Volume I

From Molecular Understanding to Innovative Applications of Humic Substances

Proceedings of the 14th International Meeting
of the International Humic Substances Society

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Dedication to the memory of Professor Dmitry S. Orlov

This book is to commemorate the memory of Professor Dmitry Orlov, who was an outstanding Soviet/Russian scientist. Dmity Orlov contributed immensely into development of humus chemistry. His works gave seeds to many contemporary ideas about genesis, structure and functions of soil humic substances. Dmitry Orlov was a system-scientist. His fundamental work: “The general theory of humification” laid foundations to quantitative description of evolution of soil humic substances. He was a bright thinker and a great teacher. His books, thoughts, and pupils are the best memorial to his deal and spirit. We were fortunate to know him personally and hoped so much to give a chance to the international community to experience his touch at the IHSS-14 conference in September 2008. This was to be the first IHSS conference in Russia, and he inaugurated the conference by accepting an offer to become an honorable head of its scientific committee. To commemorate outstanding contribution and devotion of Professor Orlov to humic science, the scientific committee of the conference has decided to dedicate IHSS-14 to his name. Let his spirit be and live with us.

Irina V. Perminova
Coordinator of CIS-IHSS
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Preface

The 20th century was the century of the petrochemical economy. With oil prices steadily climbing up, development of alternative feedstocks is critical to maintain the viability of the manufacturing industry. The 21st century is announced a century of bioeconomy based on a use of plant biomass produced by photosynthesis within biological rather than geologic time. With the sources encompassing lignites, peats, sapropels, composts, organic wastes, and others, humic materials occupy a niche between fossil rocks and fresh biomass.

The most striking feature of humic materials is constellation of unique properties such as non-toxicity, biocompatibility, resistance to biodegradation, and polyfunctionality. As a result, these materials can be competitive on the market of biobased products, e.g., biocompatible plastic, “green” chemicals (flocculants, chelators), biosorbents, detoxicants. Development of advanced fractionation can refine biomedical properties of humics inherent in the minor fractions. They can be used as elicitors, immunomodulators, and others.

To elucidate the growing economic potential of humic materials, the 14th Meeting of International Humic Substances Society (IHSS-14) was entitled “From molecular understanding – to innovative applications of humic materials”. In appreciation of the industrial importance of the emerging concepts of humic science and technology, the conference was supported by International Union of Pure and Applied Chemistry (IUPAC). The meeting was invited by the Commonwealth of Independent States (CIS) IHSS Chapter and was held on September 14 – 19, 2008, aboard the ship traveling from Moscow to Saint Petersburg (Russia) (see the web-site: www.ihss-14.humus.ru). The aim of this conference was to expand the existing attitude toward humic substances as most abundant components of organic matter in soil and water to much broader understanding of potential of humic materials as alternative feedstock for biobased economy. This aim was in line with the long standing traditions of theoretical and applied science intrinsic to the Soviet-Russian schools in humic research. The works of soil scientists (M. Kononova, L. Alexandrova, D. Orlov), laid foundations to humification theory, to understanding structure and biospheric functions of humic substances. The coal chemists (G. Stadnikov, T. Kukharenko) contributed the most into industrial production of humates and elaborated the first state standard protocol for determination of humic acid yield. The pioneering works of L. Khristeva (Ukraine) on physiological activity broadened substantially agricultural applications of humates. The Belorussian scientists (N. Bambalov, F. Puntus) achieved substantial progress in production of biologically active humics from peat and sapropel.

The main topics of the conference were: 1) Molecular understanding of humic substances (HS) and natural organic matter (NOM), 2) HS and NOM in the changing environment, 3) Physical-chemical and biological properties of HS and NOM, 4) Knowledge-based design and advanced separation of humic materials, 5) Industrial production and innovative applications of HS. The unique feature of this conference became the satellite exhibition “Humic materials – resources for the 21st century”. The purpose of organizing the scientific conference and industrial exhibition at the same venue was to bring together a league of experienced scientists with an association of engineers and business people. This was to create among the participants an awareness of the current status of research in humic science and technology. At the meeting, 84 oral and 158 poster presentations were given followed up by six round table discussions with about sixty short oral presentations. Twelve companies were presenting their products and technologies at the exhibition which are presented in the separate exhibition catalogue.

