the inlet and column effluent DOC values. Thus, the typical BDOC versus EBCT relationships as shown in Fig. 1 provides information not only on the final BDOC-level, but also on the rates of degradation.

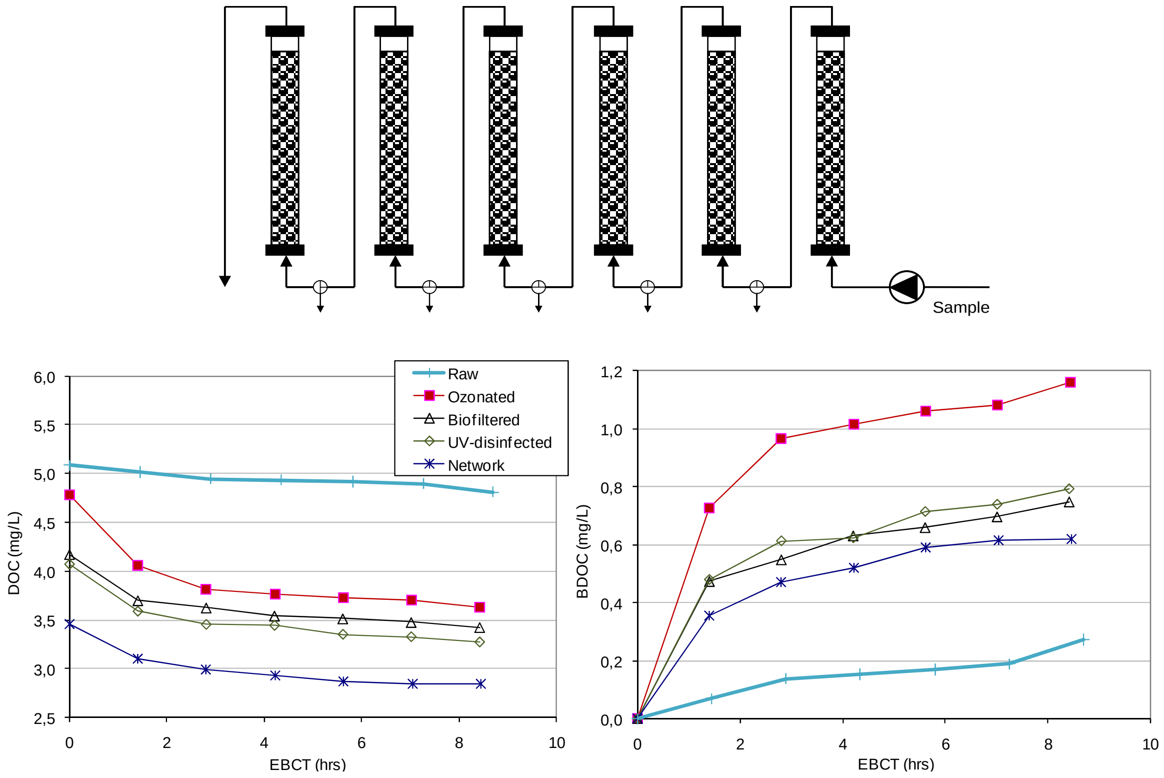

Figure 1: Scheme of the modified BDOC set-up using six columns-in-series, and illustration of typical BDOC results obtained from an ozonation-biofiltration facility (Aug 2009)

Test sites. The rapid NOM-fractionation and novel BDOC analyses described above were applied at two full-scale utilities using different treatment technologies: 1) An enhanced coagulation (EC) facility employing ferric sulfate for coagulation and contact filtration through anthracite-sand-crushed calcium carbonate, and 2) An ozonation-biofiltration (OBF) facility using activated carbon, sand and crushed calcium carbonate as biofilter media. Raw water and effluent water samples from different water treatment steps were taken at different seasons of the year, and samples from the distribution network provide information also on the links between treatment and distribution.

3. Results and Discussion

Ozonation-biofiltration (OBF). Figure 2 shows typical concentration profiles of BDOC, CHA and CHA+NEU during the OBF treatment process for samples taken in August 2009 with a raw water DOC concentration of 6.6 mg/L (DOC/BDOC degradation profiles for the same samples was shown in Fig. 1). Figure 2 demonstrates how the low concentration levels of

BDOC and hydrophilic NOM in raw water were significantly increased as a result of ozonation. Furthermore, Fig. 2 illustrates well that the biofilter with an EBCT design value of about 20 minutes is only capable of removing a fraction of the BDOC and hydrophilic NOM formed by ozonation. Thus the residuals are sent to the distribution system where they may act as substrate for regrowth. The relatively high heterotrophic plate counts (> 300/mL) often found in distributed water and the declining concentration levels from treatment to distribution (i.e. Net I and Net II) also indicates that substrate is available and is being used for regrowth. The results also show that UV-disinfection at normal dose levels (40 mJ/cm^2) does not significantly affect the concentration levels of BDOC and NOM fractions.

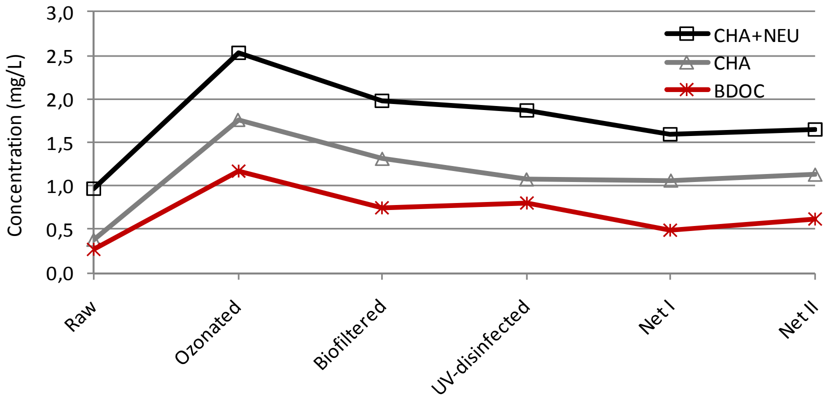

Figure 2: Concentration profiles of BDOC and hydrophilic NOM fractions during OBF treatment steps and distribution (Aug 2009)

Enhanced coagulation (EC). Figure 3 shows typical NOM fraction concentration levels in water samples from the EC facility. Raw water is dominated by the hydrophobic VHA fractions (> 70 %). Because the VHA fraction is effectively removed and the NEU fraction is not, treated water is dominated by VHA (40–50 %) and NEU (35–40 %). The residual concentrations are however very low, with the total DOC concentration reduced from about 3.5 to less than 1 mg/L. The BDOC levels were low in all samples and within the accuracy of the DOC analysis (±0.1 mg/L).

Linking NOM fraction concentrations and biostability. From a number of water samples taken at different seasons from Norwegian raw and treated waters, i.e. EC and OBF facilities, Figure 4 shows the good correlation obtained between BDOC and the hydrophilic NOM fraction concentrations (CHA+NEU). Also the BDOC and CHA fraction concentrations correlate well, while no correlation was found between BDOC and the hydrophobic NOM fractions (VHA, SHA). Thus the control of the hydrophilic NOM fractions seems to be a key element in the control of regrowth in drinking water distribution systems, in specific where disinfectant residuals are not being used for this purpose.

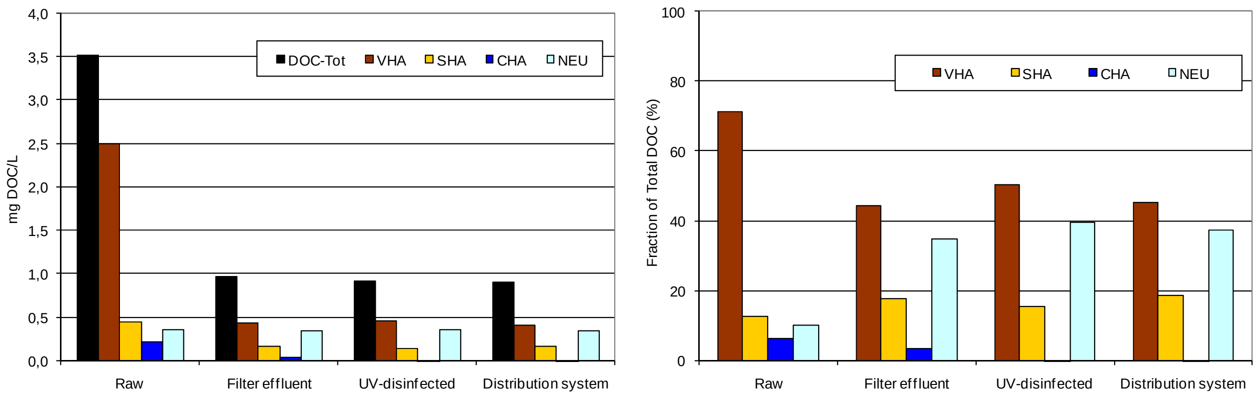

Figure 3: NOM fraction concentrations (mg/L) and relative distributions (%) at the EC facility (Aug 2009)

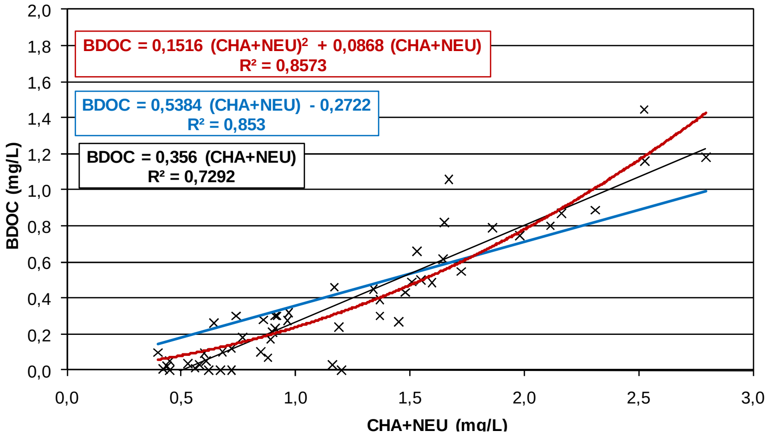

Figure 4: BDOC versus hydrophilic NOM fraction concentrations for a range of raw waters and treatments

4. Conclusions

The results from two full-scale water treatment plants in Norway show that NOM-fractionation and modified BDOC analyses can provide valuable information on water treatment process design, treatment performance and holistic optimization potentials. The results also demonstrate the treatability of different NOM fractions and the strong links between hydrophilic NOM and the regrowth potential (BDOC).

Acknowledgements

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Influence of Hydrophilic Organic Matter on Copper and Mercury Speciation and Bioavailability in Urbanized River

Gilles Varrault[a*], Benoît Pernet-Coudrier[a,b], Zeinab Matar[a], Bogdan Muresan[a], Yoann Louis[a], Marie-Héléne Tusseau-Vuillemin[c]

[a]Laboratoire Eau Environnement et Systèmes Urbain (LEESU), Université Paris-Est, 61 Avenue du General de Gaulle, F 94010 Créteil Cedex, France; [b]Department Water Resources and Drinking Water, Eawag, Überlandstrasse 133, 8600 Dübendorf, Switzerland; [c]Unité QHAN Cemagref, Parc de Tourvoie, BP 44, 92163 Antony Cedex, France
E-mail: varrault@univ-paris12.fr

1. Introduction

In aquatic systems, dissolved organic matter (DOM) constitutes a key component of the carbon cycle controlling the speciation, bioavailability and toxicity of trace metals [1,2]. Over the past few decades, many studies have been published regarding the capacity of DOM to complex copper. It is interesting to note however that the published data pertain mainly to the so called ‘‘humic substances” (HS) and demonstrate the ability of these substances to complex metals. HS are derived from oxidative and hydrolytic biodegradation of plants and animals [3] and they make up 40–60% of DOC in natural surface water [4]. In urbanized aquatic system, the hydrophobic characteristic of DOM is weaker as a result of various urban DOM discharges and the strong primary productivity induced by these discharges [5,6]. Because of the difficulty in isolating the hydrophilic fraction of DOM, very little information is available in the literature regarding hydrophilic DOM and their influence on metal complexation. The primary objective of this study is to exhaustively extract DOM from water samples then to fractionate it according to polarity criteria [7] and to further characterize the obtained fractions by means of various physico-chemical analyses. The second objective is to study binding affinity of each isolated fraction with copper and mercury. The third goal is to study the influence of every isolated fraction on copper bioavailability and toxicity.

2. Materials and methods

Three sites located in the Seine basin, upstream (located at *Méry/Marne*) and downstream Paris (located at *Andrésy*) as well as the treated effluent of the biggest wastewater treatment plant (called *Seine Aval WWTP*) of Parisian conurbation were sampled (from 250 L to 1000 L for each sample) during a dry weather period. Samples were filtered onsite through subsequent 10 µm and 0.45 µm polypropylene cartridge. The samples were then softened on sodium cation-exchange and treated by reverse osmosis in order to reduce the volume.

Sample filtration, softening and concentration were carried out in line and onsite so as to limit process duration and potential DOM biodegradation. The RO concentrate was then acidified and filtered back at the laboratory on nonionic macroporous Amberlite® DAX-8 resins (acrylate ester) and Supelite® XAD-4 (divynil benzene) combined with one another. This allows us to fractionate DOM into three fractions according to polarity criteria: hydrophobic (HPO), transphilic (TPI) and hydrophilic (HPI) fraction. Various physico-chemical analyses were carried out to characterize the isolated fractions: elemental analysis, spectrofluorescence, Pyrolysis associated to Gas Chromatography and mass spectrometry (PyGC-MS), Infra Red spectrometry (FTIR), Size Exclusion Chromatography (SEC).

Copper and mercury binding abilities of each isolated fraction were assessed by using respectively potentiometric methods (ISE) and competitive ligand exchange-solvent solvent extraction (CLE-SSE). Data were modelled respectively with the NICA-Donan model and WHAM (VI).

Influence of each isolated fractions on copper toxicity and bioavailability was assessed with respectively acute toxicity tests on Daphnia Magna (ISO 6341) and bioaccumulation tests on aquatic mosses (*Fontinalis antipyretica*). These tests were also carried out with the Suwannee River Fulvic Acid (SRFA) obtained from the International Humic Substances Society (IHSS) in order to compare properties of DOM from urban aquatic system with natural humic substances.

3. Results and discussion

Composition of DOM from urban aquatic systems. Due to WWTP discharges which contain more than 40% of hydrophilic fraction and less than 40% of hydrophobic fraction, a strong enrichment in the hydrophilic fraction was observed downstream of Paris (see Fig 1). Indeed the hydrophobic fraction represent more than 60% of DOC is upstream from Paris and only 40% downstream from Paris. The various physico-chemical analyses carried out show a lower aromaticity for DOM from urban aquatic system compared to SRFA. Molecules contained in DOM from urbanized river are smaller (Mn≈700 daltons) than SRFA (Mn≈2000 daltons). However, high contents of functional groups have been highlighted for hydrophilic fraction, particularly organic N and S groups mainly due to proteinaceous structures.

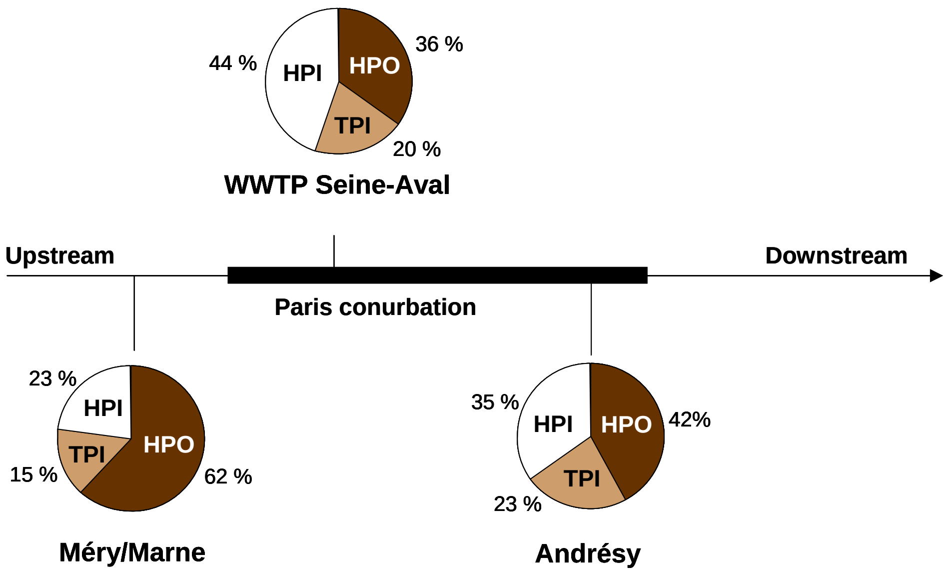

Figure 1: Evolution of DOC distribution among the different fractions from upstream Paris to downstream Paris

Influence of DOM on copper and mercury speciation. Hydrophilic fraction from WWTP effluents presented stronger binding capacities for copper and mercury than (i) hydrophobic fraction from less urbanized site (upstream from Paris) and (ii) Suwannee river fulvic acid from IHSS (see Fig 2). This feature was strongly correlated to the higher contents of organic N and S groups.

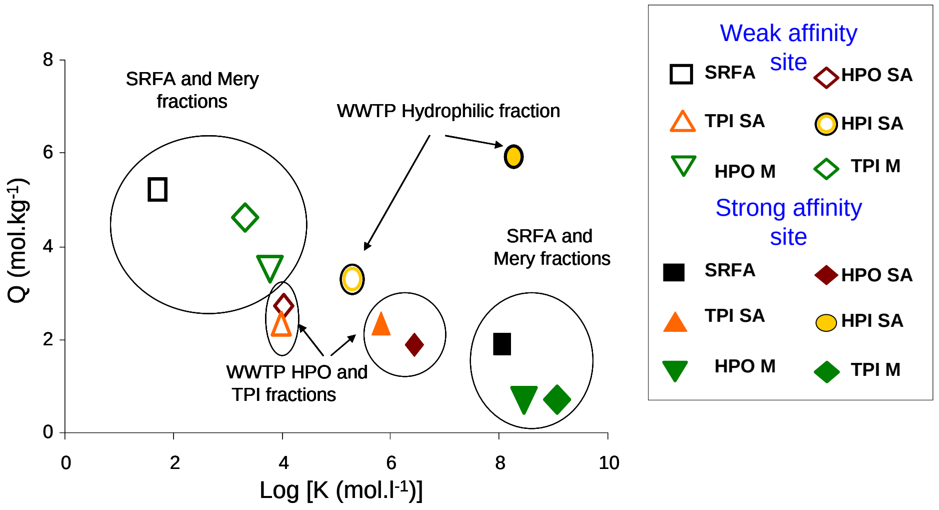

Figure 2: Binding sites amount and complexation constant of weak affinity sites and strong affinity sites of the HPO, TPI and HPI fractions from Suwannee River Fulvic acid (SRFA), Effluent of WWTP Seine Aval (SA) and Mery/Marne (M) samples

Influence of DOM on copper bioavailability. Biotests highlighted a significant copper bioavailability decrease in presence of DOM. However, the copper bioavailability predicted by the free ion activity model is much lower than that observed in our experiments. This probably highlights an important proportion of labile complexes Cu-DOM which are bioavailable.

4. Conclusions

In this work, the DOM isolated from upstream and downstream of Paris, as well as the effluents of the Parisian biggest Waste Water Treatment Plant (WWTP), was fractionated according to polarity criteria. Due to WWTP discharges, a strong enrichment in the HPI fraction was observed downstream of Paris. This hydrophilic fraction presented stronger binding capacities for copper and mercury than (i) hydrophobic fraction from less urbanized site (upstream from Paris) and (ii) Suwannee river fulvic acid from IHSS. This feature was strongly correlated to the higher contents of organic N and S groups. Furthermore, biotests highlighted a significant copper bioavailability decrease in presence of DOM partially explained by the free ion activity model.

Acknowledgements

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Dissolved Organic Matter Influence on Copper Speciation in the Stratified Oligotrophic Krka River Estuary (Croatia): Usefulness of Voltammetry and 3-D Fluorescence Spectroscopy Analysis

C. Garnier[a*], D. Omanović[b], A.-M. Blatarić[b], H. Zhao[a], V. Lenoble[a], G. Durrieu[a], N. Cukrov[b], I. Pižeta[b], S. Mounier[b]

[a] Laboratoire PROTEE, Université du Sud Toulon Var, BP 20132, 83957 La Garde, France;
[b]Division for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia
E-mail: cgarnier@univ-tln.fr

1. Introduction

Dissolved organic matter (DOM) and copper dynamics in the stratified Krka River estuary (Šibenik bay, Croatia) has been studied by combination of different analytical and modelling tools for sampling campaigns 2007-2009. Depth profiles of total dissolved copper and dissolved organic carbon (DOC) didn't show conservative trends vs. salinity in winter, increasing markedly at the freshwater/seawater interface probably due to metal release from particles and autochtonous DOM production. In summer, results showed a strong increase of Cu at the estuary surface layer, due to antifouling paint release associated to strong boat traffic, but slight DOC increase in the surface layer. Contrary to March-April period for which the dissolved organic ligands are sufficient to maintain the free Cu concentration at non-toxic level in the whole water column, in July this limit is over-passed in the surface layer for at least one order of magnitude, particularly at locations close to nautical marina.

2. Material and Methods

The Krka River estuary water column is permanently stratified because of a sheltered geography and the low tidal range of the Adriatic Sea. Due to low anthropogenic activities, this estuary is oligotrophic with ultratrace levels of heavy metals. However, during summer touristic period, the total Cu concentration increases, mainly because of Cu leaching from the antifouling paints [1]. According to depth variations of physico-chemical parameters (T/S/pH/%O2), samples were collected by scuba diving in April-2007 and March-2008, at 4 depths in front of the scientific marine station Martinska (site M). Additionally, in March-2008, samples were collected in the Krka River and the Adriatic Sea to obtain the 2 end-members of the estuarine water mixing. These samples were studied to (1) characterize the dissolved organic matter by DOC content and 3-D fluorescence spectroscopy (with PARAFAC analysis), (2) determine the dissolved Cu concentrations by DPASV, and (3) study the DOM/Cu interactions by logarithmic titration with analysis of the ASV-labile Cu fraction and further modelling to define the DOM binding parameters [2]. Furthermore, 3 field campaigns were performed in July 2008 and 2009. Water samples were collected during 3-4 consecutive days, 1-2 a day at 6 depths (according to physico-chemical parameters depth variation), at 2 distinctive sites (the "clean" site M; and a "polluted" one in a nautical marina, site S), leading to discrete and composite samples. These samples were analysed for DOM, total/dissolved Cu and DOM/Cu interactions by the previously described techniques.