This book summarizes the proceedings of the IHSS-14 and is dedicated to Professor Dmitry Orlov (deceased) – the honorable chair of the Scientific Committee of the conference. All papers have been subject to peer review by at least one referee – member of the Scientific Committee. We thank all members of international Scientific Committee and the authors for their helpful collaboration. The sincerest appreciation goes to Dr. Natalia A. Kulikova – scientific secretary of the IHSS-14 who took over responsibility for abstract submission, reviewing, and formatting. Special thanks to our sponsors who contributed greatly in organization of this meeting.

We wish the conference and all its distinctive participants full and highly deserved success – outcome of five days spent in the unique atmosphere of the ship traveling from Moscow to Saint Petersburg along the rivers of Russia!

August 2008

Irina V. Perminova
Coordinator of CIS-IHSS
Co-Chair of the Organizing Committee
of the IHSS-14 Meeting

Dear colleagues and friends of humic sciences,

It is a great pleasure for me to have opportunity to welcome to the 14th International Meeting of the International Humic Substances Society. The scientific program indicates that IHSS-14 will bring together a distinguished group of researchers, who would create a stimulating and dynamic professional meeting. It will be most enjoyable to discuss with colleagues and friends with whom we can exchange our recent experiences and research, while meeting new people who share our common interests may create new friendships. The place of the meeting is also very important. Certainly, visits to Kremlin and Armory in Moscow, as well as to Hermitage in Saint Petersburg must delight and provide plenty of impressions. Furthermore, exciting venue, such as a four-deck ship traveling from Moscow to Saint Petersburg, makes this event very special. This is my second visit to Russia. Being aware of great hospitality and cordial reception by Russian colleagues, I am convinced that gathering all participants for a week in this not too big area will result in a really very specific atmosphere. During our way, we will have occasion to visit renowned Russian historical and architectural attractions, including Uglich, Goritsy, and Kizhi Islands. The conference organizers, leading by Irina Perminova, deserve our sincerest appreciation and thanks for all these arrangements, indeed. From the other hand, none of participants can leave a ship during its way, thus conference room should be quite well occupied during scientific sessions.

Humic substances research and scientists are very special, as well. Significant benefits of soil organic matter to crop productivity have been recognized by agriculturalists since ancient times, while research on humic substances dates back two centuries. In 1798 Achard used base extraction of soil to obtain a darkly colored solution for his studies, while Berzelius published in 1806 on humic substances isolated from a spring Porla in Sweden. Although studies of humic substances in terrestrial and aquatic environments have been conducted for more than 200 years, only in the last three decades our understanding of the structure and reactivity of these complex materials has increased dramatically. The International Humic Substances Society, an organization that was conceived and brought into being 27 years ago, contributed to that progress very much. The founders of IHSS provided a forum in which scientists from very diverse backgrounds and scientific disciplines could meet to discuss their common interests. Such a gathering stimulated the formulation of ideas and sharing of knowledge, which would lead to a greater scientific understanding of humic substances nature. From small group at the beginnings in 1981, IHSS have grown to over 800 members in 58 countries. Nowadays, we have an internationally accessible reference and standard humic substances collection, and a recommended standard procedure for their extraction. Numerous contributions presented at previous 13 International Meetings of the IHSS played a crucial role in enhancing the knowledge of the structure, function and reactions of these materials. Nevertheless, we have only taken the first step on the long way towards unveiling the properties of humic matter. It is enough to say, that we are still not able to provide accurate definition of humic substances, thus IHSS Board adopted only a description of humic substances, available at IHSS website from this year.

The future of our science is in the young. Without them, the lines of investigation would disappear and much of the progress made in the last years would be dashed. Having this in mind, the Society offers financial support for students, allowing them to attend biannual meetings, and to become full partners in our achievements to increase knowledge on humic substances. The IHSS board appreciated very much a great response of students to the last call for Travel Support Award. Submitted contributions were of very high scientific level, and applying students indicated great achievements. I am especially looking forward to scientific sessions to learn from the 17 students who were awarded IHSS Travel Support Award to attend this meeting.

The 14th International Meeting of the IHSS is entitled "From molecular understanding to innovative applications of humic substances". The conference presents a promising program, enabling participants to discuss very different issues. I want to invite all of you to bring your recent findings and discuss in friendly and stimulating ambiance. The abstracts you find in this book are an excellent basis for a thorough exchange of ideas. Have a good week with us.