3. Results and Discussion

DOC and Cu concentrations measured in April-2007 and March-2008 showed non-conservative variations, with a clear enhancement at the fresh/marine water interface (Fig. 1).

NOT AVAILABLE

Figure 1: Depth profiles of measured salinity, DOC and total dissolved Cu and calculated free-Cu concentrations in March and July 2008 at the M site

The main source of fluorescent material in the estuary is due to terrestrial inputs, and the additional OM produced by biological activities did not show any specific fluorescent signature. The increasing Cu concentration at the freshwater/seawater interface is controlled by the organic ligands, leading to calculated free-Cu concentrations lower than the toxicity limit of 10^{-11} M [3] (Fig. 1). In summer, important metal contamination of the estuary surface has been recorded, due to increasing boat traffic [1]. Under such conditions, predicted free-Cu concentrations over-passed the toxicity level in the whole water column, except at the bottom (Fig. 1). Results obtained for the summer campaigns demonstrated that site S is more polluted than site M for Cu but showed quite comparable values for DOC, amplifying the effect on free Cu concentration.

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The effect of a Changing Environment on the Role of DNOM on Phosphorous Loading

Rolf D. Vogt[a*], Alexander Engebretsen[a], Brian Lutz[b], Christian Mohr[a]

[a]Department of Chemistry, University of Oslo, Norway
[b]Program in Ecology, Duke University, Durham, NC 27708, USA
E-mail: rolf.vogt@kjemi.uio.no

1. Introduction

This study is a part of a research project, EUTROPIA - Watershed EUTROphication management through system oriented process modelling of Pressures, Impacts and Abatement actions —with the aim to produce a holistic decision analysis tool for management of eutrophication problem.

The European Water Framework Directive (WFD) requires a good surface water status and that the condition of all water reserves should not deviate largely from their natural condition within the year 2015. Eutrophication is usually the main cause for not fulfilling the requirements for good ecological quality in agricultural districts. Over the past 10 years the equivalent of more than 100 million USD has been spent on measures to reduce nutrient (phosphorus and reactive nitrogen) supply to a eutrophic lake (part of the Vansjø-Hobøl watershed) south of Oslo, Norway. The reductions of anthropogenic flux of nutrients have, however, not led to any amelioration of water quality. Leaching of phosphorus (P) from forests to the aquatic environment is mainly in the form of dissolved natural organic matter (DNOM). In a rather forested study sites in Finland, the P flux in organic form averaged 47% of the total dissolved P [1]. The amount of dissolved organic P (DNOM-P) relative to the DOC shows great spatial variation, but in a Swedish study they find that in forest streams the C/P ratio is about 2000 w/w [2]. Although DNOM is believed to be an important component of P cycles in aquatic ecosystem, DNOM-P is indeed less frequently studied compared to inorganic P compounds. As a result, much uncertainty remains regarding the governing factors for natural background P loading to watersheds. This knowledge is needed in order to evaluate the role of this P-fraction for the eutropication processes in general and in the evaluation of potential changes in the bio-availability of this DNOM-P related to environmental changes.

Long-term monitoring of surface water quality has shown increasing concentrations of DNOM across large parts of the northern latitudes [3]. Specifically, assessments of long data series from lakes that serve as raw water sources in Østmarka, a forested region comprising the headwaters of the Vansjø-Hobøl watercourse, show that the colour and concentration of dissolved organic carbon (DOC) in water leaching from the forests have increased and that the properties of DNOM have changed over the last 25 years [4]. This increase has inherently also resulted in enhanced fluxes of organically bound P transported by the DNOM.

Significant reduction in acid rain and changes in climate in eastern Norway have had a strong impact on water quality and biogeochemical processes in the catchment area. As a consequence of reduced sulphur emissions, the amount of non-marine sulphate deposition over southern Norway decreased by about 60% [5]. Long term decrease in anthropogenic input of sulphate, and also to some extent fluctuations in sea-salt (chloride), has reduced the ionic strength (I) of the percolating water. The amount of inorganic labile aluminum (Ali) that is washed out by acid rain from acidic soil is reduced accordingly and thereby the solubility of coloured DNOM is increased. Fluctuations in rainfall amount and intensity as well as summer and winter temperature are intensively studied as regionally important explanatory factors for the seasonal and inter annual variation in DNOM concentration and quality [1,4,6,7,8,9,10]. Winter temperature in the region has increased by about 2 °C [14]. This increased temperature has led to more recurrent winter thaw periods since the mean temperature in January and February is only -3 °C. Melt water that flushes over forest floor and agricultural land causes elevated DNOM fluxes. Inter annual fluctuations in water colour in headwater catchments ion the region are mainly explained by variation in predominant water flow paths depending on amounts and intensity of precipitation [11], both of which are predicted to increase due to climate change [12,13].

The main objective of this study is to assess why and how changes in the environment, such as anthropogenic loading and climate, may induce temporal trends and inter-annual fluctuations in concentrations of DNOM. This is then used to assess background leaching of DNOM-P to surface waters. Biogeochemical and hydrological processes (water flowpaths) governing the significance of DNOM to background nutrient load of P and its governing drivers are investigated by studies of soil-soil/water interactions in a small forested catchment (Dalen), draining into the eutrophic Lake Vansjø.

2. Materials and Methods

The study area for the EUTROPIA project is the Vansjø-Hobøl lake and catchments, situated south of Oslo, Norway. This watershed receives considerable attention due to its eutrophication problems causing frequent blooms of cyanobacteria. The watercourse was selected by the Ministry of the Environment as a pilot case for the practical implementation of the WFD. The region possesses wide spatial gradients with regard to land-use, urbanization, pollution and geological conditions. The watershed (690 km^2) stretches from small forested catchments with dystrophic ponds (Østmarka) to larger agricultural areas downstream. Monitoring data of water quantity and quality is available for the last 25 years.

Throughfall, soil water from genetic horizons including ground water (i.e. O-, E-, B-, and C horizons) and stream water is being monitored in one catchment (Dalen) in three soil plots along a topographic gradient. Dalen is situated below the marine limit after the last glacial period. The bedrock is covered with glacifluvial sands and some till. In the valley bottom there is a shallow minerothropic bog lying over a layer of clay.

Water samples are analyzed for absorbency at UV and Visible range, pH, conductivity, P fractions (free PO_4^{3-}, total P, particulate P, DNOM-P), alkalinity (only samples with pH>5.5) and aluminum fractions (only samples with pH <5.5), and major anions and cations. P fractions are determined by measuring free PO_4^{3-}, total P (after oxidation), dissolved P (after 0.7 μm filtration) and UV oxidizeable organic P (by exposure to UV light). The P bound to DNOM (DNOM-P) is determined as the difference between Dissolved P and free PO_4^{3-}. Particulate bound P is determined by difference between total P and filtered P. All sampling, handling and analysis (except P- and Al fractionation) are conducted in accordance to ISO standard methodology.

3. Results and Discussion

Ongoing analysis of water chemistry in Dalen shows that pH is rather low. This was not anticipated as the whole catchment is situated below the marine limit. Typically such areas possess high base saturation of the soil which is therefore well buffered. In the soil water on the slopes the pH increases from about 4 in the O horizon to only 5.5 in the C horizon of the Podzol profile, while in the riperian deposits along the valley floor the pH increases from 5.2 to 7.2 with depth in the soil profile. The DOC in throughfall and forest floor O horizons on the slope varied between 23 and 80 mg C/L, while in the riperian minerothrophic bog in valley bottom the DOC was only between 5 and 12 mg C/L. The lower values in the bog are likely due to that the soils are frequently water saturated due to an impermeable clay layer below 30 cm depth. Recharged mineral soil water from the slopes flushes therefore through the organic peat soils. In the mineral soil layers the DOC was less than 5 mg C/L and decreased with depth, as commonly found. Total P concentrations were surprisingly high (up to 259 μg/L) in throughfall and water percolating freely from the organic forest floor, though dropped down to typical value of 20 μg/L in the mineral soil. Significant amounts of labile organic and inorganic aluminum (Alo and Ali, respectively) were found in soil water samples with pH <5.5. Highest concentration of Alo (2 mg/L) is found in the E horizon of the Podzol, though typically on the slope the concentration down through the soil profile decreased from 1 mg/L to below detection limit in the C horizon. In the valley bottom significant amounts of Alo (250 μg/L) are only found in the organic surface layer. Samples with more than 800 μg/L Ali were found in the acid organic and AE layers of the midslope Podzol, while this declines to around 400 μg/L in the B horizon. The levels of Ali in the C horizon, with pH around 5.5, were below the detection limit. In the surface horizons in the riperian deposits the Ali concentration ranged from 60 to 180 μg/L.

The small brook draining the forested catchment possesses a dark brown colour due to DOC values of more than 20 mg C/L. Exposing stream water samples to strong UV irradiation reduces the DOC concentration with about 20%. Despite the rather higher pH in the riverine soils the water is commonly rather acid with pH values as low as 4.5. Total P levels in the stream (mainly organically bound) were about 25 μg/L. There is still a considerable leaching of aluminum with typical values of 350 and 125 μg/L for Alo and Ali, respectively. It is likely that the reduction in acid rain over the past

25 years has reduced this supply of reactive aluminum to western Vansjø. Assuming roughly that the 60 % reduction in sulphate leaching through the watershed has led to a similar reduction of aluminum, the concentration of inorganic labile aluminum during the worst acid rain period would have been more than 400 μg/L. Due to a very low solubility of $AlPO_4$ ($pK_{sp} \not\subset -20$) this Ali would remove a nearly equivalent amount of orthophosphate. The reduction in acid rain has therefore reduced an effective cleanser of phosphorus in the rivers. This may be one of several reasons that measures to reduce the supply of phosphorus apparently have not had the anticipated effect.

4. Conclusions

It is clear that reduction in acid rain and climatic factors such as increased frequency and intensity of precipitation as well as increased winter temperature play an important role in governing increased background DNOM and nutrient fluxes from forested watersheds.

Increased loading of DNOM causes inherently an increased flux of DNOM-P, which is believed to be partly potentially bio-available by mineralization through photooxidation in the lakes and via bacterial grazing. Reduced leaching of inorganic labile aluminum may be important in explaining some of the increased leaching of DNOM. Moreover it is likely that it has played an important role in removing orthophosphate during the acid rain epoch.

Acknowledgements

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Extraction of Arsenic, Iron and Uranium from Industrial Effluents by Natural Organic Matter

Anumaija Leskinen[a*], David Read[a,b], Peter Warwick [a]

[a]Loughborough University, Chemistry Department, Leicestershire, LE11 3TU, UK
[b]Enterpris Ltd, The Old Library, Lower Shott, Great Bookham, Surrey, KT23 3DW
E-mail: a.a.leskinen@lboro.ac.uk

1. Introduction

Purified kaolin (China Clay) is used extensively in the paper, ceramics, paint, and rubber industries. When freshly mined, China Clay is coloured cream through yellow to brown owing to the presence of impurities, primarily iron oxides, which affect its usefulness as an industrial coating and hence its commercial value [1,2]. Treatment to remove coloration by acidification and reduction of Fe(III) to Fe(II) can also release contaminants such as arsenic and uranium into aqueous effluents. Conventionally, re-oxidation and iron hydroxide precipitation is used to lower the concentration of contaminants in effluents to acceptable levels. However, the resultant sludge has to be disposed of, typically to lagoons. The objective of this investigation is to assess the viability of using natural organic matter (NOM) as an alternative to iron precipitation as a method for removing arsenic, iron, and uranium on the industrial scale.

2. Materials and Methods

The chemistry, mineralogy and particle size distribution of the selected NOM has been characterised in detail [3]. Samples of industrial effluent were collected over a one year period and analysed by ICP-OES and ICP-MS. The average pH of the effluents was 2.7 ± 0.2 and they contained 24 – 132 µg dm^{-3} arsenic, 65.4 – 75.8 mg dm^{-3} iron and 18.6 – 18.8 µg dm^{-3} uranium. The kinetics of extraction was determined by placing 0.5 g, 1.0 g or 10.0 g of NOM in contact with 100 cm^3 of solution. The mixtures were constantly stirred before sampling for analysis after intervals from 1 – 60 min (0.2 µm filter). The maximum extraction capacity (mol g^{-1}) for each contaminant was measured by placing 0.2 g of NOM in contact with 20 cm^3 of solution containing varying concentrations of arsenic, iron or uranium. After 24 hours, the concentration of each contaminant remaining in solution was measured. The ionic strength of the effluent was determined and thereafter control experiments were repeated using arsenic, iron and uranium in 0.1 mol dm^{-3} sodium nitrate solution.

The possibility of using NOM in a dynamic flow system was investigated by conducting a column experiment in which 50 g of NOM was placed into a glass column (2.6 cm diameter) and 100 cm^3 of effluent were pumped through the column with a flow rate of 0.2 cm^3 min^{-1}. The output from the column was fraction collected and the arsenic (ICP-OES), iron (ICP-OES) and uranium (ICP-OES or ICP-MS) concentrations were measured in each fraction.

3. Results and Discussion

The kinetic experiments showed that arsenic (100% within 20 min) and uranium (100% within 30 min) were quickly bound to NOM. The kinetic results for iron are shown in Figure 1. Within 60 minutes, the extraction of iron reached 100% in samples containing 1:10 and 1:100 solid liquid ratios. However, only 40% of iron was extracted in 60 minutes from samples with a solid to liquid ratio of 1:200.

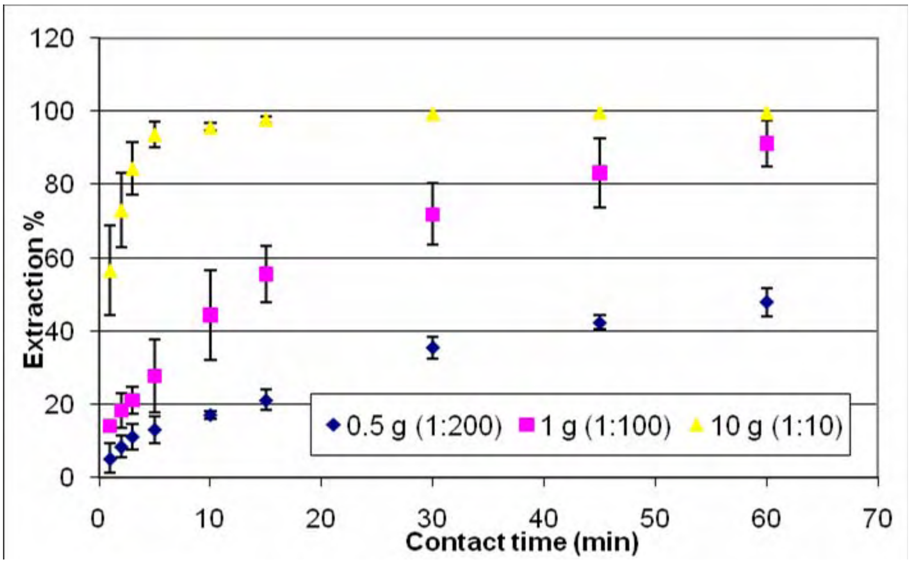

Figure 2: Extraction of iron from effluent by NOM. 100 cm^3 of industrial effluent was placed in contact with 0.5 g, 1 g and 10 g of NOM giving solid: liquid ratios of 1:200, 1:100 and 1:10, respectively

The extraction capacities of the NOM in electrolyte and industrial effluent are shown in Table 1. It should be noted that the value for arsenic extraction is lower than the actual value for the NOM extraction capacity because saturation of the NOM was not achieved.

The results of the column experiment are shown in Figure 2. The extraction of arsenic and iron was greater than 99% and greater than 90% for uranium.

Table 2: Arsenic, iron and uranium extraction capacities of NOM in electrolyte and industrial effluent

Solution	*Arsenic (mol g^{-1})*	*Iron (mol g^{-1})*	*Uranium (mol g^{-1})*
0.1 mol dm^{-3} $NaNO_3$	1.2E-05	5.2E-04	1.4E-04
Industrial effluent	≥ 3.4E-04	5.1E-04	1.8E-04

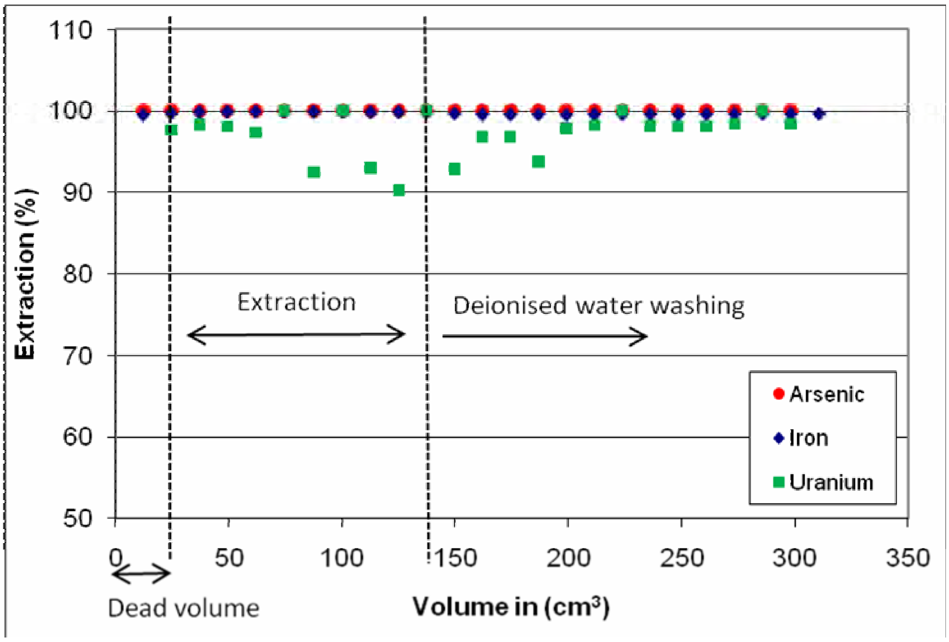

Figure 3: Extraction of arsenic, iron, and uranium from industrial effluent by 50 g NOM column

4. Conclusions

Batch and column experiments have shown that the NOM used in the experiments described above has potential for industrial use in effluent clean up even where the effluent is strongly acidic. This is unusual and appears to be a specific property of the source material. Similar experiments using other sources of humic matter were ineffective in removing metals below pH4.

Acknowledgements

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Absorption of different types of DOM to two marine phytoplankton species and its effects on Pb bioavailability

Paula Sánchez-Marín[a,b]*, Ricardo Beiras[a,b]

[a]Departamento de Ecoloxía de Bioloxía Animal, Facultade de Ciencias do Mar, Universidade de Vigo, Estrada Colexio Universitario s/n. E-36310 Vigo, Galicia, Spain; [b]ECIMAT, Universidade de Vigo, Illa de Toralla, E-36331 Vigo, Galicia, Spain
E-mail: paulasanchez@uvigo.es

1. Introduction

Dissolved organic matter, as humic and fulvic acids, has surfactant properties that explain its tendency to accumulate on abiotic and biotic surfaces [1]. DOM adsorbed to cell surfaces can modify cell membrane characteristics, as surface charge and membrane permeability [2], which can influence metal uptake by the organisms [3]. Despite these considerations, the most considered effect of DOM on metal bioavailability has been its metal binding capacity, which controls free metal ion concentration in aquatic environments [4]. It has been observed that Pb bioavailability for marine invertebrates is enhanced in the presence of humic acids (HA) [5] despite formation of Pb-HA complexes and decrease of inorganic Pb concentrations.

For three types of DOM: humic acids, fulvic acids, and suwanee river DOM, we have studied: (1) their binding properties with Pb in seawater, (2) their adsorption to two marine phytoplankton species and (3) their effects on Pb adsorption and internalization by these microalga. The chosen organisms, the diatom *Thalassiosira weissflogii* and the flagellate *Isocrysis galbana*, present very different cell surface structure: the first one is covered with a silica frustule while the second presents a naked cell membrane. We investigate if these differences can modify the effects of adsorbed DOM on Pb uptake.

2. Materials and Methods

Pb complexation with humic acids (HA) (Fluka, Aldrich; Steinheim, Germany), fulvic acids (FA) (Frediks research products, Amsterdam, Holland) and Suwanee River DOM (SRDOM) (IHSS) in artificial seawater was measured by Anodic Stripping Voltammetry (ASV), and complexation capacity (L) and conditional stability constant (K') of the complexes were obtained by fitting obtained data to a simple complexation model [6].

Thalassiosira weissflogii (clone ACTIN, strain CCMP 1336) and *Isochrysis galbana* (ECIMAT collection) cells were exposed to different concentrations of Pb, DOM or a combination of both, during 1 h at 25ºC.

After exposure, algae were isolated from solution by centrifugation (4000 rpm) and metal adsorbed to algal surfaces was extracted with EDTA 0.01 M to determine intracellular metal ($\{M\}_{int}$). Non-EDTA washed algae was used to determine total Pb (adsorbed + intracellular) associated with algae ($\{M\}_{tot}$). Metal contents in the algae were measured by ICP-MS after heat-acid digestion of the organisms.

DOM absorption to cell surfaces was evaluated by measuring the loss of DOM concentration (absorbance at 340 nm) in solution after algae exposure. DOM adsorption to the algae was described by a Henry adsorption isotherm: $\{DOM\}_{ads} = K_H \cdot [DOM]_{diss}$; being $\{DOM\}_{ads}$ the quantity of DOM adsorbed to cells (expressed in mg of HA/cm^2) and $[DOM]_{diss}$ the concentration of HA remaining in solution after algae exposure, i.e. in equilibrium, expressed in mg/ml.

3. Results and Discussion

Pb complexation by DOM. Pb was complexed in seawater by the three types of organic matter, decreasing the strength of complexation in the following order: HA>FA>SRDOM. Logarithm of the apparent conditional stability constants of the complexes (*log K'*) was 5.65 ± 0.11 for Pb-HA, 5.13 ± 0.23 for Pb-FA and 4.55 ± 1.12 for Pb-SRDOM. As an example, at 10 mg/L of DOM and 1 µM of total Pb, a 45% of the Pb was in the inorganic form if the DOM was either HA or FA and around a 30% in the presence of SRDOM.

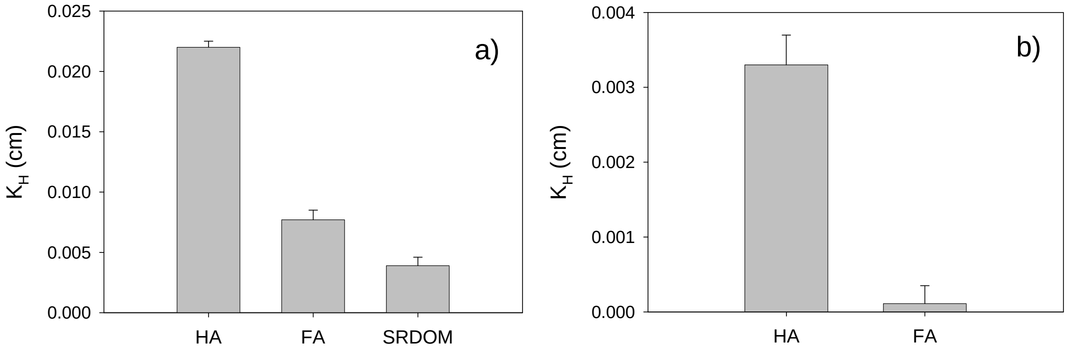

Figure 1: Henry adsorption constants of HA, FA and SRDOM adsorption to *T. weissflogii* (a) and HA and FA adsorption to *I. galbana* (b)

DOM adsorption to phytoplankton. The three types of DOM adsorbed to surfaces of *T. weissflogii* and the quantity of adsorbed DOM per cm^2 of algal surface was one order of magnitude higher for this alga than for *I. galbana*. HA were much more adsorbed to algal surfaces than FA or SRDOM, from 3 to 30 times higher depending on the organism considered (Fig. 1).

Effects of DOM on Pb adsorption and internalization. Pb accumulation by both organisms increased with Pb concentrations in solution showing a saturation plateau at dissolved Pb concentrations around 0.8 µM. Of the total metal accumulated by the algae during the 1 h exposure, the majority was adsorbed to surfaces, and only a 18% for *T. weissflogii* and a 13% for *I. galbana* corresponded to intracellular metal.

The presence of DOM did not affect significantly the quantity of $\{Pb\}_{tot}$ accumulated by *T. weissflogii* (fig 2.a) but it had an effect on Pb internalization (fig 2.b). $\{Pb\}_{int}$ decreased with increasing concentrations of humic or fulvic acids in solutions, in accordance with Pb complexation properties of theses substances. $\{Pb\}_{int}$ by *T. weissflogii* in the presence or absence of DOM was successfully predicted according to the labile Pb concentration in solution measured by ASV.

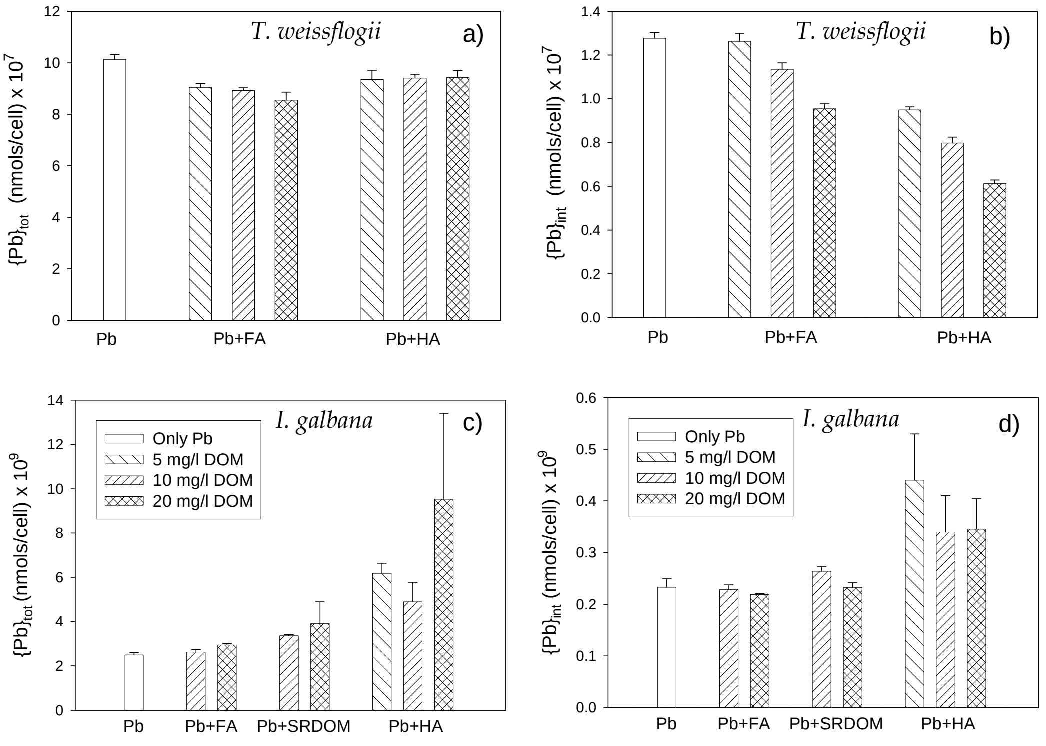

Figure 2. Total (intracellular + adsorbed) (a) and c)) or intracellular (b) and d)) metal determined in *T. weissfloii* (a) and b)) or *I. galbana* (c) and d)) after 1 h exposure to 0.4 µM of total Pb in the absence or presence of different types and concentrations of DOM

In the case of the flagellate *I. galbana*, none of the three DOM types tested had a protecting effect against Pb accumulation by the algae. On the contrary, $\{Pb\}_{tot}$ increased in the presence of DOM, especially for HA (fig. 2.c), the DOM type with higher adsorption to cell surfaces. Also Pb internalization increased in the presence of HA for *I. galbana*, in concordance with previous results with marine invertebrates [5].

The contrary effect that HA exerted on Pb internalization by both organisms leads to the idea that it is HA adsorption on cell membrane what causes an increase in Pb uptake by the organisms. HA adsorbed to the diatom may be adsorbed to the silica frustule and probably does not reach the cell membrane, and therefore HA effects on Pb uptake by the diatom are restricted to the effects on the chemistry of the external medium (Pb complexation).

4. Conclusions

DOM adsorbs to marine phytoplankton, and HA adsorption is from 3 to 30 times higher than that of FA or SRDOM. The accumulation of Pb on cell surfaces increases also in the presence of HA, and to a lesser extent in the presence of the other two types of DOM. However, DOM effects on Pb internalization are contrary for the two organisms tested. While Pb internalization by a diatom decreases according to Pb complexation, Pb internalization by a flagellate with naked membrane increases in the presence of adsorbed HA. It is hypothesized that the diatom frustule protects against direct effects (e.g. permeability changes) of DOM on cell membrane.

Acknowledgements

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Temporal and Spatial Variations of NOM in the Upper Mississippi River and Impacts on Membrane Filtration

Raymond M. Hozalski*and Bethany M. Brinkman
University of Minnesota, Department of Civil Engineering, 500 Pillsbury Dr. SE,
Minneapolis, MN, 55455 USA
E-mail: hozal001@umn.edu

1. Introduction

The concentration and composition of natural organic matter (NOM) in a water body at a given location and time results from a complex series of processes including inputs from the land surface of allochthonous NOM, production of autochthonous NOM within the water body as well as transport, transformation, and loss processes [1,2]. Natural organic matter concentration and composition varies spatially due to differences in climate, topography, land use, precipitation, and other factors. Temporal variations in NOM concentration and composition have been linked to precipitation events and daily cycles in the short term (hours) and seasonal weather changes at intermediate time scales (weeks to months). Changes in land use and climate are the likely drivers at longer time scales (years to decades). Natural organic matter is of particular relevance for the water treatment community because it has many possible effects including: fouling of membranes used for filtration, consumption of chemicals including coagulants, and reaction with disinfectants such as chlorine to form disinfection by-products.

The overall goal of this research is to investigate temporal (both short-term and seasonal) and spatial (resulting from land use) variations in the concentration and composition of NOM in the upper Mississippi River and the effects on membrane fouling. The results of this research could be used to optimize membrane selection and process operation, to develop models for real-time operation and control of water treatment systems, and possibly to develop guidelines for watershed management to minimize inputs of 'problematic' NOM.

2. Materials and Methods

Seasonal variations in water quality were monitored using on-line instrumentation installed at a water treatment facility in Minneapolis, MN, USA. Dissolved organic carbon (DOC) and ultraviolet light absorbance at 254 nm (UV_{254}) and 280 nm (UV_{280}) were measured in near real-time for eleven months in 2007. Specific UV absorbance (i.e. SUVA = UV_{254}/DOC) was computed to serve as an indicator of NOM composition.

NOM concentrations and composition for the upper Mississippi River and selected tributaries

(53 samples total) were sampled during base flow conditions in late summer 2008. Flow information was recorded and water samples were analyzed for total organic carbon (TOC), DOC, and ultraviolet absorbance from 200 to 400 nm. Selected water samples were analyzed by fluorescence spectroscopy to create excitation-emission matrices to better characterize the NOM composition in the water samples. Watersheds were delineated for each sampling site and overlaid with land use data using GIS software to determine the fraction of land area in the sub-watersheds devoted to each land use.

Eight of tributaries were selected for more intensive sampling over nine months in 2009. Two tributaries were representative of Minnesota’s four dominant land uses (forest, agriculture, wetland, and urban). Monthly sampling was performed to determine flow and measure TOC, DOC, ultraviolet absorbance, and fluorescent emission/excitation.

Finally, batch membrane fouling experiments were performed with stirred-cell filtration units using flat-sheet membranes.

3. Results and Discussion

From the near real-time monitoring, we observed short-term increases (over the time scale of hours) that were correlated with increases in river discharge during storm and snowmelt events. In addition, distinct seasonal trends in NOM concentration and composition were observed. Dissolved organic carbon concentrations were the highest in the summer months (June – August) and lowest during the winter months (December – February) when the river had relatively low flows and was covered by ice (Table 1). Specific Ultraviolet Absorbance values were greatest in the winter and decreased in the summer suggesting an increase in the fraction of autochthonous NOM in the summer months. The highest variability in NOM concentration and composition, as indicated by relative standard deviation, occurred in the spring and autumn for all measured parameters. Spring and fall are the seasons with the highest variability in water inputs to the river because of snowmelt and/or rainfall. The increases in DOC during spring, summer and fall are attributed to runoff water carrying allochthonous NOM into the river via overland flow or percolation through the riverbanks. Coincident increases in DOC and discharge during summer storm events has been reported for other studies of rivers and streams in the temperate United States [3,4]. SUVA positively correlated with river flow during the spring and autumn, but not in the winter and summer. As aromaticity and molecular weight of NOM are positively correlated with SUVA, it is expected that SUVA would increase as spring snow melt and autumn storms mobilize allochthonous NOM from the upper soil layers [5, 3].

Table 1: Mean (± standard deviation) DOC concentration, UV_{254}, and SUVA as a function of season for the upper Mississippi River

Season	**DOC (mg/L)**	**UV_{254} (1/cm)**	**SUVA (L/mg-m)**
Winter	7.08 ± 0.38 (*n* = 7.870)[1]	0.1534 ± 0.0111 (*n* = 2.029)	2.18 ± 0.16 (*n* = 1.826)
Spring	8.31 ± 2.24 (*n* = 16.488)	0.1609 ± 0.0544 (*n* = 5.063)	1.92 ± 0.55 (*n* = 4.050)
Summer	9.52 ± 0.81 (*n* = 7.099)	0.1384 ± 0.0714 (*n* = 13.263)	1.45 ± 0.42 (*n* = 4.969)
Autumn	8.31 ± 1.48 (*n* = 9.336)	0.1231 ± 0.0456 (*n* = 5.297)	1.48 ± 0.72 (*n* = 3.111)

n is the number of data points

TOC and DOC concentrations along the length of the Mississippi River remained relatively constant between 7 and 9 mg/L despite high concentrations (>20 mg/L) in several tributaries (Fig. 1). A preliminary analysis of the watershed information suggests that TOC and DOC are positively correlated with fraction of wetland area in the contributing watershed. For small streams and rivers, land use tends to be more important for NOM inputs than in-stream production [6]. Specific Ultraviolet Absorbance was positively correlated with fraction of forested land in the watershed and negatively correlated with grassland area. This is in agreement with other researchers who have noted that surface waters originating in forests have more terrestrially derived organic matter that is typically more aromatic [7]. Excitation-emission matrices showed no distinct differences for water samples obtained from forest-dominated versus agriculture-dominated tributaries, but peaks associated with microbial-derived NOM (i.e. proteinaceous material) were observed in the sample of effluent water from a large wastewater treatment plant. Fluorescence intensities for the samples ranged from 1.29 to 1.72, indicating that the organic matter was derived from both terrestrial (~1.4) and microbial (~1.9) sources [8].

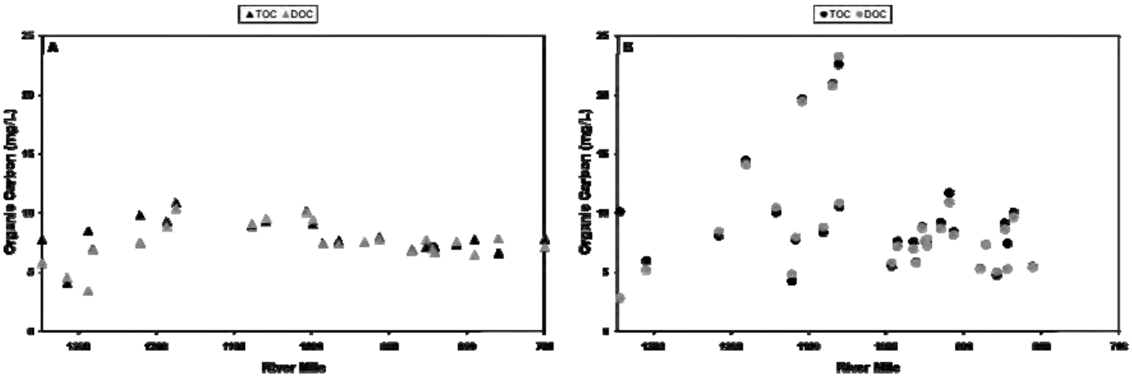

Figure 1: Total and dissolved organic carbon concentrations of the upper Mississippi River (A) and tributaries (B)

Monthly sampling of eight selected tributaries to the upper Mississippi River was performed to evaluate temporal changes in NOM in the sub-watersheds. DOC concentration was relatively stable over the March to November sampling campaign (Fig. 2). Specific Ultraviolet Absorbance exhibited a large peak in response to the spring snowmelt. In general, urban watersheds had lower SUVA values, suggesting that the NOM emanating from urban watersheds is more aliphatic in character. This is consistent with the negative correlation between SUVA and grassland area mentioned above.

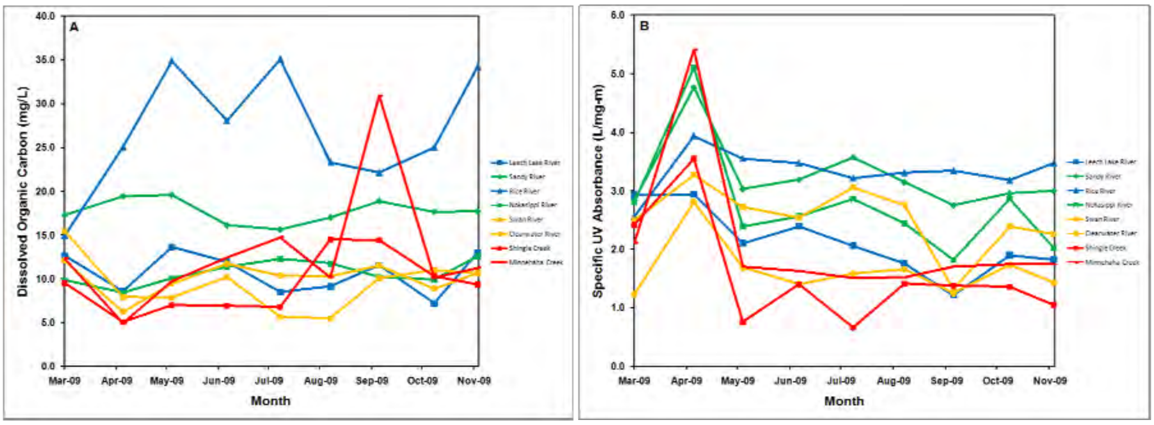

Figure 2: DOC concentration (A) and SUVA (B) for eight tributaries of the upper Mississippi River from March to November 2009. Land use is represented by colors: blue (wetland), green (forest), yellow (agriculture), and red (urban)

4. Conclusions

On-line analyzers were used to monitor NOM concentration and composition of the upper Mississippi River over eleven months in near-real time. Significant short-term variations were observed that would have been missed by typical weekly grab sampling regimes. Strong seasonal changes corresponding to spring snowmelt, summer production, and autumn rainfall were also observed. Although the investigation of spatial variability in NOM is ongoing, a preliminary analysis suggests that wetlands tend to increase DOC concentrations and that forests tend to increase the aromaticity and molecular weight of the NOM (i.e. SUVA). Membrane fouling experiments with Mississippi River samples and IHSS reference materials (Suwannee and Pony Lake) are ongoing.

Acknowledgements

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Unexpected Low Contribution of Litter Carbon to Dissolved Organic Matter. A Process Study Apply New LC-IRMS Techniques

Andrea Scheibe, Lars Krantz and Gerd Gleixner*

Max Planck Institute for Biogeochemistry, 07749 Jena, Box 100164, Germany
E-mail: gerd.gleixner@bgc-jena.mpg.de

1. Introduction

The origin and fate of dissolved organic matter (DOM) like humic and fulvic acids in soil depth profiles is still unclear. The general assumption that decreasing DOM concentrations with soil depth are related to selective degradation and sorption of individual compounds and increasing recalcitrance of the remaining compounds could not be proven by detailed chemical and isotopic investigations [1,2]. However, these investigations are very time consuming and more efficient methods are need to generalize these results are needed. Here, we introduce for the first time liquid chromatography on line coupled to isotope ratio mass spectrometry (IRMS) via wet chemical combustion to measure isotope ratios of dissolved organic carbon. We used this new technique to quantify the contribution of litter derived carbon to DOM collected in 5 cm soil depth over the season.

2. Materials and Methods

In November 2009 a litter exchange experiment started at 56 plots of an old growth beech forest in the National Park Hainich, Germany. Native litter of 28 plots was exchanged with identical amounts of ^{13}C labeled litter and glass suction plates (1 µm pore size, UMS, Munich, Germany) were installed at 5 cm soil depth. Water samples were biweekly collected with a suction pressure of 200 mbar below atmosphere collecting mainly free water from the soil [2].

^{13}C content of dissolved organic carbon was measured in a commercial LC-IRMS system (isoLink Delta XL plus, Thermo Finnigan, Bremen, Germany) with necessary modifications introduced earlier [3]. We first evaluated the conversion efficiency of the wet chemical combustion for the use dissolved organic carbon (see below) and applied the developed method to the samples from the experiment. All samples were free of dissolved inorganic carbon. 25 µL of soil solution was injected in the direct mode using a Surveyor HPLC system at 500µL/min flow of HPLC grade water (Milliprep). 1.7 M phosphoric acid and 0.55 M sodium peroxodisulfate solution were added at a rate of 30 µL/min and all organics were combusted at 100 °C to CO_2 that was on line transferred to the IRMS system.

3. Results and Discussion

We used a set of chemical of different stability to prove that the amount of CO_2 formed from the wet chemical combustion is quantitative (Fig. 1). Our results demonstrate that both conversion efficiency and isotopic content of the dissolved organics meet the reference values (Fig. 2). Consequently the new method can be applied to determine the isotopic content of dissolved organic carbon in soil solutions.

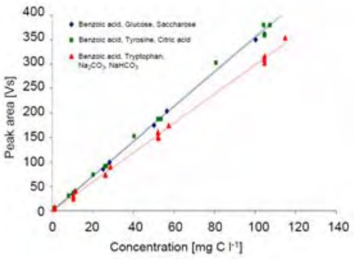

Figure 1: Conversion efficiency of different chemicals

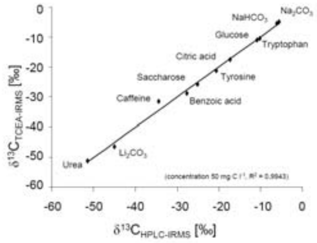

Figure 2: Comparison of isotope ratios from reference measurements

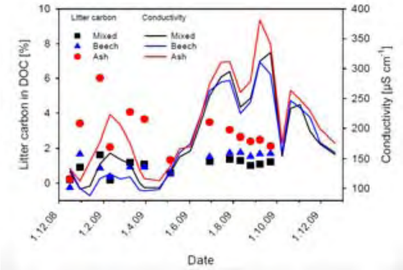

Figure 3: Conductivity and calculated litter-derived carbon of soil solution

Most interesting our results show only very low contribution of ^{13}C to dissolved carbon (Fig. 3). Only in the first month after application up to 6 % of DOM was labeled by ash litter derived carbon. This suggests that carbon derived from leave litter is mainly oxidized (data not shown) and only some carbon enters the soil organic pool. The introduction of new carbon removes older carbon from the mineral surfaces, similar to solid/liquid phase distributions, and liberates them to the liquid phase.

4. Conclusions

Our results highlight that LC-IRMS can be routinely applied to measure ^{13}C content of dissolved organic carbon at µL amounts of natural waters. Furthermore our results suggest that DOM is not related to plant carbon and that it might origin from transformed carbon like soil microbial carbon.

Acknowledgements

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SESSION 8: HS INTERACTIONS

More Than Fifty Years of Studies on Trace Metal Complexation by Humic Substances. What comes next?

Montserrat Filella
Institute F.-A. Forel, University of Geneva, 10 route de Suisse, CH-1290 Versoix, Switzerland
E-mail: montserrat.filella@unige.ch

It is currently accepted –and repeated in the introduction of many articles and books– that humic substances are largely responsible for the complexation of trace elements by natural organic matter (NOM) in waters and soils. For this reason, the study of trace element complexation by humic substances has been the focus of considerable research and an impressive amount of literature exists on the topic, with the earliest studies dating back to the late 1950s. However, in spite of the amount of work done and the fact that the use of speciation codes embedding humic binding models that imply a high degree of fitting is increasingly observed in some fields (e.g. bioavailability studies), trace element complexation by humics has been, and essentially remains, a controversial issue.