August 2008

Jerzy Weber
President of the International Humic
Substances Society (IHSS)

On behalf of the International Union of Pure and Applied Chemistry (IUPAC) I would like to extend a warm welcome to all of you to this IUPAC sponsored 14th Conference of the International Humic Substances Society. The biannual IHSS meetings provide a great opportunity for humic researchers, chemical engineers and humate producers of many countries to come together and to learn about the latest discoveries in humic science and technology. The unique peculiarity of the current meeting lies on the technological focus reflected already in the title “From molecular understanding – to innovative applications of humic materials” strengthened by the Satellite Exhibition “Humic materials – resources for the 21st century”. This will be the first time when this kind of exhibition will take place in the framework of the conference, which demonstrates the growing economic potential of humus-containing raw materials and humic-based products.

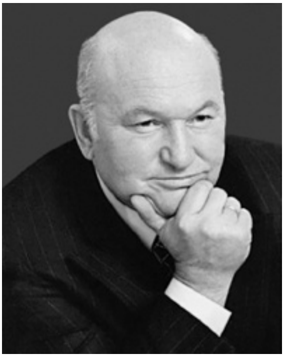
I believe that IHSS-14 will draw attention of industrial chemists and policy-makers to the prospect of conversion of huge resources of humified biomass into alternative feedstock for bio-based products. The conference and exhibition will provide a great networking opportunity for scientists and commercial sector worldwide. In addition, this event is a perfect and unique opportunity for young scientists to learn about the emerging humic-based technologies which will surely become a significant part of “green” chemistry industry.

The special atmosphere of the meeting organized by the Russian hosts of IHSS-14 aboard the ship traveling from Moscow to Saint Petersburg will stimulate brain storming and exciting discussions on the newest discoveries and prospects of humic science and technology. This book is a great survey of the latest research and achievements in the field of humic science and technology, which provides a reader with a snapshot of newest trends in this exciting area. I wish all the success to this meeting. And may it carry along the spirit of IUPAC who serves to advance the worldwide aspects of the chemical sciences and to contribute to the application of chemistry in the service of Mankind.

My special appreciations to the organizers, especially to Prof. Irina Perminova and welcome to IHSS-14 in Russia!

August 2008

Jung-Il Jin
President of the International Union of
Pure and Applied Chemistry (IUPAC)



On behalf of the Government of Moscow and of myself I am glad to welcome organizers, participants and guests to the 14th Meeting of the International Humic Substances Society – the pre-eminent forum in the field of fundamental and applied humic research!

It is fair and deeply symbolic that the Lomonosov Moscow State University became the venue of the Opening Session of this conference held for the first time in Russia. The Lomonosov Moscow State University is a cradle of many scientific schools of Russia. MSU was also alma mater of Professor Dmitry Sergeevich Orlov – the founder of modern Russian school of humus chemistry.

Studies on the role of humic substances in global climate change belong to the newest branches of environmental chemistry. Of particular importance is the satellite exhibition of the conference «Humic materials – resources for the 21st century».

I am deeply convinced that this conference will contribute to further developments in fundamental research as well as broaden practical applications of scientific developments.

My wish to all participants and guests of the conference is that your interactions are intense, energizing, and creative, that new scientific contacts evolve and of course I wish you success in realizing all of your plans!

September 14, 2008

Yuriy Mikhailovich Luzhkov
The Mayor of Moscow

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Section I

Novel methods and approaches to molecular understanding of humic substances and natural organic matter

Agent-Based Modeling of Natural Organic Matter

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Keywords: humic, fulvic, model, stochastic, agent-based model

1. INTRODUCTION

Two types of models for natural organic matter (NOM) are commonly in use- structural and functional. Structural models are atomic connectivity maps- chemical structures- which may have been obtained from the experience and creativity of a researcher or group of researchers (1) or calculated from a set of analytical and structural constraints (2). Functional or predictive models are sets of equations which represent a specific aspect of NOM behavior, for example metal complexation (3) or the production of reaction oxygen species (4). Functional models are typically calibrated with experimental data from multiple NOM samples, and contain only average structural information (e.g. UV absorbance, MW) but no specific structures.

In this talk, I will describe a third type of model, which represents individual molecules or pseudo-molecules as software agents. Each molecule behaves according to a set of chemically reasonable rules, interacting with its environment and with other molecules over time.