As a result of more than 50 years of data measuring, a relatively high number of studies that contain binding data for humics exists. However, these data have been obtained for humic substances which have been isolated by using different fractionation techniques, not always adequately characterised, under different conditions, for a wide variety of titration windows and often only for high concentrations. Moreover a wide range of interpretation models for the representation and quantification of the binding properties of humics has been developed, which adds a further difficulty for the practical application of existing data. Review papers on the topic do exist, though unfortunately some of them cannot be considered particularly useful either because they just reflect the opinion of the author of one of the models, which inevitably is biased in favour of it, or they just contain a compilation of models without any further analysis. No comprehensive compilation of published data exists and attempts at data compilation have usually been guided by the need to supply particular models. Citation practices show a strong ‘inbreeding’ referencing pattern as well as the endless referencing of a few papers. Very few elements have repeatedly been studied while most of the Periodic Table remains largely unexplored. Finally, the implementation of quality assurance practices in humic complexation measurement has never been attempted.

Significant advances in the field require: (i) the exhaustive evaluation of what is already known, which implies the compilation of all published data; (ii) the development of quality assurance criteria that could be applied both to the critical assessment of existing data and to the production of new one; (iii) the development of model-free data normalisation procedures that allow the comparison of existing and new data. It is important to stress that collection of binding data in the laboratory should be guided by their possible application conditions (e.g., extremely low trace element concentrations in non-polluted waters, high ionic strength in some industrial applications, etc.) but that raw data are intrinsically independent from any model (needed, on the other hand, for any practical application) and that as such should always be also published.

A similar situation exists, and a similar approach is suggested, in a companion field: the study of humic sorption onto mineral surfaces and particles. This type of studies is experiencing a renaissance with the current boom of work on the environmental behaviour of engineered nanoparticles. Undoubtedly, this field would also benefit from a better knowledge of what is already known and improved experimental practices.

Supramolecular Complexes of Humic Acids Including Complexation of Nano-particles. Spectrophotometric, Spectrofluorimetric, Capillary Electrophoretic and Mass Spectrometric Study

Josef Havel[a,b*], Eladia M. Peña-Méndez[c]

[a]Department of Chemistry; [b]Department of Physical Electronics, Faculty of Science, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic; [c]Department of Analytical Chemistry, Faculty of Chemistry, University of La Laguna, 38071 La Laguna, Tenerife, Spain
E-mail: havel@chemi.muni.cz

1. Introduction

Humic acids (HA), the most important natural part of soil/waters organic matter, are low molecular weight (MW) compounds as proved by MALDI TOF mass spectrometry [1] but easily forming high MW aggregates—evidenced e.g. by vapour pressure osmometry and conductivity [2]. HA are complexing almost all metal ions but also many organic compounds and/or xenobiotics forming supramolecules [3–5]. This is important for the fate of xenobiotics in nature and explanation of adsorption of pharmaceuticals and personal care products in soils [6]. HA are also strongly interacting with minerals and nano-particles [7]. As nanotechnology is increasing production of a variety of consumer products, release of nano-materials into the environment is raising. Therefore, this work deals with the study of the supramolecular complex formation of HA with organic compounds but also with various nano-particles, namely with nano-gold and nano-diamonds [8] — as both are nowadays extensively used as markers of the biomolecules. The formation of nanogold from ionic forms of Au (III) was also investigated.

2. Materials and Methods

Auric acid, $HAuCl_4.3H_2O$, and nano-diamond powder (<10 nm, purity 95%) were purchased from Sigma-Aldrich (Steinheim, Germany). Soil HA standard (IS102H) and Leonardite HA standard (IS101H) were from IHSS and coal-derived Czech HA standard was also used [9]. Mass spectra were measured using Kratos Shimadzu (Manchester, UK) and/or Auto-flex mass spectrometer of Bruker Daltonics (Bremen, Germany). The CE was carried out on the SpectraPhoresis 2000 TSP (Fremont, CA, USA).

3. Results and Discussion

Spectrophotometry/spectrofluorimetry proved that HA reduce Au (III) rather fast to nanogold particles of various size. An example of spectrophotometric kinetic spectra is given in Fig. 1.

The kinetics of nano-gold formation was studied under various conditions, from alkaline to slightly acid solutions and it was found that the reaction goes in several steps (3-5). By CE it was proved that nanogold (NG) particles interact with different HA fractions. Different types of the interaction were also found with nano-diamonds.

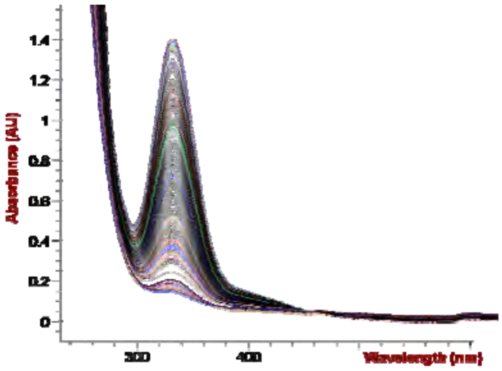

Figure 1: Spectrophotometric kinetic spectra showing the nano-gold formation

4. Conclusions

Supramolecular complex formation of humics includes not only various xenobiotics and/or organic compounds but also nano-particles. Gold nano-particles are stabilized in aqueous solution of HA and are bound to HA in different way– chemically to sulphur containing group and weakly when forming {HA, Au_m} supramolecular complexes, where Au_m is a gold cluster. Similarly, nanodiamonds are forming several different {HA, C_n} supramolecules (C_n is a nano-diamond cluster). The structures of the supramolecules are discussed. Supramolecular complex formation of nano-gold with soil HA might be important for the migration of gold in the environment.

Acknowledgements

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Influence of Lanthanide Concentration and the Presence of Competing Metal Ions on Europium and Gadolinium Speciation with Humic Acid Analyzed by CE-ICP-MS

Ralf Kautenburger[a]*, Christina Möser[a], Horst Philipp Beck[a]

[a]Institute of Inorganic and Analytical Chemistry and Radiochemistry, Saarland University,
Campus C4.1, 66123 Saarbrücken, Germany
E-mail: r.kautenburger@mx.uni-saarland.de

1. Introduction

For the safety assessment of a future waste disposal in deep and stable geological formations it is important to understand the radionuclide migration in the near- and far-field of a repository caused by an incident [1]. In this environment, natural organic matter (NOM) such as humic substances can play an important role by their complexation behavior for metal ions [2]. Capillary electrophoresis hyphenated with inductively coupled plasma mass spectrometry (CE ICP-MS) has been used to study the complexation behavior of Eu and Gd (as homologues of the actinides americium and curium) with humic acid. The influence of lanthanide concentration as well as the presence of competing cations like Ca, Mg and Al on the HA-complexation has been analyzed. The lanthanide speciation by CE-ICP-MS reveals weak and strong HA binding sites for the used trivalent lanthanides subject to the given lanthanide concentration. The influence of the competing alkaline earth ions can be assumed as relevant at very high concentrations only while aluminium at already low concentration represents a strong competitor to Eu and Gd in HA-complexation, and may affect toxic metal speciation and thus metal mobility in the geological barrier of a future disposal.

2. Materials and Methods

The HA used in the experiments is commercially available from Aldrich (St. Louis, USA; HA sodium salt). Milli-Q deionized water (18.2 MΩ) was used to prepare all solutions. The CE electrolyte buffer (100 mM acetic acid / 10 mM Na-acetate) was filtered and degassed before use. The single element standards of Cs, Eu, Gd, Ho as well as the used competing cations Mg, Ca and Al (as nitrates, Certipur or Suprapur quality) were obtained from Merck (Darmstadt, Germany). For the complexation experiments we used a mixture of different concentrations of Eu or Gd respectively, 25 mg L^{-1} HA and 10 mM $NaClO_4$. The experiments with competing cations are performed by adding Mg, Ca and Al together with the Ln and HA. The samples (10 ml) were mixed in a rotator (Stuart SB 3, Bibby Sterlin LTD., UK) for 72 h at 25°C before CE-injection. To reach the complex formation equilibrium between iodinated

HA and the Ln, a contact time of at least 48 h was necessary. CE was hyphenated by a homemade interface to ICP-MS to obtain a high sensitivity for the determination of the rare earth element species of europium and gadolinium with HA [3].

3. Results and Discussion

With CE-ICP-MS as speciation method both the free metal and metal humate can be detected in one analysis step. Furthermore, with this method it is possible to investigate the heterogeneity in HA-complexation for metal ions by evaluating the stability of metal-humates during the CE-separation. The speciation reveals strong and weak binding sites in the HA for the used Ln. The kinetically labile lanthanide humates (LnHA) partially collapse during the CE-separation due to the applied separation voltage of 30 kV and interactions of the sample with the acetate in the electrolyte buffer [4]. Due to this complex kinetics during the CE-separation, the influence of different Ln concentrations and the influence of competing cations were analyzed and the preliminary results are communicated here.

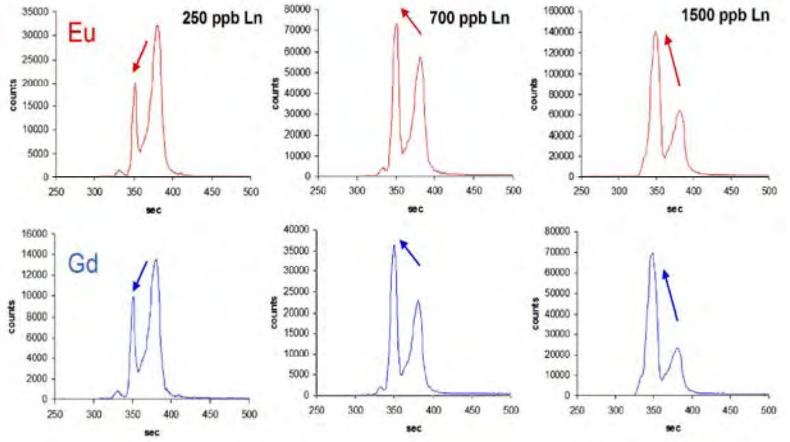

Figure 1: CE-electropherograms of different Eu and Gd concentrations: 250 µg L^{-1}, 700 µg L^{-1} and 1.5 mg L^{-1}, pH 5, ionic strength of the samples (I): 10 mM $NaClO_4$, c(HA): 25 mg L^{-1}

Different concentrations of Ln can cause different behavior in HA-complexation as shown in Fig. 1. CE-ICP-MS runs were taken at Eu- and Gd-concentrations between 50 and 3000 µg L^{-1} (0.32-19.7 µmol L^{-1}) at pH 5. With quantification of the Ln-signals in the CE-electropherogram, conditional stability constants for EuHA and GdHA could be calculated [5]. In the concentration range up to 250 µgL^{-1} the larger part of both Ln is HA-complexed, represented by the two rear signals (shown on the left side). For this low metal ion concentrations, mainly stronger binding sites of the HA are occupied by Ln. Thus, only a little part of the metal is extracted from the HA-complex during the CE-separation and the second signal is relatively small. At higher Ln concentrations (e.g. 300-1500 µg L^{-1}) more and more weak binding sites of the HA are occupied. During the CE-separation higher amounts of this weak HA-complexed Ln dissociate from the HA-complex (results in a significantly higher second signal, as shown in the middle and on the right side) until a nearly constant amount of the LnHA-complex (shown as peak 3 in the figure representing the amount of Ln complexed by strong binding sites) survived the applied high voltage of the CE-separation. At metal concentrations above 1500 µg L^{-1} a precipitation of the LnHA-complex can be observed. Consequently, one can state predications about the stability of the HA-complexes through qualitative analysis of the second signal in our CE-separations.

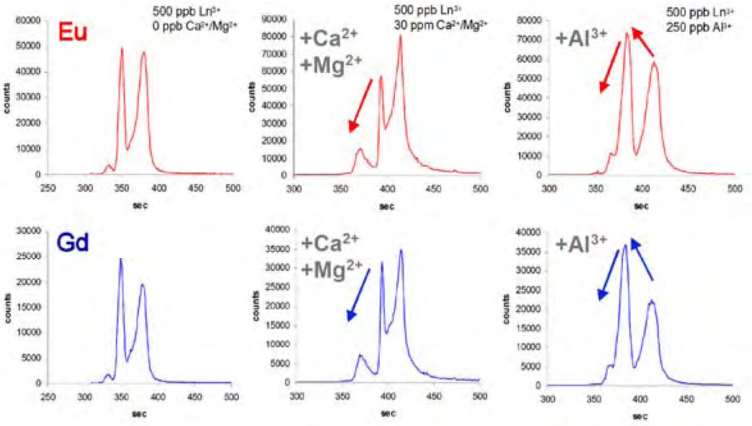

Figure 2: CE-electropherograms of Eu and Gd with different competing ion concentrations present, $c(Ln^{3+})$: 500 µg L^{-1}, pH 5, (I): 10mM $NaClO_4$, c(HA): 25 mg L^{-1}

Another effect is the influence of naturally occurring metals on the HA complexation by lanthanides. The influence of alkaline metals on the complexation behavior of Eu and Gd with HA is very weak because of the low charge and big size of alkaline ions. Ca and Mg are usually found in groundwater and at higher concentrations the effect on the HA complexation is more distinct than for Na and K because of their bivalency. Al may be present due to clay decomposition. Comparing the electropherograms shown in Fig. 2 without the influence of competing cations (on the left side) and the ones with 30 mg L^{-1} Ca and Mg (in the middle), a significant increase of the first signal can be observed. Lanthanides are replaced by alkaline earth metals particularly in the weak binding HA fractions. The second peak decreases for the benefit of the non HA-complexed metal represents by the first signal. This is due to the higher stability of the Ln^{3+}-complexes in comparison to Ca- and Mg-humate complexes. For 250 µg L^{-1} Al present (on the right side) the ratios are different. The first and the second peak increase at the cost of the third one. This means that Al is able to displace Ln from stronger binding sites to the weak binding sites of the HA and to uncomplexed metal. However, the almost completely amount of the displaced Ln is found in the second peak, which shows that it is still HA-complexed metal before the CE separation. With other analysis methods, such as ultrafiltration, the changes in complexation behavior could possibly not have been detected because these methods only distinguish between complexed and uncomplexed metal, no matter how stable the complexes really are in presence of competing ions.

The replacement of Eu and Gd from the HA complexes correlates with the alkaline earth concentration (not shown here). However, even at a 200fold excess concentration of Ca and Mg the HA-complexed amount of the Ln is still over 75 %. In contrast to these findings, aluminium as a small, trivalent cation represents a strong competitor to Eu and Gd (and therefore also for trivalent actinides) in HA-complexation. At only 250 µg L^{-1} Al, which is e.g. a concentration that can be detected due clay decomposition when working at low pH-values and 0.1 M ionic strength, a large amount of the Ln are displaced from stronger binding complexation sites of the HA. Thus, it is advisable to work at preferably natural conditions when performing sorption and complexation studies involving clay minerals in standardized laboratory experiments. In nature, the presence of strong competing metal ions like Al e.g. due to weathering of clays can cause a (re)mobilization of HA-complexed heavy metal pollutants such as actinides into the aquifer.

4. Conclusions

In summary, the results of our studies clearly indicate the need for the knowledge about the exact geological conditions at a potential nuclear waste disposal site. Many parameters can influence toxic metal migration, metal concentration, pH-value and presence of competing cations as well as NOM like humic substances being the most important factors which can lead to a migration of the toxic heavy metal pollutants through the geological barrier.

Acknowledgements

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Modeling the Humification Process and the Humic Acid Molecule

Marios Drosos, Yiannis Deligiannakis*

Lab of Physical Chemistry, Dept. of Environmental & Natural Resources Management ,
University of Ioannina, Seferi 2, 30100 Agrinio, Greece.
E-mail: ideligia@cc.uoi.gr

1. Introduction

The structural origin of the properties of humic acids (HA) is of great importance. Natural HAs consist of several major functional groups, predominantly carboxylic (R-COOH) and phenolic (R-OH), as well as carbonyl (C=O) and quinoid (1). Carboxylic and phenolic functionalities determine the H-binding, metal binding and charge properties of HA (1). In addition, all HA of diverse geographic and climatic origins contain stable organic radicals (2) which are considered to play a key-role on the transformation of HA itself (1,2). Presently, the oxidative polymerization of polyphenols in soils is thought to be among the major processes of formation of natural humic substances (1). Given the acknowledged structural complexity and heterogeneity of natural HA (1), an approach adopted by various groups was the production of ‘‘synthetic HA” (3,4,5). Recently we have presented a method for performing a HA-Like Polymer (HALP) at significant yields with no use of a catalyst (5). Herein we present a detailed study off the mechanism of the polymerization in conjunction with S.E.C. data of the evolution of the molecular size of the HALP. Finally the relation of the structures with physicochemical properties is discussed.

2. Materials and Methods

The reagents used were 3,4,5 trihydroxybenzoic (gallic) acid (Lot. G7384), benzoic acid (Lot 812548-124), 4-hydroxy-benzoic acid (Lot 505151-443). Redox potential of the solution Eh was measured with a Metrohm platinum redox electrode (type 6.0401.100). The electrode was calibrated using a reference solution of 10 mM K3Fe3(CN)6: 10 mM $K_2Fe_2(CN)_6$ having Eh = +228 mV vs. Ag/AgCl, saturated with 3 M KCl. O2 concentrations in solution were measured by a O2-meter (OXI-340-B, Germany) with a O2-selective electrode type OX-325. Elemental composition (C, H, N, S) of freeze-dried sample was determined with a Perkin Elmer Series II CHNS/O 2400-Analyzer. Electron paramagnetic resonance (EPR) spectra were recorded at liquid nitrogen, with a Bruker ER200D spectrometer, equipped with an Agilent 5310 A frequency counter. g-Values were calibrated versus DPPH, g = 2.0027, which was also used as spin standard for radical concentration. The polymerization was carried out in aqueous solution bubbled with air, at controlled pH 10.5. The redox potential was continuously monitored by the redox electrode immersed in the solution. For a 1:1 mol solution 31.47 g of gallic acid (GA) and 28.53 g of protocatechuic acid (PA), were suspended in 3 L of milli-Q water. The mixture was continuously stirred for 12 h at 26 _C in a closed glass jar, under controlled air bubbling. Then the pH was adjusted at pH 10.5 with NaOH and then the mixture was further bubbled with a continuous flow of natural air under stirring for up to 10 days. During that period the, initially transparent, reaction mixture, turned to green and then to a dark-brown/black color. Finally, the IHSS procedure for HA isolation was applied (6). In brief, the pH was adjusted to >1 with HCl and the solution was allowed to precipitate for 72 h. We found that this time was optimum for obtaining maximum precipitation. Then, the precipitate obtained after centrifugation at 4000g/15 min, was washed with Milli-Q water to remove monomer residues and Cl^-, freeze-dried at -66 ^oC with a CHRIST-ALPHA 1-2LD freeze drier, and stocked until further use.

3. Results and Discussion

During polymerization the redox potential E_h of the reaction solution varied, depending on the reactants, see Figure 1. In the presence of GA, E_h decreased from an $E_{initial}$ = +125 ± 5 mV towards an equilibrium value of _143 ± 3 mV after 8 days. This can be attributed to the known interaction of GA with O_2 to form radicals (7,8) at alkaline pH, at the expense of O_2 oxidizing equivalents (7,8). Indeed, the O2 measurements showed that dissolved O_2 concentration was decreasing from an initial maximum value of 4.9 ± 0.1 to _0mg L_1. PA behaved differently: after 90 h, E_h was practically constant near _15 ± 5 mV with a small O_2 consumption. The binary system GA:PA showed a $E_h(t)$ profile intermediate between GA and PA with a final E_h and O_2 value comparable to that of GA. Thus the present $E_h(t)$ data show that (a) GA and GA:PA appear to be able to consume oxidizing equivalents, while PA is not. (b) GA alone does not produce a HALP.

From the Elemantal Analysis of Table 1, we notice that the O content of HALP is at the upper O-percentage encountered in natural HA.

Table 1: Elemental Analysis of HALP

C	H	O	N
44	2.6	53.4	-

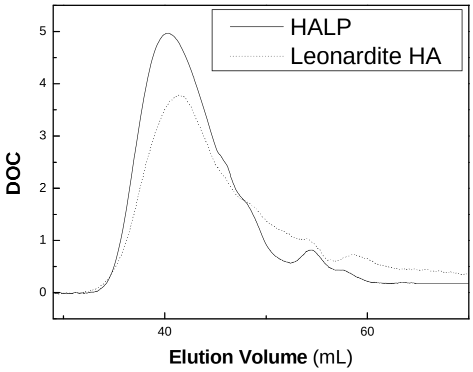

Figure1: Time dependence of solution potential Eh during polymerization, for GA:PA = 1:1, GA = 32 mM, PA = 32 mM

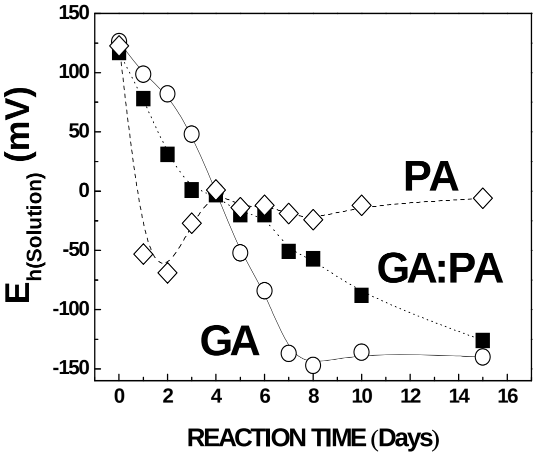

Figure 2: SEC-OCD of HALP and Leonardite HA

From the SEC-OCD chromatography we see that the chromatographs of the standard IHSS Leonardite humic is almost identical to the HALP. The nominal Molecular Size is found to be 5000Da for both materials using PEGs for calibration standards, linear between 200 and 20.000Da. The SEC-OCD chromatography of the HALP has been followed for Reaching time 76hr up to 200hr (Figure 3).

From Figure 3 several Molecular Size fractions can be resolved. These match the Molecular Size of multiples of P-G units. The time evolution of these PG oligomers is presented in Figure 4. Initially a product is formed rapidly resulting from Gallic (G) and Protocatechuic (P) acid to form a Protocatechuic-gallic acid dimer (PG). This dimmer

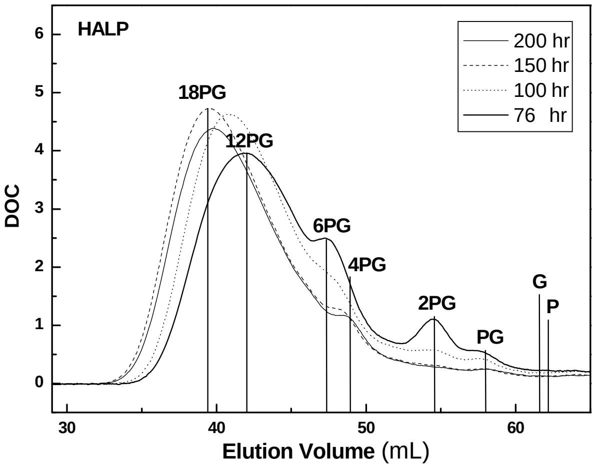

Figure 3: SEC-OCD of the Kinetic of HALP production versus time. P is indicator for protocatechuic acid, G is indicator for gallic acid and PG is indicator for the dimmer protocatechuic-gallic acid

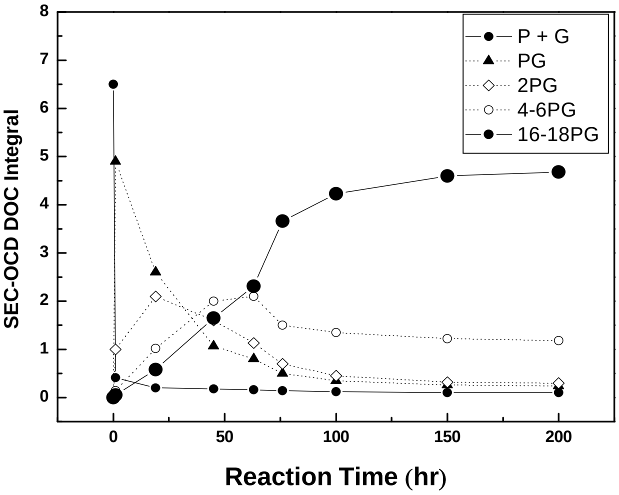

Figure 4: Time-evolution of the abundance of the PG oligomer formed during formation of the PG-HALP

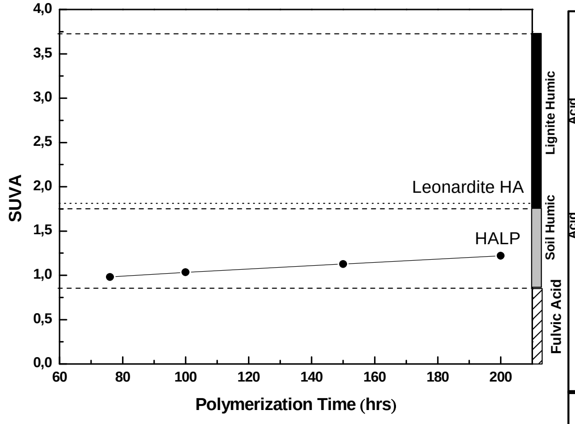

Figure 5: SUVA rates of the HALP fractions during polimerization as revealed by SEC-OCD

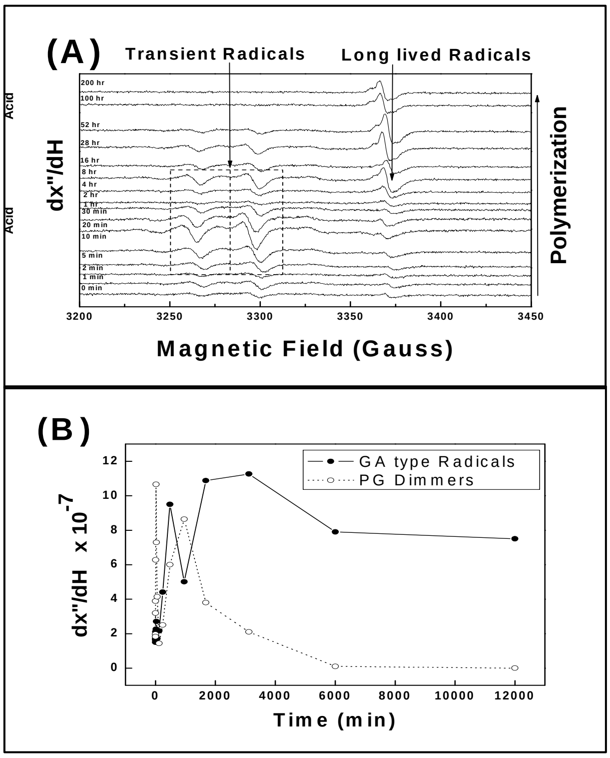

Figure 6: A) EPR Signals of HALP during polymerization. B) Percentage of the radicals of 6A

continues to polymerize until 150hrs. A 2PG unit attains maximum at 20hrs, then forms fractions of 4 and 6PGs. These fractions are maximized at 65hrs. Finally the major HALP fraction which consists of 16-18 PGs is maximized at 150hrs with no further evolution.

In Figure 5, we present the SUVA ratio of HALP during the polymerization process. We observe that SUVA tends to increase according to the time of polymerization. This shows that during polymerization the aromaticity of the HALP is increasing.

The EPR signals of the Reaction mixture (at pH12, Eh 150mV) are shown in Figure 6. We observe two types of Radical Species (I) A transient Radical species, which is maximized at time reaction ~ 10min, then disappearing at t > 52hr. (II) A long lived Radical formed at t >8h The long lived EPR signal has a g value 2.0047±0,0002 and ΔH=6Gauss. This EPR signal is typical for phenolic π type radicals and resembles strongly with the indigenous radicals of the natural HAs (3,8,9). The g value indicates that the molecular identity of the radicals is similar in gallic acid, HALP and natural HA.

The concentration of the stable free radicals at pH 12 is 8.9×10^{17} Spins/g for HALP and 2.4×10^{17} Spins/g for Leonardite HA. At pH 5 the EPR signal decreases to 1.26×10^{16} Spins/g. The same phenomenon happens in natural HA as well (9). This phenomenon is reversible. This reversibility is not observed in gallic acid solution. This is a unique property of polymeric matrices such as natural HA and HALP.

4. Conclusions

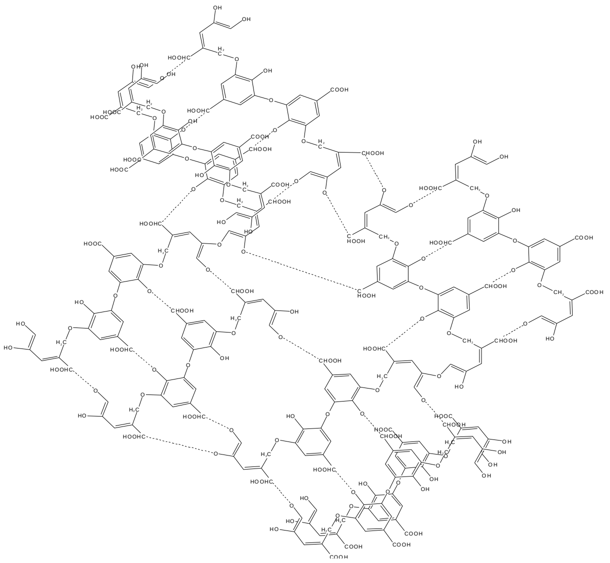

Figure 7: A Plausible structural model for GP-HALP

Oxidative copolymerization of Gallic acid and protocatechuic acid at 1:1 ratio provides a water soluble humic-acid-like polycondensate (HALP) which mimics fundamental physicochemical and spectroscopic properties of natural HA. This material can be used as a model for HA molecule. The polymerization procedure can be used to reveal the mechanisms of humification. It is based on stable radicals' formation. The conditions used were oxidative. In the GP-HALP model humification tends to create more aromatic structures via polymerization of polyphenol acids coupled together to form esters. These HA precursors bind together via hydrogen and π-π bonds, to form HA. A model is outlined in Figure 7.

Acknowledgements

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SESSION 9: HS AND NOM PHYSIOLOGICAL ANDBIOLOGICAL EFFECTS

Mechanisms involved in the beneficial action of humic substances and natural organic matter on plant development

J. M. Garcia-Mina

CIPAV- Roullier Group
Department of Chemistry and Soil Chemistry, Faculty of Sciences, University of Navarra.
E-mail: jgmina@timacagro.es

A number of studies have shown the ability of natural organic matter (NOM) in general and humic substances (HS) in particular, to affect the development of plants and microorganisms in many different natural ecosystems and agro-ecosystems. Regarding plants, these NOM and HS effects were expressed in both root growth and architecture, and shoot growth. However, these effects were different in intensity and quality depending on several intrinsic and extrinsic factors associated with HS structure and concentration, plant species and soil properties. Two main mechanisms have been proposed to explain the beneficial action of NOM and HS on plant growth. An indirect effect expressed through the improvement of plant nutrition by increasing soil nutrient availability, principally some micronutrients (mostly Fe); and a possible direct action affecting the transcriptional and post-transcriptional regulation of several enzymes and molecular transporters in the root.

In this communication the relationships between the effects of HS on root development, shoot development and plant nutrition, are discussed. This study is developed in the context of the links existing between the signal role of some nutrients and the hormonal balance in both root and shoot.

Microbial Carbon Turnover and Dynamics from Biochemically Diverse Microbial Groups in Temperate and Tropical Forest Soils

Heather M. Throckmorton[a], Jeffrey A. Bird[b], William R. Horwath[a], Mary K. Firestone[c]

[a]Dept. Land, Air and Water Resources, UC, Davis, One Shields Ave., Davis, CA 95616, United States; [b]School of Earth and Environmental Sciences, Queens College CUNY, 65-30 Kissena Blvd., Flushing, NY 11367, United States; [c]Dept. Environmental Policy and Management, UC, Berkeley, 137 Mulford Hall #3114, Berkeley, CA 94720, United States.
E-mail: hthrockmorton@ucdavis.edu

1. Introduction

Microorganisms represent an important source of actively cycling carbon (C) in terrestrial ecosystems, yet little is known of the relative importance of microbial biochemistry as a factor influencing C stabilization across different microbial groups. This project utilized uniformly ^{13}C-labeled, biochemically diverse, non-living microbial residues as substrates in a reciprocal transplant experiment in Blodgett Forest (BF), a temperate forest in the Sierra Nevada, and Luquillo Forest (LF), a tropical forest in Puerto Rico to examine the stability of unique microbial biochemistries. These sites represent diverse ecosystems that are known to support substantially different microbial communities, and provide an excellent opportunity to look at the effects of climate, parent material, and microbial community on factors affecting humification processes.

2. Materials and Methods

Temperate and tropical microorganisms from four biochemically contrasting groups (fungi, actinomycetes, bacteria Ggram (+), and bacteria Ggram (-)) were isolated from BF and LF and grown in 99-atom-percent ^{13}C media. Enriched microorganisms were autoclaved and lyophilized lypholized and lysed microbial cells were added back to soil at both sites. Treated soils were excavated at 5 time points over a span of 3 years at BF and 2 years at LF. Soils were separated by ultrasonic/liquid density fractionation into light, aggregate-occluded, and mineral-associated fractions. Whole soils and fractions were analyzed for total C and ^{13}C using combustion gas chromatography-isotope ratio mass spectrometry (GC-IRMS). Soils treated with temperate fungi were analyzed using Curie point pyrolysis- gas chromatography-mass spectrometry- isotope ratio mass spectrometry (Py-GC-MS-IRMS) to determine compound-specific turnover.

3. Results and Discussion

Microbial C dynamics differed substantially between the two sites, with microbial C levels stabilizing at 35% of input C after 12 months in BF, while in LF microbial C did not to begin to stabilize until about 16 months at less than 10% of initial input C. Average mean residence time (MRT) was 5.21±1.11 years in BF and 2.22±0.45 years for LF soils.

Although microbial treatments did not differ in their relative partitioning among soil physical fractions, there was some evidence for slower overall decomposition of bacteria Gm+ and fungi relative to bacteria Gm- and actinomycetes; however, the effect was not substantial. In BF soils, there was a significant difference among microbial groups recovery in soils (*P*p = 0.0384), whereby more C derived from bacteria Ggram+ and fungi were recovered relative to bacteria Ggram-, while recovery of actinomycetes-C did not differ from any of the other groups (Ffig. 1).

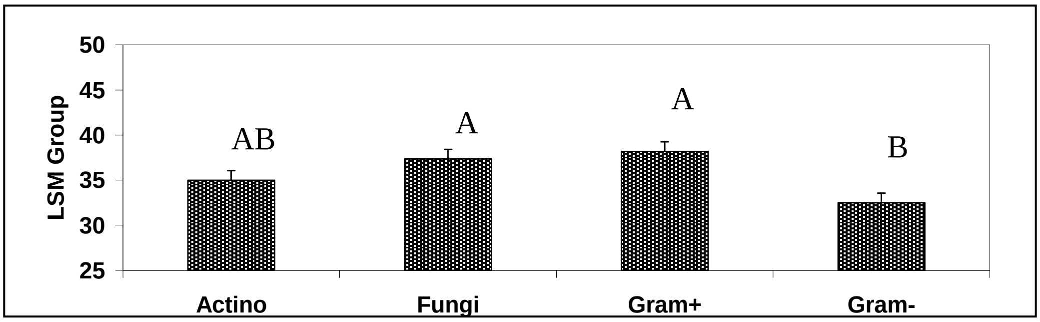

Figure 1: California (BF): least squares means for percent microbial C recovered in soils for microbial groups throughout the course of the study

For LF, there were also significant differences in microbial C recovery; however, in this case the origin (temperate vs. tropical) of the microbial group influenced whether this effect was significance. Results indicate that significantly more temperate fungi, temperate Ggram+, tropical actinomycetes, and tropical gram- were recovered relative to temperate bacteria Ggram-, and the remaining three groups (temperate actinomycetes, tropical fungi, and tropical Ggram+) did not differ from any of the other groups (Ffig. 2).

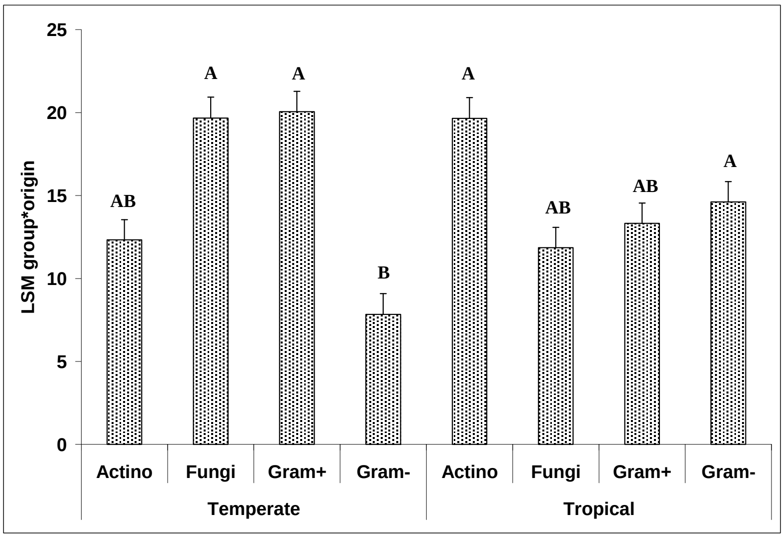

Figure 2: Puerto Rico (LF): least squares means for percent microbial C recovered in soils for microbial treatments (by microbial group*origin) throughout the course of the study

Physical fractionation of soils indicate that, despite the substantial difference in microbial C turnover between the two sites, microbial C turnover in the fraction occluded within aggregates and in the fraction associated with mineral surfaces did not differ between the two sites; the only physical fraction where microbial C turnover differed between the two sites was the light fraction, which is unassociated with the mineral matrix (Ffig. 3).

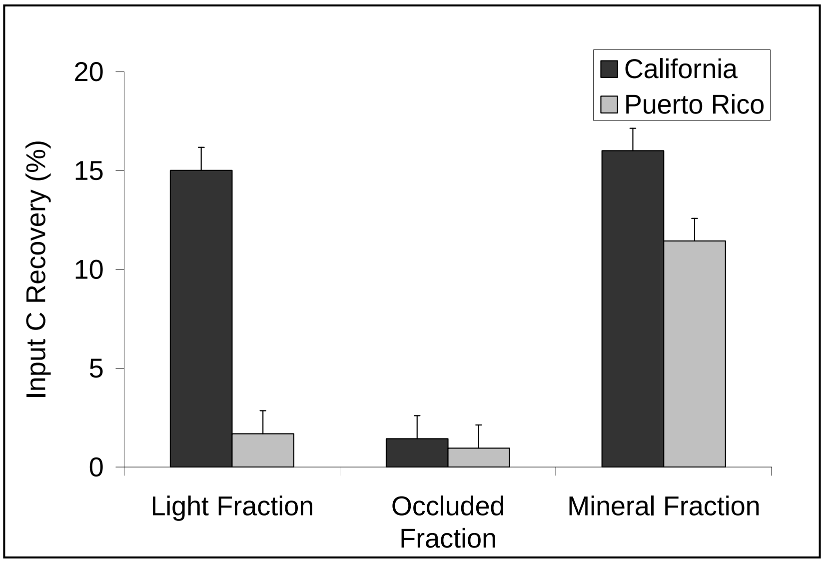

Figure 3: Plot of the least squares means for the site by fraction interaction

Pyrolysis- results indicated that there were differences in the turnover of different compound classes. Polysaccharide and protein biomarkers exhibit a rapid initial decline in enrichment, and begin to stabilize after the second mesocosm excavation for both sites. For California soils, proteins exhibit an increase in relative recovery of input microbial C from the third to fourth mesocosm excavation. For both sites, lipids were relatively stable throughout the course of the study, and for the tropical site exhibit an initial increase in enrichment from the input microbial C, which may be explained as microbial resynthesis of new compounds (figs. 4a and 4b). These results agree with other studies that suggest that proteins and polysaccharides turnover rapidly, while lipids become relatively more prominent over time in microbial C cycling (Baldock et al, 1989, Hopkins et al, 1997).

4. Conclusions

There were substantial differences in microbial C dynamics between the two sites, with faster turnover in the tropical site. However, the only soil fraction where microbial C turnover differed between the two sites was the light fraction, while there was no difference between the occluded and mineral fractions. These results suggest that the effect of climate on microbial C turnover is limited to organic matter unassociated with the mineral matrix. Although there were no differences in the partitioning of microbial C among soil fractions, there were some differences in turnover among microbial treatments; however, the differences were not substantial.

Pyrolysis results indicated that there are differences in the turnover of compound class biomarkers, which highlights the importance of examining these dynamics at the molecular level. Py-GC-MS-IRMS proves to be a valuable tool with the potential to follow the dynamics of specific compound classes in isotope tracer experiments.

Acknowledgements

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Soil Humic Substances Inhibit Fibril Formation and Cellular Internalization of the β Form of the Prion Protein

Maurizio Polano [a], Alessandro Corsaro[b], Tullio Florio[b], Maria De Nobili[a*]

[a]Dipartimento di Scienze Agrarie ed Ambientali, Università di Udine, via delle Scienze 208, 33100 Udine, Italy; [b]Laboratorio di Farmacologia, Dipartimento di Oncologia, Biologia e Genetica, Università di Genova, viale Benedetto XV 2, 16132 Genova, Italy
E-mail: denobili@uniud.it

1. Introduction

Persistence of the infectivity of prions, the causative agents of transmissible spongiform encephalopaties, which include bovine spongiform encephalopathy in cattle, scrapie in sheep and chronic wasting disease (CWD) in cervids, was demonstrated first by Brown and Gadjusek (1). More recently, Seidel *et al.* (2), showed that oral administration of contaminated soil can induce scrapie in Syrian hamsters. Notwithstanding the long persistence of infectivity in soils and the large spread of potentially infected lands, prion diseases have become endemic only in geographically limited regions. Reasons for this remain unknown. Our work helps to elucidate a possible mechanism by which, interaction with humic substances, could contribute to reduce risk of horizontal transmission via soil ingestion.

2. Materials and Methods

We examined the interaction among the 90-231 fragment of the recombinant human prion protein, produced as previously described (3) and soil HAs (Elliot soil HA standard 1S102H), and soil FAs (Elliot soil FA standard 1S102F). Controlled thermal denaturation was used to induce the conversion of HuPrP from α-helix to a β-sheet. HuPrP was incubated for 1h at 53°C. SH-SY5Y human neuroblastoma cells were treated in DMEM/2% FCS for 24 hours with a single administration of either HuPrP in native conditions and HuPrP denatured for 1 hour in absence or in presence of FAs (0.2μg/μl) directly to the cell medium. After 3 washings in PBS, cells were lysed and supernatant protein concentration was evaluated using the Bradford assay. Presence of HuPrP in both soluble and insoluble fractions was detected by Western blot, using the 3F4 monoclonal antibody (Signet Lab).

3. Results and Discussion

Thermally denatured HuPrP showed a higher binding to Thioflavin T, confirming an increment of β structures. In the presence of FAsoil (0.2μg/μl) thermal denaturation caused a strong inhibition of the acquisition of β structures as testified by decrease of the tryptophan intrinsic fluorescence of HuPrP in the presence of (up to 90–99%, as compared to HuPrP in the native conformation). This clearly demonstrates that HS interact with HuPrP either quenching fluorescence or inducing a conformational change that may favor protein aggregation in amorphous complexes. To further demonstrate that the HS-HuPrP interaction leads to an impairment of β structuration and the fibrillogenic pathway, we investigated the effects of FA on fibrillar aggregation by ThT epifluorescence microscopy. The presence of FAs strongly reduced the number of fibrils formed during incubation for 5 days at 37°C, while some macro-aggregate were detected. Particle length distribution analysis showed that not only the number of fibril-like aggregates was reduced but also their length: in the absence of HS proto-fibrillar structures grouped from 1.2 to 2.1 μm, conversely, in the presence of FA, the length of the aggregates was limited to 0.75–0.87 μm.

βHuPrP neurotoxic effects are related to its cell internalization that, in turn, depends on an oligomeric β-rich conformation. Thus, we analyzed, whether the presence of FA during HuPrP conversion may interfere with its internalization efficiency in the SH-SY5Y human neuroblastoma cells. The rate of internalization of HuPrP thermally denatured in the presence of FAsoil was significantly reduced as compared to the β structured peptide. These data strongly suggest that, in the presence of HS, HuPrP is prevented from the acquisition of properties that, through intracellular accumulation may lead to neuronal death.

4. Conclusions

Our data suggest that HuPrP-HS complexes, being unable to be internalized in living cells should prevent or at least limit the oral transmission of the infective particles. These effects may play a relevant role in reducing prion infectivity in soils.