2. MATERIALS AND METHODS

The agent-based model treats time as a discrete variable, advancing in steps of 0.1 hours and using a pseudo-random number to determine whether a molecule reacts and, if so, how. Each molecule is represented by its elemental and functional group composition and by a series of reaction probabilities and properties calculated from the composition data and environmental variables. Environmental variables which influence the reaction probabilities include pH, moisture, dissolved oxygen, temperature, light, exo-enzyme activities, and bacterial density. The algorithm, including equations for probability calculation, has been previously published (5), as have estimation equations for various chemical properties (6). The AlphaStep desktop version of the model was used in the simulations; it was programmed in Delphi version 6, and typical simulations of ~10,000 molecules for 5 months of simulated time require <5 minutes on a Pentium laptop computer. Ancillary programs (e.g., for metal complexation calculations) are also programmed in Delphi 6.

3. RESULTS AND DISCUSSION

Using this agent-based model (ABM), we can simulate many aspects of NOM behavior, including elemental and functional group composition, size distribution, bioavailability to microbes, metal complexation and reactive transport. In addition, we can examine how specific environmental factors can affect these behaviors- for example, how differences in precursors lead to differences in bioavailability, or how exposure to sunlight affects metal complexation.

One advantage of the ABM is that we can simulate the evolution of humic substances from specific precursor molecules and observe not only changes in bulk properties (%C, MW_n, etc.) but also the reactions leading to these changes. For example, Figure 1 shows how the production of –COOH groups lags the photolysis of conjugated alkenes in a simulation of protein and lignin fragments exposed to sunlight.

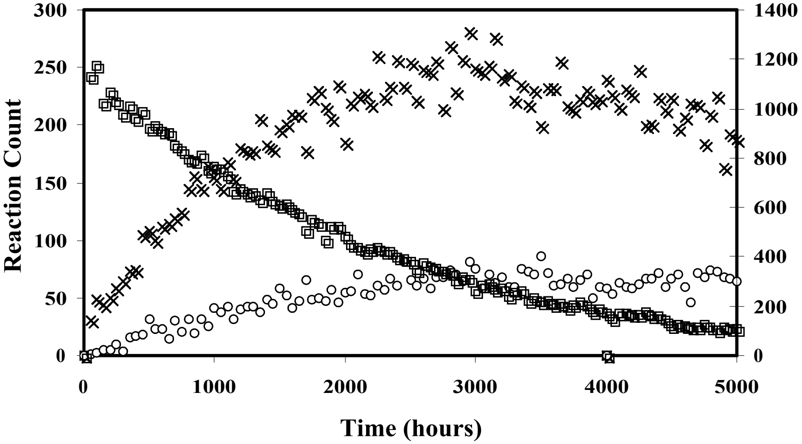

Figure 1. Surface water incubation of biopolymers: Reaction counts per 50 hour interval for C=C oxidation to diol (squares, right Y axis), alcohol oxidation to aldehyde or ketone (X), aldehyde oxidation to -COOH (circles).

Metal complexation is simulated *a priori* (without calibration on NOM) by combining the AlphaStep output (molecular structure data) with a quantitative structure-property relationship (QSPR) to predict a metal binding K' for each molecule in the assemblage. These K' values can then be used to calculate binding in solution of known NOM content (Fig. 2). The results are semi-quantitative, which is quite remarkable given that the model is not calibrated using any NOM complexation data.

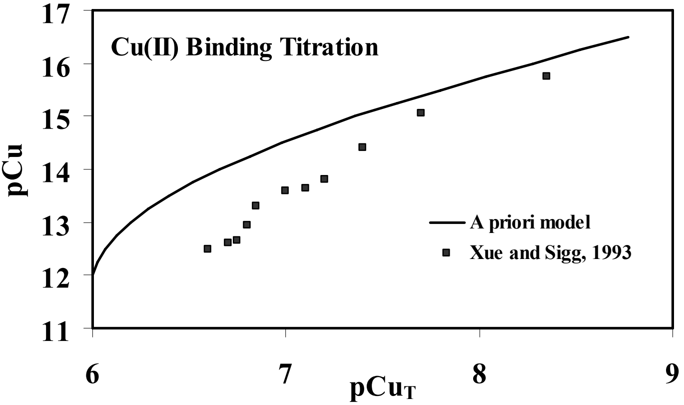

Figure 2. An *a priori* simulation of metal complexation by aquatic NOM compared with CSV data from a Swiss lake (7).