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SESSION 10: OM STABILIZATION AND HUMIFICATION PROCESSES

Evolution of Modern Concepts of the Compositions of Humic Substances

Michael H.B. Hayes

Chemical and Environmental Sciences, University of Limerick, Ireland
E-mail: Michael.H.Hayes@ul.ie

1. Introduction

In a recent review Hayes (2009) has outlined some of the evidence which shows that the importance of soil organic matter (SOM) has been recognised since the 'dawn of agriculture'. It was known in antiquity that the more recalcitrant components of SOM, which we would now regard as humic substances (HS), arose from the transformations of plant and animal debris in the soil environment. Because developments in chemistry were well ahead of those in microbiology, emphasis in the 18th and 19th centuries focused on chemical processes that would give rise to HS.. Advances were impeded by the difficulties encountered in isolating HS and in fractionating the components. At the first international meeting of IHSS Hayes (see Clapp et al., 2005) reviewed the development of procedures for the isolation of HS and outlined the principles involved in the solvation of humic molecules.

The modern era in HS studies had its beginning in 1786 when the German chemist, Achard, obtained an amorphous dark precipitate when a potassium hydroxide extract from a peat was acidified. There was considerable emphasis in the first half of the 20th century on Browning reaction products formed from condensation reactions between reducing sugars and amino acids (the Maillard Reaction), and between glycine and compounds such as methylglyoxal, as formulated by Enders. Some reaction products could match, as outlined by Clapp et al. (2005), the elemental compositions, cation exchange capacities, and thermochemical properties of HAs. Nuclear magnetic resonance (NMR) techniques were not then available to show that the aromatic components were different from those in soil HAs.

The ligno-protein theory by Waksman and Iyer (see Clapp et al. 2005), in the 1930s, had adherents for a generation. That indicated that oxidized lignin when reacted with protein (casein) gave a product similar to HAs. This author repeated that work during the 1950s (Hayes, 2009; Clapp et al., 2005) and obtained a product with properties similar to those described by Waksman and Iyer. However, the Differential Thermal Analysis (DTA) data showed that the products were mixtures of the oxidized lignin and casein, and the thermograms were not comparable with those for HAs isolated from a sapric histosol.

2. Isolation and Fractionation of Humic Substances

The HA and FA fractions of organic matter are still widely recognised. After Bremner (see Clapp et al., 2005) had pointed out in the middle of the 20th century that organic isolates from soil in base undergo oxidation in alkaline media it has became general practice to carry out such extractions in an atmosphere of dinitrogen gas. Then, after the foundation of the IHSS in 1981, and following the first International IHSS Conference at Estes Park, Colorado, in 1983, it became accepted that the classical FAs should be regarded as the FA fraction. When that fraction is passed on to a (poly)methylmethacrylate resin column the polar components elute through and the FAs are recovered from the materials sorbed on the column. The polar components are retained by and recovered from styrenedivinylbenzene resins placed in tandem. Humic acids when dissolved in base, diluted to < 50 ppm, and acidified to pH 2 can be subjected to the resin fractionation, as described in Clapp et al. (2005). This process is akin to that used to isolate HAs from waters. In this way polar components associated with the HAs can be removed, at least partially.

The author and his colleagues have used exhaustive extractions at increasing pH values, (pH 7.0, 10.6, and 12.6 (0.1 M NaOH), followed by exhaustive extraction in 0.1 M NaOH + 6 M urea. Finally the residue is thoroughly washed with water, dried, and extracted, exhaustively, with dimethylsulphoxide (DMSO) + concd H_2SO_4 (6%). This extract is diluted with water to pH 2. The dilute base-insoluble precipitate formed can be regarded as humin. Graded porosity gels, introduced in the 1960s enabled size fractionation

3. Emergence of Concepts of Sizes of Humic Molecules

Humic substances were traditionally considered to be macromolecular and polyelectrolytic. Over 40 years ago an extensive study led by Swift and Posner (see Cameron et al., in Clapp et al., 2005), using gel filtration and membrane fractionation procedures, carefully fractionated a HA from a sapric histosol into several cmponents and subjected the isolates to ultracentrifugation. A plot of molecular weights (MW) obtained against the frictional ratio (f/f_{min}) values (shown in Figure 1) gave a linear relationship for fractions with MW values of the order of 400 000 Daltons. Larger components had MW values > 1.2 x 10^6 Daltons. The relationship shown in Figure 1 was considered to be indicative of random coil conformations depicted by A (Figure 2). More recently Simpson et al. using DOSY NMR spectroscopy have shown that HAs consist of associations of relatively low MW materials. The association concept has also been promoted by the work of Wershaw, Piccolo, von Wandruszka and others (all referenced in Clapp et al., 2005)

4. Emergence of Concepts of Compositions

From the 1960s, and later, Morris Schnitzer and his colleagues have provided GCMS data that identified a vast array of digest products from the chemical degradations of HS. In the mid-1970s Hayes and Swift (see Clapp et al., 2005) predicted, based on chemical degradation mechanisms, structural types in the humic molecules that could give rise in the digest products. It was, however, the development of NMR techniques that has given the major stimulus to our awareness of compositions of HS. Careful examination of the spectra in Figure 3 shows compositional differences between the fractions isolated at the different pH values. Note the similarity between the sample isolated in base + urea and that isolated at pH 12.6. The urea released HAs sterically trapped, or hydrogen bonded in the humin matrix.

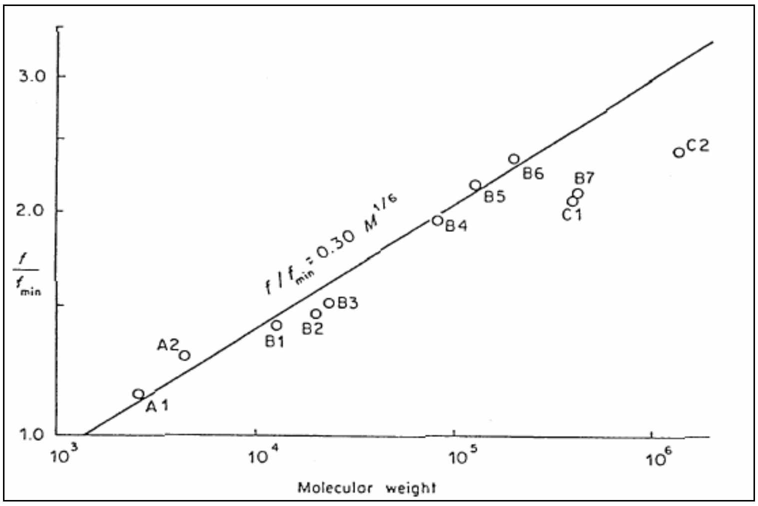

Figure 1: Relationship between the frictional ratio (f/f_{min}) and the molecular weight for different humic acid fractions isolated from a Sapric Histosol. The line is the theoretically derived rerlationship between frictional ratio and molecular weight for a randomly coiled polymer (after Cameron et al., 1972; see Clapp et al., 2005).

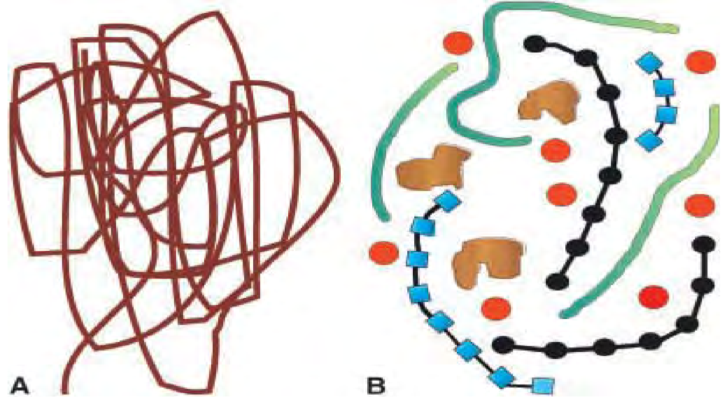

Figure 2: A, Depiction of the traditional concept of HS as randomly coiled marcomolecules, and B newer concepts indicating that the major components found in alkaline extractable soil HS are relatively low molecular weight (<2,000 Da) associations of structures in the presence of metals to form aggregates. The spheres represent generic metal cations, the linked spheres polysaccharides, the linked squares polypeptides, the wavy lines aliphatic chains, and theirregular structures aromatic lignin fragments

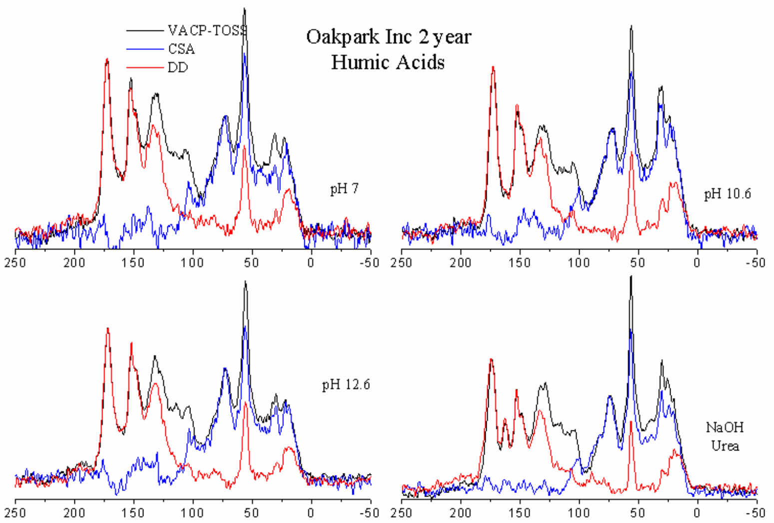

Figure 3: Solid state ^{13}C NMR of HAs isolated from soil at pH 7, pH 10.6, pH 12.6, and 0.1 M NaOH + urea. The soil was amended with Zea mays two years prior to sampling

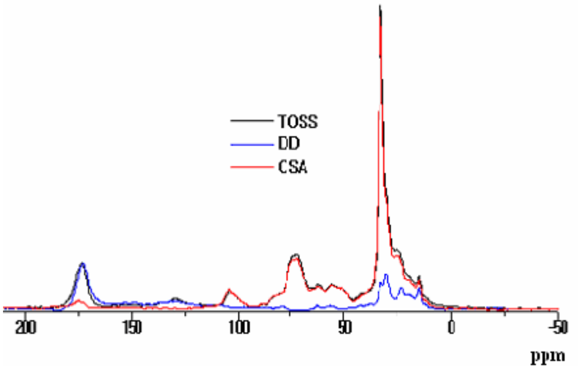

Figure 4: Solid state ^{13}C NMR of humin isolated from the DMSO-H_2SO_4 following base extractions

Sugars and amino acids abundances and ratios were different for all of the fractions, and it was clear that the isolates at the higher pH values had greater inputs from plant residues.

Comparisons of Figures 3 and 4 highlight the differences between the compositions of humin and HA materials . The major humin components are aliphatic hydrocarbons, long chain acids/esters, cutins/cutans, waxes, and significant amounts of carbohydrates and peptides. Hayes and Swift discuss in detail in this symposium the compositions of the components isolated in the DMSO-acid system, and that contribution emphasises that humin is composed largely of biological molecules, mainly of plant, and also of microbial origins. Their recalcitrance can be attributed to protection by the inorganic colloids.

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Controls on the Stability of Soil Humic Substances

William R. Horwath*, Heather Throckmorton, Julie Bower, Garrett Liles

Dept. Land, Air and Water Resources, Univ. California, Davis, CA, USA
E-mail:wrhorwath@ucdavis.edu

1. Introduction

Our current understanding of the role of vegetation diversity on microbial and humification processes is limited. The importance of microbial processes in controlling humification pathways is receiving increasing attention (Kogel-Knaber et al. 2008). Previous research has established that soil and plant management likely effect the size of the microbial community and in some case its' composition (Horwath 2008). It is generally considered, however, that microbial communities mediate decomposition and humification processes in two main ways: 1. through their decomposer role including C uptake, transformation, resynthesis and respiration, and 2. through their composition and turnover, which provide a broad suite of macromolecules with a broad range of chemical compositions and reactivity. Our current understanding of the role of vegetation diversity on microbial humification processes in soils is imprecise at best. We present a set of studies showing the importance of microbial inputs and alteration of litter inputs through management that affect the stability of soil organic matter (SOM) in forest systems.

2. Materials and Methods

Complimentary studies and analytical methods were used to reveal the fractions and stability of SOM contributing to soil productivity. These studies utilize a range of analytical techniques including stable isotopes to pyrolysis-Gas Chromatograph/Mass Spectrometry/Isotope Ratio Mass Spectrometry (PyGC/MS/IRMS) to Differential Scanning Calorimetry (DSC). The studies were located at various sites in the Ponderosa Pine (*Pinus Ponderosa*) biome in the Sierra Nevada of California (Table 1). Study 1 compared removing understory shrubs (Understory Absent; UA) to leaving them intact (Understory Present; UP) to explore the effect of vegetation diversity. The understory typically contains ceanothus *(Rhamnaceae spp.)* an N fixing shrub. Study 2 examines microorganisms as a source of C input to soils in an 11-year-old pine forest.

Study 1. Ponderosa pine seedlings were uniformly labeled in the greenhouse with $^{13}CO_2$ (8 atom %), litter residues collected upon senescence and air-dried. The pine litter residues were applied to soils at the Challenge Experimental Forest and Feather Falls.

Study 2. Microorganisms consisting of fungi, gram- and gram+ bacteria and actinomycetes were cultured and uniformly labeled with ^{13}C at 99.9 atom%. The labeled microbial groups were applied to microcosms at the Blodgett Research Forest.

Table 1: Site characteristics of the various forest research sites

Study	Site	Elevation (m)	Annual ppt. (cm)	Treatment	Isotope addition	Soil N (g kg^{-1})	Soil C (g kg^{-1})
1	Challenge Experimental Forest	790	1,730	UA	^{13}C pine litter	2.1	58.4
				UP		3.1	69.8
1	Feather Falls	1220	1780	UA	^{13}C pine litter	2.06	56.8
				UP		2.34	69.6
2	Blodgett Research Forest	1,230	1,660	UA	^{13}C microbes	5.4	123.7

Soils were sampled from 0 to 15cm over a 7-year period. Soil ^{13}C and N were measured on an Isotope Ratio Mass Spectrometer (IRMS, Europa Scientific Ltd., UK). Soils were pyrolyzed at 590° using a Curie Point pyrolyzer and separated on a Thermo GC/C system using a Trace GC Ultra gas chromatograph (GC) coupled on line to a Varian 3800 Analytical Mass Spectrometer (MS) and a Europa IRMS through a GC/C-III interface. The enthalpic character of soils was determined by DSC (Netzsch Instruments N.A. LLC., Burlington, MA)

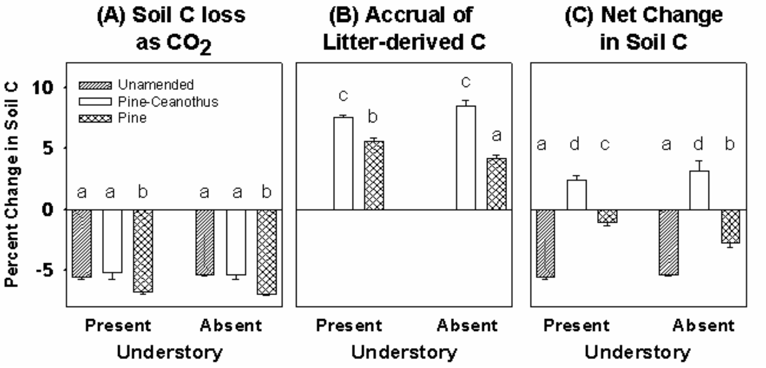

Figure 1: The soil C loss (A), accural of C (B) and change in soil C (C) in different understory treatments

3. Results and Discussion

Study 1- ^{13}C-pine litter approach. We hypothesized that diverse litters, especially those containing N-fixing species would lead to greater SOM. A mass balance of soil C stocks showed that a net accrual of new, litter-derived soil C only occurred in the pine-ceanothus treatments (Figure 3C). Both pine treatments showed a net loss of soil C, with UA pine losing significantly more C (Fig. A and B). The presence of ceanothus, an N fixer, enhanced the stabilization of pine-derived C in SOM. The primary influence of ceanothus on the fate of pine-derived C was to increase its’ conversion to SOM. The result appears due to changes in the type of litter availability not in microbial community composition (results not shown). Other studies support the positive influence of N-fixing species on C and N accrual forests (reviewed by Binkley et al. 1992).

Energetic relationships in SOM in the different treatments were altered by litter inputs. Figure 2 illustrates soils of similar C content (4.8%) express vastly different stored energy signatures. The herbicide (UA) treatment had the lowest energy (W g^{-1} soil) compared to the control (UP) or fertilized (UP) soil. The results indicate that SOM associated with less diverse litter inputs has less overall energy storage and a greater proportion of more thermally resistant SOM. This corroborates the ^{13}C litter study where greater C loss occurred with pine litter only.

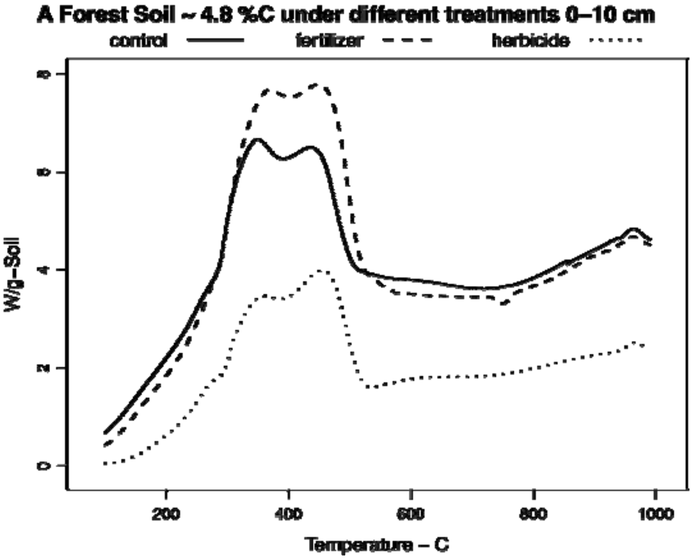

Figure 2: DSC thermograms for soils under fertilizer (UP) and herbicide (UA) treatments

Study 2-Blodgett Forest ^{13}C-microial residue approach. We hypothesized that different microbial groups would contribute differently to SOM maintenance due to varying cellular components. Results suggest a pattern of long-term stabilization of fungi compared to gram (-) bacteria (Fig. 3).The PyGC/MS/IRMS analysis shows similar outcomes to total microbial group recovery in the loss of specific microbial biomarker compounds (data not shown). Interestingly, the accumulation in certain biomarker compounds may provide more information on SOM maintenance (data not shown).

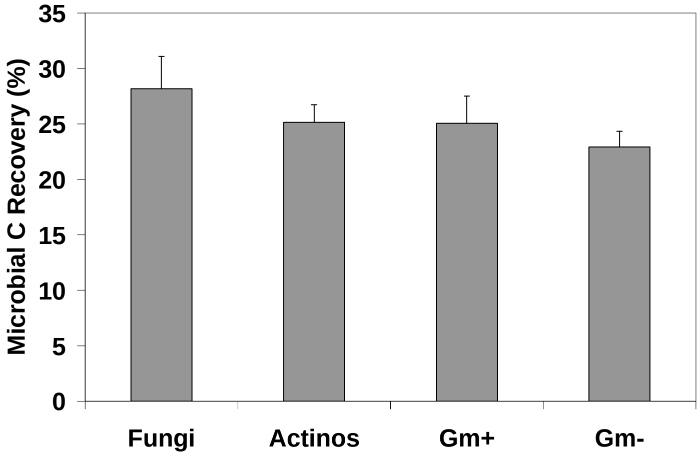

Figure 3: The amount of microbial C (%) after 3y in the field

4. Conclusions

The following points are raised by this research:

1. Diversity of litter inputs, especially including N-fixers, increases SOM.
2. Fungi were a more stable source of SOM compared to gram (-) bacteria.
3. Lack of litter diversity appears to reduce soil energy storage.

On balance, the impact of vegetation management is likely more important since the microbial community diversity is minimally affected by vegetation management or fertilization. There is evidence that certain microbial groups may contribute more to humic substances. Our results show that vegetation management influences the stability of SOM and is likely important to sustaining long-term soil productivity.

Acknowledgements

We thank Robert Powers and the USDA Forest Service for access to research forests.

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Linking Abiotic Characteristics of Functional SOM Fractions to Biotic Parameters and to Microbial Use

Elke Schulz[a*], Marc Breulmann[a], Scott Demyan[b], Tatjana Boettger[b], Frank Rasche[c], Georg Cadisch[c], François Buscot[a]

[a]Department Soil Ecology & [b]Department Isotope Hydrology, Helmholtz Centre for Environmental Research - UFZ, Theodor-Lieser-Str.4, D-06120 Halle, Germany; [c]Department of Plant Production and Agroecology in the Tropics and Subtropics, Universität Hohenheim, 70593 Stuttgart.
E-mail: elke.schulz@ufz.de

1. Introduction

Soil organic matter (SOM) is heterogeneously distributed among soil compartments into pools of different quality and stability. Approaches based on chemical and/or physical principles of fractionation techniques enable measuring the quantity and quality of the various SOM pools. They enable to distinguish between labile and stabilized carbon pools that rely to soil functions. Previous research on agricultural soils of temperate European regions allowed discriminating the impact of management practices on a hot water extractable fraction (HWE) of the SOM [1] which is assumed to be highly degradable. In contrast, SOM associated to clay particles and to soil fractions with high densities is of special importance for stability and resilience of soil structure and properties [2]. Long-term field experiments are among the best means to predict impacts of soil management on soil organic carbon (SOC) storage. Recent studies use natural isotope $^{13}C/^{12}C$ ratios as an *in situ* marker to characterize storage and turnover of relic and newly formed SOC pools [3]. The soil microbial community regulates all transformation processes of SOM and can be considered to be a key component of the active SOM pool. The main objective of the presented work is to link abiotic characteristics of SOM fractions to the biological processes by analyzing the age, source and availability of C in SOM fractions as well as the structure of microbial community associated to the fractions.

2. Materials and Methods

The presented results are based on data sets of two different ecosystems:

(1) Semi-natural extensively managed grassland in Central Germany (part of the BIOLOG-Europe Programme, called DIVA and funded by BMBF). The soil was a brown soil formed from mainly schist and greywacke bedrock. Average annual precipitation was above 950 mm and the average temperature was 5.0 °C. Archived air dried soil samples taken in 2004 from the entire profile of different plots were used for the study.

(2) Static Fertilization Experiment at Bad Lauchstädt, initiated in 1902. The mean annual

precipitation and temperature were 483 mm and 8.8 °C, respectively. The soil type was a Haplic Chernozem derived from loess. The texture was loamy with about 21.0% clay. For the study we used archived air dried soil samples (0–30 cm) taken in 1956 and 2004 from these respective treatments: (a) 30 t ha^{-1} farmyard manure (FYM) per ha every 2 years + NPK , (b) 30 t ha^{-1} FYM per ha every 2 years, (c) NPK and (d) without any fertilization.

A size-density fractionation of soils was done following a modified protocol of Shaymukhametov [4]: (i) separation of SOM associated with an easily dispersible clay fraction (CF, particles < 2 µm) by applying ultrasonic energy to a soil/water suspension; CF was subdivided into CF1: < 1 µm and CF2: 1–2 µm. (ii) from the remaining sediment two light fractions (LF) of LF1: < 1.8 g cm^{-3} and LF2: 1.8–2.0 g cm^{-3} were stepwise separated by using bromoform/ethanol mixtures. Hot water extractions (HWE) of bulk soil and SOM fractions were performed by using an accelerated solvent extraction device (ASE 200, Dionex, Germany). Hot water extractability of C in the solid physical fractions served as stability indicator of SOM in these fractions. A set of abiotic parameters was applied to characterize the properties of C in bulk soils and SOM fractions: content of soil organic carbon (SOC) and its natural $\delta^{13}C$ values bulk soils and SOM fractions, the conventional ^{14}C age and spectroscopic properties (DRIFT-MIRS) were measured. The functional and structural composition of the soil microbial community as biotic characteristics of SOM fractions was described by measuring a set of soil microbial enzyme activities (alkaline phosphatase, protease, β-glucosidase, and xylanase) and extraction of PLFAs. The utilization of C in bulk soil samples and the respective isolated SOM fractions (kinetic process measurements - C fluxes) was measured by incubating at 22 °C in an automatic respirometer (RESPICOND) for different time intervals and CO_2 evolution was measured hourly.

3. Results and Discussion

For understanding the fate and dynamics of SOM, three characterization steps in the investigation of the major fractions should be considered: the age and the origin of the SOM, its quality and availability and the directly associated microbial community. Once these mosaic stones are known, it becomes possible to depict their integration to a functional entity. Our approach was a combination of physical and chemical separation techniques, including size-density fractionation and extraction of a readily decomposable organic carbon pool by hot-water extraction. Additionally, C content in hot water extracts can also be used as a stability indicator of C in the size-density fractions of SOM. The effects of management on these pools will be discussed.

Depending on land use and management, SOM undergoes certain changes related to its stability. The DRIFT-MIRS was utilized to study total SOM and the relationship of specific peak regions as related to SOM fractions and stabilities. A significant correlation of the MIRS band at 2930 cm^{-1} (Fig. 1) to the C content in HWE of bulk soils (0.92 at $P<0.001$) and to C in the light fraction LF1 (0.95 at $P<0.1$) was found.

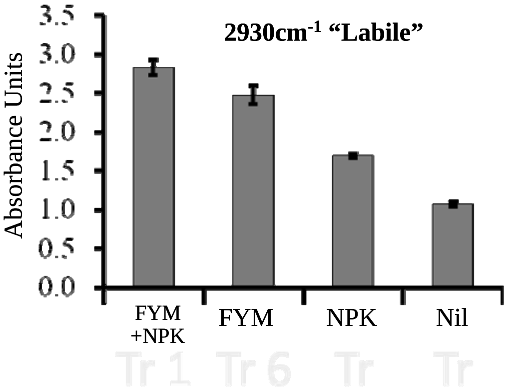

Figure 1: Management influence on a MIRS band of "labile" C

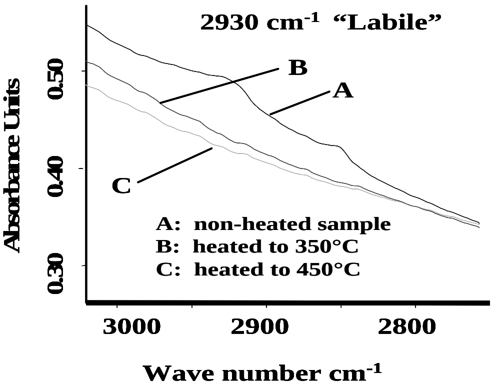

Figure 2: Thermal response of the MIRS band of "labile" C

Thermal analysis has been used to identify spectral regions which correspond to different organic compound stabilities. The relatively low thermal stability of the spectral region at MIRS band 2930 cm^{-1} indicates that this band corresponds to more labile organic compounds (Fig. 2).

Measurements of the natural $^{13}C/^{12}C$ isotope ratios as an *in situ* marker indicated management influence and characterized storage and turnover of relic and recent SOC fractions in connection with their radiocarbon age (Fig. 3).

Management effects on hot water extractability of C in SOM fractions will be demonstrated. The effects of this labile pool on phospholipid fatty acids and enzyme activities will be shown. Most of the detected soil microbial parameters were significantly correlated to the HWE pool. The activities of all enzymes were positively correlated with C_{HWE} and of N_{HWE} bulk soil. A close relationship of enzyme activities and PLFA parameters among the C_{HWE} pool was observed. The labile C pool could be seen as a key driver of the microbial community composition and its activity.

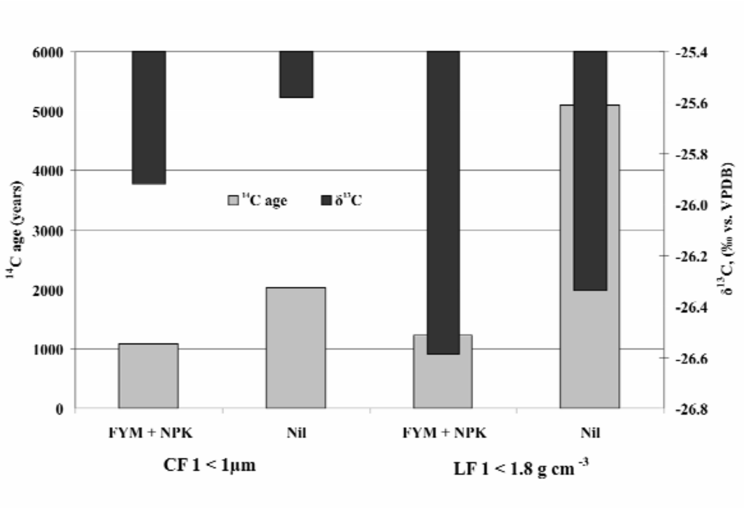

Figure 3: Management influence on the ^{14}C age and δ^{13}C values of SOM in a clay (CF1) and specific light fraction (LF1)

Microbial use of the C in SOM fractions as a key process for the flow of energy was tested in a respiration experiment. Corresponding to abiotic parameters like hot water extractability, δ^{13}C values, and MIRS band correlating to labile SOM as well as the thermal lability of this band the decomposability and respective microbial use of C in a LF of a density < 1.8 g·cm^{-3} was most intense compared to the C source in bulk soil and in clay fraction.

4. Conclusions

Quality and decomposability of SOM is the driving force of the microbial community composition. A close relationship of enzyme activities and PLFA parameters among the C (HWE) pool was observed. The labile C pool could be seen as a key driver of the microbial community composition and its activity. In future, soil respiration process studies, CO_2 fixation form SOM pools and its release due to microbial decomposition also require monitoring of SOM fraction-specific fungal and bacterial communities by DNA fingerprints and of functional diversity by biopolymer-degradation mRNA fingerprints – thus identification of candidate key players.

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Structural and Statistical Analysis of Diverse Analytical Techniques Spectra from Diverse Source Organic Matter in Solution

R. Baigorri[a*], M. Fuentes[a], F.J. González-Vila[b], J.M. García-Mina[a]
[a]CIPAV-Roullier Group, Orkoien, Spain; [b]IRNAS-CSIC, Sevilla, Spain
E-mail: rbaigorri@timacagro.es

1. Introduction

Although many studies indicate that the qualitative and quantitative interpretation of the mass spectra of humic substances (HS) is frequently very difficult, recent studies have demonstrated that the use of sophisticated statistical tools, such as principal components analysis (PCA) or multilayer neural networks, could permit the adequate classification of humic samples as a function of their origin and sampling places (1).

These studies showed the usefulness of statistical methods like principal components analysis (PCA) for the quantitative or semi-quantitative analysis of very complex data set (2). Among these statistical methods the use of a specific method of principal components analysis (PCA), Pareto analysis, has proven to be very promising (3). This statistical technique uses the Pareto principle: a large majority of consequences (80%) are produced by a few key causes (20%). Thus, the use of this complementary statistical approach can allow the analysis of the totality of the data derived from the use of the different analytical techniques (4). Likewise, it facilitates the identification of those structural features that characterize and discriminate each humic fraction.

In this context, the employment of PCA-Pareto for the analysis of the data derived from the application of different analytical techniques (UV-Visible, FTIR, ^{13}C-NMR, fluorescence and Pyrolysis-GC/MS) will permit us to evaluate which region (or regions) of the spectrum is more relevant in order to define each HS fraction. Finally, we have carried out a Discriminant Analysis (DA) of PCA-Pareto data to assess the capacity of these specific structural markers for grouping the different HS fractions. In this way, we also have applied PCA-Pareto and DA to biological raw materials, their composted outputs, humic acids (HA) and fulvic acids (FA) to find structural markers and to evaluate, if it is possible, the humification grade of composted materials.

2. Materials and Methods

Humic materials. Four soil HA, including two IHSS standards, two FA from IHSS standards, four biological raw materials (NON-COMP) and their composted outputs (COMP) were used this work.

Extraction procedure. Humic fractions were isolated following the IHSS procedure. HA were fractionated in gray humic acid (GHA) and brown humic acid (BHA) fractions according to Swift (5).

Instrumental Analysis. Samples were analyzed by solid-state ^{13}C NMR; UV-visible, FTIR and synchronous fluorescence spectroscopy (SFS), and Pyrolysis-GC/MS.

Statistical software. The analytical data were treated to obtain Pareto analysis PCA by MarkerViewTM 1.1 software (Applied Biosystems). Discriminant analyses were performed by SPSS 12.0 software (SPSS Inc., Chicago).

3. Results and Discussion

The results of Pareto analysis showed that very useful structural information can be obtained (Table 1). Thus, the GHA fraction is characterized by an important degree of aliphaticity (^{13}C-NMR and FTIR), a certain aromaticity (according to SFS) but simpler than in BHA, and some conjugation in polyens and cianines (UV-Visible). The BHA fraction presents a greater and more complex aromatic character than GHA and FA (^{13}C-NMR, FTIR and UV-Visible analyses), involving polycyclic aromatics of about seven fused benzene rings (SFS) and heterocycles, such as benzothiophenes (SFS and Pyrolysis-GC/MS). Finally the FA fraction is the most heterogeneous one. It contains simpler, less degraded and more functionalizated organic molecules, such us polysaccharides, alcohols, ethers (^{13}C-NMR); aromatic amino acids and some aromatic groups (SFS); carboxylic groups (FTIR); furfurals and furans (Pyrolysis-GC/MS) and finally, conjugated systems that may involve complexed metals (UV-Visible) as Simpson et al. have previously hypothesized (6).

Table 1: Different fractions in humic substances and their characteristic structural groups by ^{13}C-NMR, SFS, FTIR, Pyrolysis GC-MS and UV-Visible

Humic substance	^{13}C-NMR	SFS	FTIR	Pyrolysis GC-MS	UV-Vis
Gray humic acid	methyl methylene	naphthalene and derivates	aliphatic C–H	alkanes and alkenes (C16–C28) terpenes	polyenes cyanines
Brown humic acid	aromatic	7 benzene units fused	aromatic C–H	anthracenes benzotiophenes phenantrenes	aromatic systems
Fulvic acid	saccharides alcohols ethers carboxylic groups	amino acids simple benzenes 5 benzene units fused	Carboxylic groups	furfurals furanes	conjugated systems that involve transition metals

Besides the structural information inferred from this statistical analysis, the principal components extracted from the Pareto analysis have been used as variables in a discriminant analyses (DA). As revealed by DA, the Pareto-PCA analysis is able to gather and reduce the information about the whole spectra of the samples, with few loose of information. In this way, the samples were grouped reasonably well, without misclassification into another HS class (Figure 1).

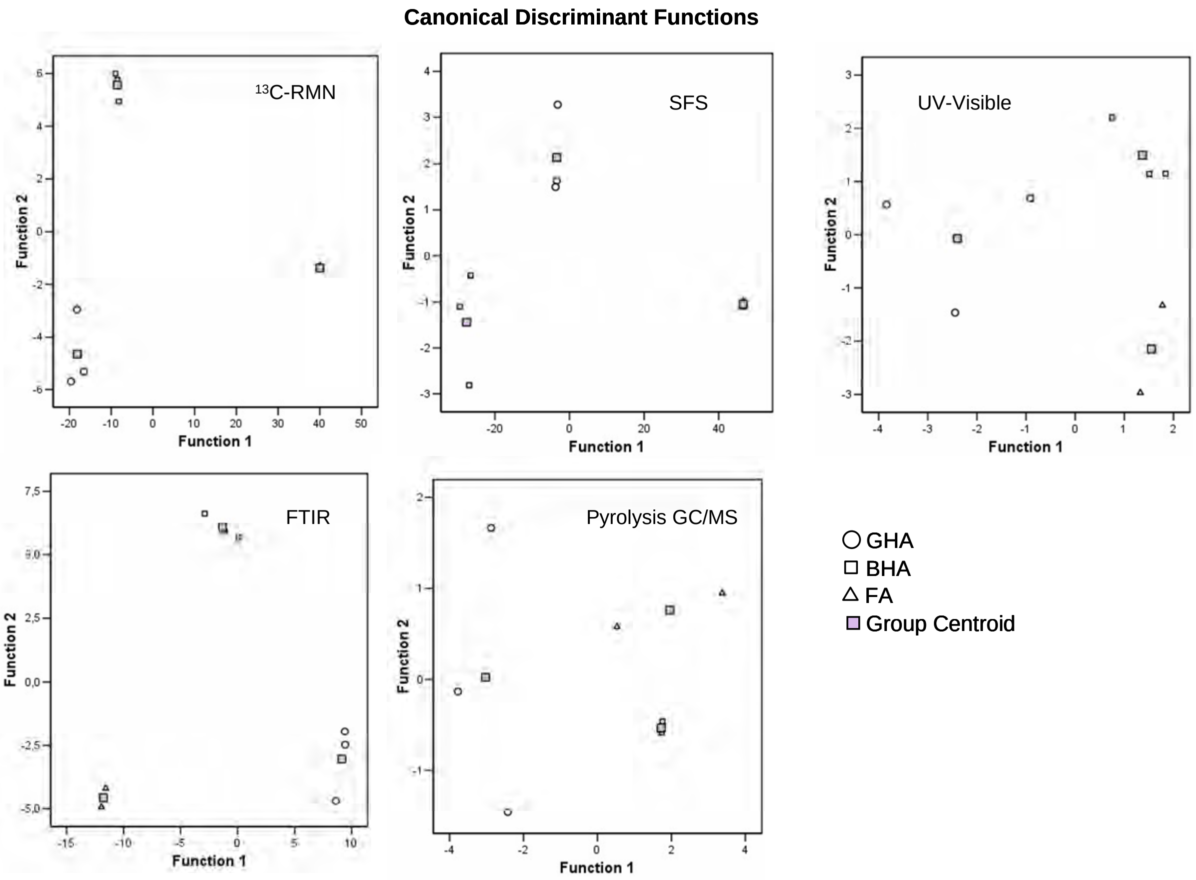
Canonical Discriminant Functions

Figure 1: Grouping by Discriminant Analysis of gray humic acid (GHA), brown humic acid (BHA), and fulvic acid (FA) for ^{13}C-NMR, SFS, FTIR, Pyrolysis-GC/MS and UV-Visible

However, when Pareto analysis was applied to the data obtained for the HA, FA, and the humic-like extracts from composts and from their corresponding initial organic wastes; no structural conclusions could be straightforwardly inferred. Notwithstanding this fact, the subsequent DA analysis of the principal components showed statistically certain proximity among raw materials and their composted outputs indicating that composting is an evolution of organic matter but not so complex than humification process. This could be supported because the most generic techniques ^{13}C-NMR, SFS, FTIR, and UV-Visible showed a better discrimination among raw materials and their composted output than Pyrolysis-GC/MS, a more descriptive technique, indicating that a first step in the process of chemical differentiation has been reached but not a more complex differentiation involving certain humification.

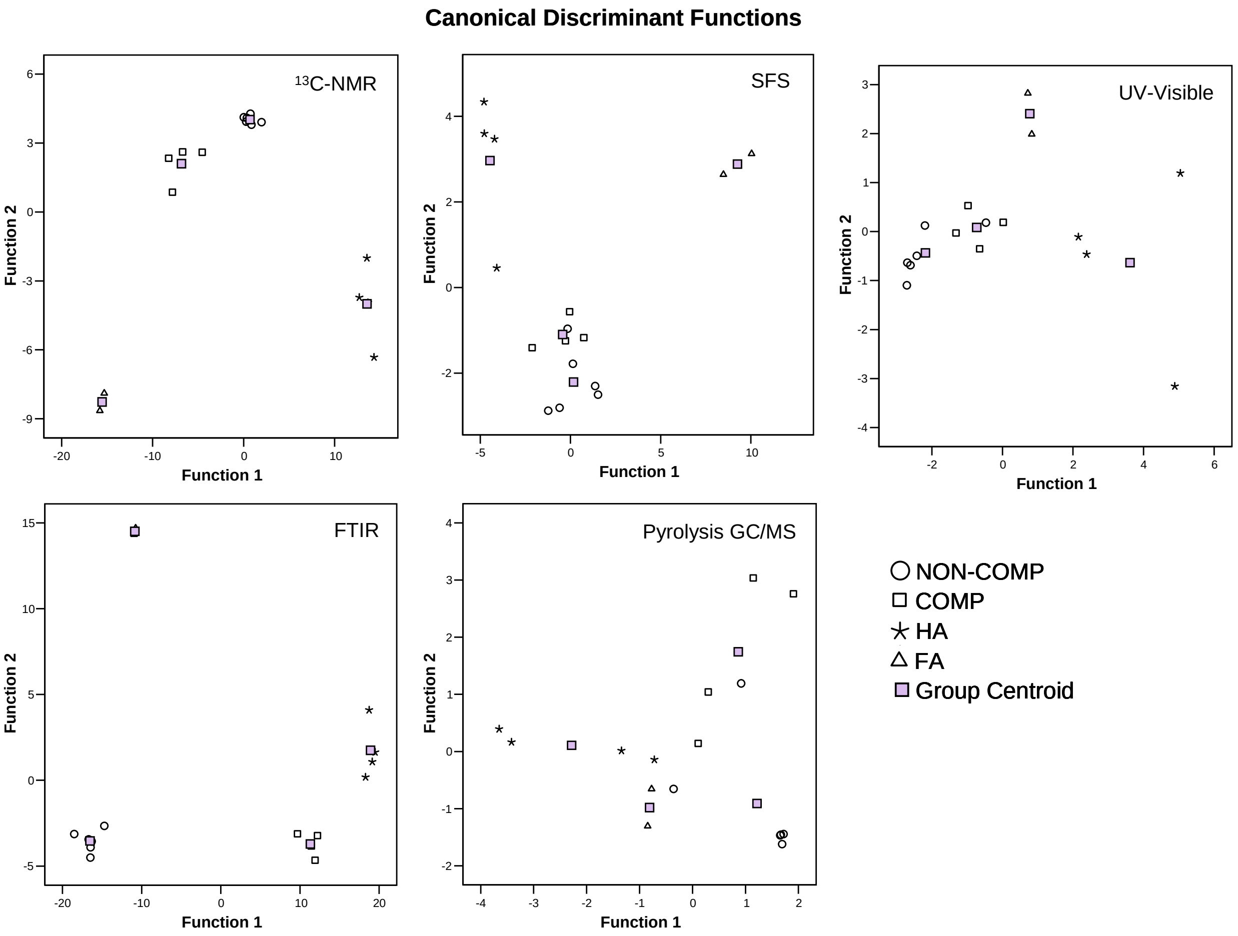

Figure 2: Grouping by Discriminant Analysis of biological raw materials (NON-COMP), their composted outputs (COMP), humic acids (HA) and fulvic acids (FA) for ^{13}C-NMR, SFS, FTIR, Pyrolysis-GC/MS and UV-Visible

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New Approaches for Analysis Ultrahigh-Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Irina Perminova[a*], Alexey Kononikhin[b,c], Anton Grigoriev[b,c], Igor Popov[b,c],
Andrew Garmash[a], Eugene Nikolaev[b,c]

[a]Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia:
[b]Institute for Energy Problems of Chemical Physics RAS, Moscow, Russia;
[c]Emanuel Institute of Biochemical Physics RAS, Moscow, Russia
E-mail: iperm@org.chem.msu.ru

1. Introduction

The particular features of humic substance (HS) are polydispersity and extreme structural heterogeneity. Application of such a powerful technique as Fourier transform ion cyclotron resonance mass spectrometry (FTICR) to HS enables to obtain useful molecular level and structural information. FTICR mass spectrum of HS contains several thousand peaks with dozens of molecules matching the same nominal mass (Fig1).

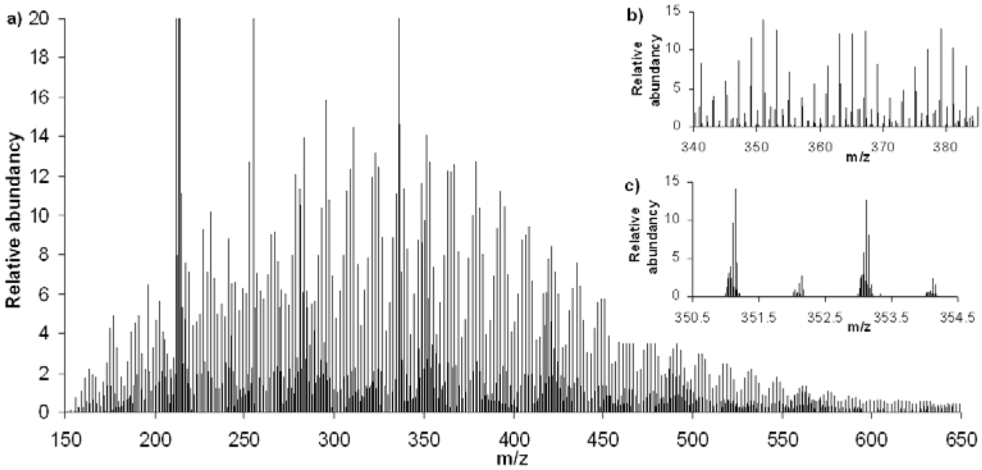

Figure 1: Mass spectrum of Suwannee River fulvic acid in negative ionization mode. (a) Broadband spectrum for m/z between 150 and 650 amu; (b) negative ions between nominal masses 340 and 385; (c) negative ions between nominal masses 351 and 354

Such a complexity poses a significant challenge for automatic data interpretation, in which the most difficult task is molecular formula assignment, especially in the case of heavy and/or multielement ions [1]. Molecular formulas cannot be assigned to such heavy peaks directly by solving Diophantine equations, which is the traditional way of compound identification in FTICR mass spectra. To determine molecular formulas of these peaks, extra information carried over by the data structure should be employed. Formula extension approach is usually used for identification for molecular formula assignment to a heavy ions based on a predefined set of mass differences related to functional group relationships (or small building blocks) typically present in NOM (CH_2, H_2, C_2H_4O, CH_4O_{-1}, and so on) [2]. This approach is very effective for formula assignment but can only be applied to a limited set of repetitive systems with predetermined functional group relationships. Recently it was shown that application of Kendrick mass defect analysis to the peaks producing trend lines in the van Krevelen diagrams facilitated identification of prevalent low mass differences corresponding to structural units like CH_2O, C_2H_2, and C_2H_4 between the peaks seen in an ultrahigh-resolution mass spectrum of NOM [3]. This approach demonstrated clearly the huge potential of mass difference analysis for data processing of high-resolution spectra. However, van Krevelen analysis did not resolve the problem of high mass compounds identification, because it requires all individual ions to be previously assigned a molecular formula.

In this study a new approach for automatic treatment of FTICR mass spectra of NOM and humic substances based on total mass difference statistics (TMDS) has been developed and implemented [4].

2. Materials and Methods

ESI FTICR mass spectra of PSS 2200 and SRFA were acquired using a commercial 7 T Finnigan linear quadrupole ion trap-Fourier transform (LTQ FT) mass spectrometer (Thermo Electron Corp., Bremen, Germany) equipped with Ion Max electrospray ion source located at the facilities of the Emanuel Institute of Biochemical Physics RAS (Moscow, Russia). The following conditions were used for electrospray: flow rate 1 μL/min, negative ion mode; needle voltage 3.4 kV; no sheath and auxiliary gas flow; tube lens voltage 130 V; heated capillary temperature 250 °C. The FTICR mass spectra of PSS 2200 and SRFA were converted into mass lists using Qual Browser (Thermo Electron Corp., Bremen, Germany).

Further mass list processing was performed offline using self-designed software for fully automated analysis of mass spectrometry data. It included ion charge detection, data filtering based on the results of charge detection; calculation of total mass difference statistics (TMDS), chemical building blocks identification, and assigning molecular formulas to individual peaks in mass spectra. The specific feature of software is its ability to assign formulas to heavy and/or multielement molecules using “virtual elements” approach. In this case, identified mass differences can be included into the Diophantine equation as masses of “virtual” chemical elements instead of masses of individual chemical elements. The

corresponding Diophantine equation may look like the following:

$m_{CcHhXxYyZzWw} = cm_C + hm_H + xm_X + ym_Y + zm_Z + wm_W$, where c, h, x, y, z, and w are integer numbers (stoichiometric coefficients), mC and mH are masses of individual chemical elements (atoms of carbon and hydrogen), whereas mX, mY, mZ, and mW are masses of "virtual elements" corresponding to mass difference formulas identified using the TDMS algorithm.

3. Results and Discussion

A new approach enables a blind search for unknown building blocks (instead of a priori known ones) by revealing repetitive patterns present in spectra. In this respect, it differs from all previously developed approaches. The principal novelty of the developed data processing software is a conversion of the measured mass spectra into differential mass spectra. The approach was tested on mass spectra of sodium polystyrene sulfonate (PSS, Mw = 2200 Da) which produce heavy multielement and multiple-charged ions. A use of differential data processing approach was shown to be very efficient in identification of the monomer fragment in the molecules of regular synthetic polymer like PSS. The most intensive peak was observed for the fragment with molecular weight of 206.001 – which corresponds exactly to the mass of $C_8H_7SO_3Na$-fragment. Application of TMDS allowed unambiguous formula assignment to all multiple-charged peaks including the heaviest peak in PSS spectrum at mass 1843.12827 with charge state –3 (mass bias +0.37 ppm). Application of TMDS-algorithm to processing data on the Suwannee River Fulvic Acids (FA) has proven its unique capacities in analysis of spectra with high peak density: it has not only identified the known small building blocks in the structure of FA such as CH_2, H_2, C_2H_2O, O, but the heavier unit at 154.027 amu (see Fig1). The latter was identified for the first time and assigned a formula $C_7H_6O_4$ consistent with the structure of dihydroxyl-benzoic acids. The presence of these compounds in the structure of FA has so far been numerically suggested but never proven directly.

4. Conclusions

The presented results on application of the proposed approach both to synthetic and natural polyelectrolytes provide a convincing proof on the large potential of the TMDS algorithm in interpretation of complex mass spectra. It was capable of identifying monomer units in both model polymers as well as of assigning unambiguous molecular formulas to multiply-charged and heavy ions. Application of the TMDS algorithm to mass data on Suwannee River fulvic acid greatly studied by other researchers has allowed for the first time the identification of a new repetitive structural block with a formula $C_7H_6O_4$ assigned to a mass of 154.027 amu. Of particular importance is that the identified repetitive unit may be assigned to dihydroxyl-benzoic acid, which is consistent with the expected structural blocks of fulvic acids stemming from oxidized lignin structures. The application of the TMDS-algorithm opens new horizons in unfolding molecular complexity of NOM and other natural products. Further modification of developed software is discussed.

Acknowledgements

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SESSION 11: CLOSING

Overview of Subsurface Peat Fires and the 2009-2010 Fire in Las Tablas de Daimiel National Park

Guillermo Rein

BRE Centre for Fire Safety Engineering, University of Edinburgh, EH93JL, UK
E-mail: G.Rein@ed.ac.uk

1. Introduction

Only after the 1997 extreme haze event in South-East Asia, the scientific community recognised the economic, ecological and public threats posed by subsurface fires. This was caused by the spread of vast biomass fires in Indonesia, burning below the surface for weeks during the El Niño climate event. The fires destroyed 0.79 x 106 ha of forested peatlands [1] and produced a smoky haze that covered large parts of South-East Asia (extending to Northern Australia and Southern China). This induced a surge of respiratory emergencies in the population and disrupted shipping and aviation routes for weeks. It also led most Southeast Asian countries to sign the ASEAN Agreement on Transboundary Haze Pollution in 2002. It has been calculated that carbon released was equivalent to 13–40% of the mean annual global carbon emissions, and contributed to the largest recorded increase in atmospheric CO_2 concentration at that time [1]. The 1997 fire episode was not an isolated case, but the only one studied in any detail.

Smouldering fires in peatlands have also been reported in tropical, temperate and boreal forests, e.g., Northern America, Siberia, the British Isles, Falkland Isles, Southern Africa, and others [2]. Peatlands, the accumulation of partially decayed vegetation matter, are one of the largest reserves of terrestrial organic carbon (one third of the world's soil carbon). Their total carbon pool exceeds that of the world's forests and is comparable to that of the atmosphere [3].

2. Dynamics of smouldering fires

Peat is not the only organic material involved in subsurface fires. Coal seams and coal mines also burn in the subsurface. These are the longest continuously burning fires on Earth. It is believed that Burning Mountain in Australia has been burning for 6000 years [4].

These fires are driven by smouldering combustion which is defined as a slow, low temperature flameless form of combustion sustained by the heat evolved when oxygen directly attacks the surface of a condensed phase fuel [5]. The characteristic temperature, spread rate and heat released during smouldering are low compared to those in the flaming combustion of a solid. Smouldering propagates at a low speed governed primarily by the rate at which oxygen can diffuse through the ground to the reaction zone, the heat losses and the thermal properties of the fuel [5]. Typical smouldering temperatures of coal and peat are in the range 700–1000 ◦C and 550–650 ◦C respectively while the propagation speed is on the order of 0.1 mm/min [2]. Material properties such as moisture content, organic fraction, density and flow permeability are important parameters dictating the ignition and spread of subsurface fires.

Subsurface fires can be ignited by natural causes such as wildfires, lightning strikes, self-heating or anthropogenic factors e.g., slash and burn, mining activities or waste incineration. Once these fires have been ignited, they are difficult to detect, locate and extinguish. Despite extensive firefighting attempts, to date, no successful extinction has been confirmed by human intervention. By propagating undetected in the subsurface, it offers the means for flaming wildfires to re-establish at unexpected surface locations (e.g. across a fire break) and at unexpected times (e.g. long after burn out of the flame front). Figure 1 is an artistic representation illustrating the propagation of a smouldering front in the subsurface. A subsurface fire removes layers of soil and the prolonged heating kills roots, seeds and plant stems.

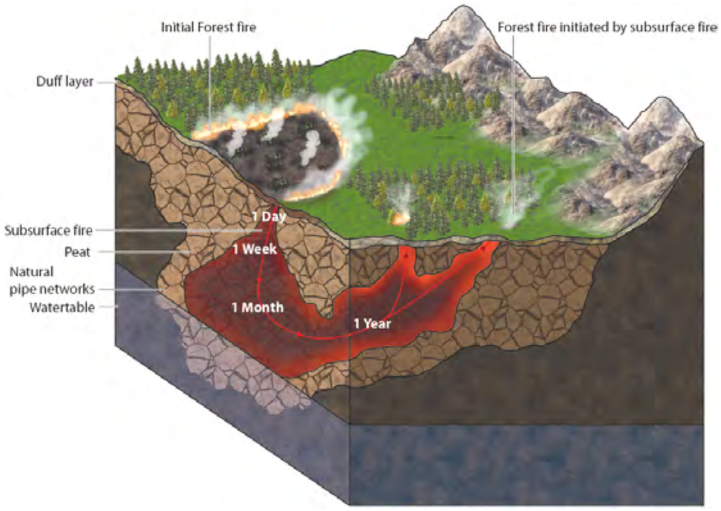

Figure 1: Illustration of a subsurface fire initiated at the surface that propagates into the peat

3. Emissions to the atmosphere

The combustion reaction in smouldering is characteristically incomplete [5] and thus it emits partially combusted gases at a higher fraction than flaming fires, e.g., volatile organic compounds, CO and polyaromatic hydrocarbons. The field studies by [6] of natural biomass fires in the tropical savanna show that the smouldering phase releases on average 130% more CO and 670% more hydrocarbons, but 15% less CO_2 than the flaming phase and no NO_x.

The only available literature on gas emissions from peat fires is scarce. Biogenic and anthropogenic sources of greenhouse gas emissions have received much attention and have been characterized well in the last two decades. Nevertheless, the non-conventional sources such as peat fires and wildfires remain unknown. Coarse estimates based on the only study available show that annual global carbon emission by peat fires can reach between 13 to 40% of the current global CO2 emissions from fossil fuels and industrial processes [1].

4. The 2009 fire in Las Tablas de Daimiel

The wetlands of the National Park of Las Tablas de Daimiel, Ciudad Real, Spain have been low of water for several decades and very dry (less than 1% of its surface with water) for at least four years. This has led to very dry conditions of the peat to (water content bellow 10 to 20% dry weight). Under these dry conditions, peat smoulders readily (smouldering fires are known to spread at water content bellow 55% dry weight) [2]. Visually, weak plumes of smoke can be appreciated in holes distributed over a surface area of 5 ha inside the Park and of 40 ha outside the Park.

The fire was detected first inside in Park in September 2009. But smoking signs of smouldering activity (see Fig 2 right) were detected earlier than that just outside the Park limits. On November 2009, a large suppression, prevention and compartmentation programme was initiated. This included novel large-scale prevention work compacting of the soil and local flooding from the surface. About 30 ha had been treated like this, stirring and compacting the soil down to a depth of 3 or 4 m with heavy equipment to prevent the spread of the fire (see Fig 2 left). This prevention technique aims at cooling down possible hot spots and disrupting the dense network of natural pipes feeding oxygen to the deeper fire seats and carrying the smoke from the subsurface to the atmosphere. In January 2010, large amounts of water started to arrive from the Tagus river at Guadalajara (more than 150 km away from the Park) via the 1979 Tagus-Segura transfer and a special pipe built in a rush by the authorities. The prolonged drought of the Park came to an end and the peatland started to be flooded again.

The flooding comes on top of the suppression activities and a wet winter. All these may led to the extinction of the fire at some point that would also allow regenerating the flora and fauna of the Park.

Figure 2: Nov 2009 in Las Tablas: left) peat compacted for fire prevention; b) smouldering peat

5. Final remarks

Subsurface fires have important implications for climate change. Warmer temperatures at high latitudes are already resulting in unprecedented permafrost thaw that is leaving large soil carbon pools exposed to natural degradation and susceptible to fires. Because the CO_2 flux from peat fires has been measured to be about 3000 times larger that the natural degradation flux [7], permafrost thaw is a risk for greater carbon release by fire and subsequently influence carbon-climate feedbacks.

However, research in smouldering fires in general modest and little is known. The literate record is very rather poor and not proportional to the threat posed. Two main reasons for this are the lack of understanding on the fundamental of smouldering combustion, and the frequent confusion between flaming and smouldering fires.

The possible links between smouldering fires and ancient char layer residues found in organic soils will be discussed.

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Extraterrestrial Organic Matter and its Relationship to the Origin of Terrestrial Life

Norbert Hertkorn[a], Zelimir Gabelica[b], Régis D. Gougeon[c], Philippe Schmitt-Kopplin[a]

[a]Helmholtz-Zentrum Muenchen, German Research Center for Environmental Health, Institute for Ecological Chemistry, Ingolstaedter Landstrasse 1, D-85764 Neuherberg, Germany; [b]Université de Haute Alsace, ENSCMu, Lab. LPI-GSEC, 3 rue Alfred Werner, F-68093 Mulhouse Cedex, France; [c]Equipe EMMA EA 581, Université de Bourgogne, Rue Claude Ladrey, F-21078 Dijon, France
E-mail: hertkorn@helmholtz-muenchen.de

1. Introduction

Delivery of extraterrestrial organic matter onto the primordial earth and its implications for the evolution of life are subjects of a lively scientific debate [1, 2]. Recently, novel opportunities for direct observation of extraterrestrial planetary disks have constrained our current models of planetary formation considerably and earth-like planets within habitable zones around stars have been newly discovered. Remnants of the chemical evolution in the early solar system can be found recorded in carbonaceous chondrites. A recent study of the famous carbonaceous chondrite “Murchison” revealed the presence of likely millions of organic molecules, rivaling and possibly exceeding terrestrial biological complexity [3]. This manuscript intends to assess the contributions of chemical and biological evolution to organic matter composition and structure.

2. Materials and Methods

The findings described here are based upon high field (12 T) FTICR mass spectra and 500 MHz NMR spectra as described in ref. [3].

3. Results and Discussion

Terrestrial natural organic compounds divide into functional biomolecules eventually deriving from a genetic code, and complex biogeochemical non-repetitive materials which are formed according to the general constraints of thermodynamics and kinetics from molecules of geochemical or ultimately biogenic origin. While biomaterials are amenable to successful separation into defined molecular fractions, complex non-repetitive materials cannot be purified in the conventional meaning of purity because of even more extensive intricacy. In fact, the molecular signatures of these supermixtures often approach the limitations imposed by the laws of chemical binding [4, 5]. Nevertheless, modern organic structural spectroscopy is capable of revealing meaningful molecular detail out of most complex natural mixtures [6-9].

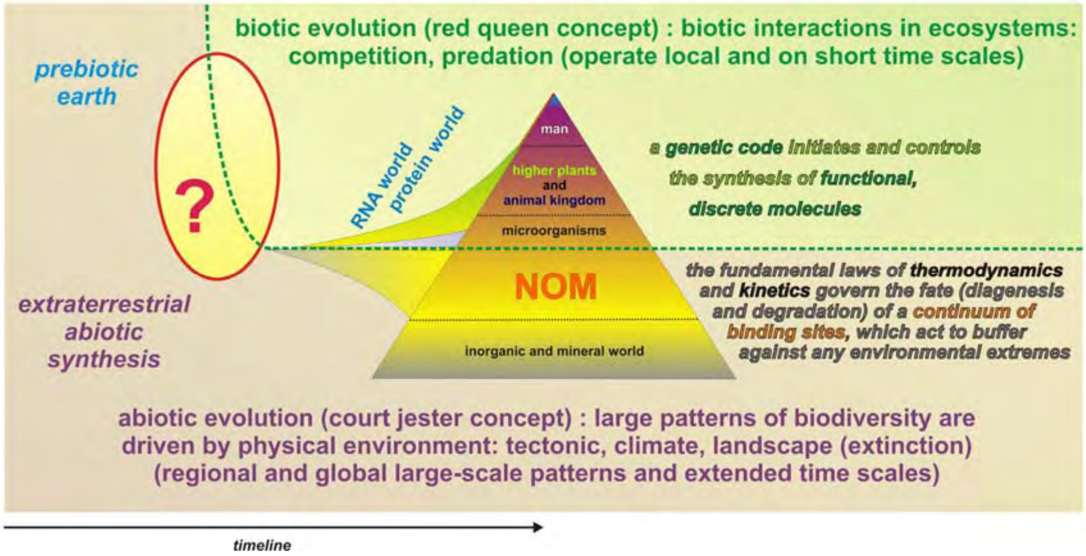

Figure: The formation of natural organic matter (NOM) in space and on earth preceded the evolution of terrestrial life. Later in the earth's history, coevolution occurred between prebiotic/abiotic molecules, NOM and primitive and higher forms of life. Rather local and short-tern biotic evolution interacts with large-scale and long term abiotic evolution in the design of ecosystems [10]. However, the origin of complex and stereochemically homogeneous biomolecules from abiotic chemistry remains unsolved (this Figure is derived from ref. [5])

An in-depth characterization of the Murchison meteorite, which fell to Earth on September 28, 1969, has revealed that it comprises probably several millions of different organic compounds [3]. This finding extends the previously known molecular diversity of carbonaceous chondrites by a wide margin and demonstrates that our primordial solar system already contained an extremely large variety of organic chemicals that might equal and even surpass the molecular diversity found on the planet Earth.

Murchison meteorite originates from the earliest days of the solar system and, probably, even before the Sun formed 4.65 billion years ago. During its trajectory of formation, Murchison meteorite has sampled across a huge variety of spatial, compositional, temperature and irradiation regimes which initiated considerable spatial (physical and chemical) heterogeneity on nano-, micro- and macroscales. Here, primitive meteorites fundamentally deviate from almost all terrestrial materials, which display more uniform histories of formation.

Life on earth is based on an enormous diversity of large molecules, assembled from a relatively small subset of universal precursors. These include the four nucleobases, the 20 proteinaceous amino acids and two kinds of lipid building blocks. Life exploits the specificity inherent in the three-dimensional qualities of organic chemicals (stereochemistry). These then lead to some readily identifiable and measurable generic attributes that would be diagnostic as biosignatures, such as (1) enantiomeric excess, (2) diastereoisomeric preference, (3) structural isomer preference, (4) repeating constitutional sub-units or atomic ratios, (5) systematic isotopic ordering at molecular and intramolecular levels, (6) uneven distribution patterns or clusters (e.g. C-number, concentration, $\delta\ ^{13}C$) of structurally related compounds (direct quotation of Ref. [11]). These characteristics apply to biomacromolecules as well as to current small molecular natural products and their related fossil derivatives (biomarkers).

Organisms build biomass suited to specific physical environments, habitats and their ecological imperatives and synthesize an enormous variety of accompanying product molecules (i.e. natural products) [11]. These evolutionary selected organic molecules are frequently synthesized by enzymes and often bind efficiently to biomacromolecules. Natural products offer prevalidated subsets of the chemical structure space explored by nature so far. A structural classification of natural products (SCONP) with annotation of biological origin and pharmacological activity has provided a structural rationalization and categorization of natural product diversity, termed “the periodic table of the natural products” [12]. Evolutionary but also exclusively structure-based relationships then have been successfully employed for library design to probe biological activity and to explore the biologically relevant chemical space.

On earth, the majority of NOM molecules ultimately originates from biomolecules; exceptions like NOM derived from graphite weathering [13] can be deduced from isotopic signatures. The combined action of biotic and abiotic reactions characteristic of the respective ecosystems progressively attenuates original biosignatures and the resulting NOM molecular signatures approach the limits defined by the laws of chemical binding [4]. The dynamic equilibrium of NOM generation and decomposition ranges across several orders of magnitude (from microseconds to millennia). Terrestrial and freshwater-derived NOM contain a larger proportion of aromatic structures, derived from lignified tissue in vascular plants, than marine organic matter [8, 9, 14, 15]. The availability of aromatic structures expands the compositional space of chemically relevant NOM molecules to significantly lower H/C values than accessible solely on the basis of open-chain unsaturation (olefinic and carbonyl) and alicyclic double–bond equivalents [5, 15]. While all NOM on earth differs in evolutionary history, the general trajectory of formation and decomposition is constrained by the respective ecosystem conditions. Extreme environments like hydrothermal deep-sea vents or hot springs deviate from common environmental (temperate aerobic) regimes but support rich and diverse communities of life with chemical energy [16]. Associated NOM will likely deviate from currently known NOM composition, analogous to extraterrestrial NOM.

Any NOM on earth shows an enormous molecular complexity which appears masked by rather narrow margins of bulk properties obtained from low-resolution analytical characterization [17]. Modern organic structural spectroscopy has matured enough to accurately depict NOM composition and structure out of natural environments and exciting prospects emerge to explore the intricate relationships between abiotic organic chemistry and origin of life on earth and in the universe.

4. Conclusions

Abiotic, biogeochemical and biological organic molecules occupy vastly different subsets of the chemical space. While the abiotic evolution of natural organic matter often follows entropy-driven trajectories that maximize chemical diversity, carbon based life is confined to a rather restricted biologically relevant chemical space. However, an enormous diversity of large molecules is assembled from a surprisingly small subset of universal precursors and the three-dimensional qualities of these molecules are critical for the sustenance of basic and higher life. The conditional relationships between abiotic organic synthesis and terrestrial life are not yet understood.

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