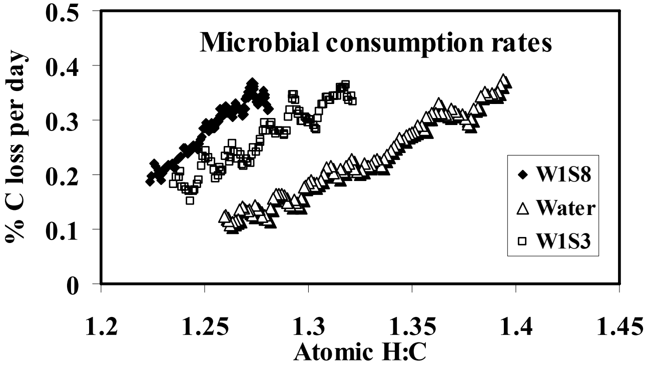

Figure 3. Simulated microbial consumption from simulated aquatic NOM (Water) and NOM containing a mixture of aquatic and soil NOM (W1S8, W1S3).

The agent-based model can also be used to examine ecological problems- for example, the counter-intuitive observation that reduced (high H:C) NOM can be more bioavailable to microbes than more oxygenated NOM (8). In the logical extreme, this suggests that hexane should be more bioavailable than sucrose! Another puzzling observation is the finding that in some ecosystems larger NOM appears more bioavailable than the smaller NOM (9). Possible explanations of these phenomena include higher energy content in larger, reduced NOM and lack of enzymes to degrade carbohydrates.

Assuming that bacteria can only utilize small, at least partially oxygenated molecules, model simulations nonetheless show that in some cases more reduced NOM gives faster uptake rates (Figure 3) and that high MW NOM may be taken up more quickly than low MW material (data not shown). This demonstrates the presence of emergent properties arising from the complex humic mixture- overall behavior which appears to contradict the individual model rules.

4. CONCLUSIONS

The ABM approach to the evolution of humic substances over time provides a comprehensive way of linking individual structure and reactivity with macroscopic measurements and behaviors.

ACKNOWLEDGEMENTS

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Probing Local pH in Hydrophobic Domains of Humic Acids: An EPR Based Method

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Keywords: humic, radical, local pH, spin probe, EPR

1. INTRODUCTION

Despite the focused efforts by many labs during the last 30 years, vital aspects of the structure and fundamental physicochemical aspects of Humic Acids (HA) are largely unknown. It is currently accepted that humic acids contain both hydrophilic as well as hydrophobic domains which are the locus of sequestration of xenobiotic molecules. However vital information on the detailed nature, the accessibility of the hydrophobic domains is lacking.

On the other hand, HA behave as polyelectrolytes which bear variable charge modulated by pH. It is considered that this pH-dependent charge is modulated by the local pKa of the chargeable structural groups i.e. carboxylates and phenolics. Traditionally only the water accessible hydrophilic fraction of the chargeable groups can be probed by H-binding or metal binding studies. However the function of chargeable groups in hydrophobic domains, local pKa, and their geometrical location are largely unknown.

In this context we have developed a novel methodology by using the Spin-Probe method with Electron Paramagnetic Resonance (EPR) spectroscopy. A series of specific spin-probes with varying hydrophobicity as well as pH sensitivity were used and studied with EPR spectroscopy. The experiments were carried out in well characterized HA samples of varying molecular weight providing information of the local environment of the spin-probes, their mobility, and local pKa values.

2. MATERIALS AND METHODS

The interaction between three pH-sensitive nitroxide spin probes with humic acid was studied by X-band EPR spectroscopy. The humic acid sample was isolated using standard extraction procedures recommended by the International Humic Substances Society (2). The EPR experiments were carried out at acidic, neutral and alkaline pH values. At each pH a spin probe with appropriate pK value was used. The time dependence of the EPR spectrum of the spin probe was monitored at room temperature.

3. RESULTS AND DISCUSSION

Several humic acid samples were prepared and incubated at different pH values. A detailed pH titration was performed for each nitroxide spin probe and studied with EPR spectroscopy in the presence and absence of humic acid. In the absence of humic acid, buffered solutions were used to fix the pH at the desired values. A pKa shift of the spin probe was observed in the presence of humic acid in two of the three cases studied. Figure 1 shows a detailed pH titration of a spin probe in the presence and absence of humic acid. The shift of pKa of the spin probe molecule from pKa = 8.8 to pKa = 9.2 in the presence of humic acid was observed. This result illustrates the difference of the local pH value in the hydrophobic interior of humic macromolecule with respect to the pH value of the aqueous solution.

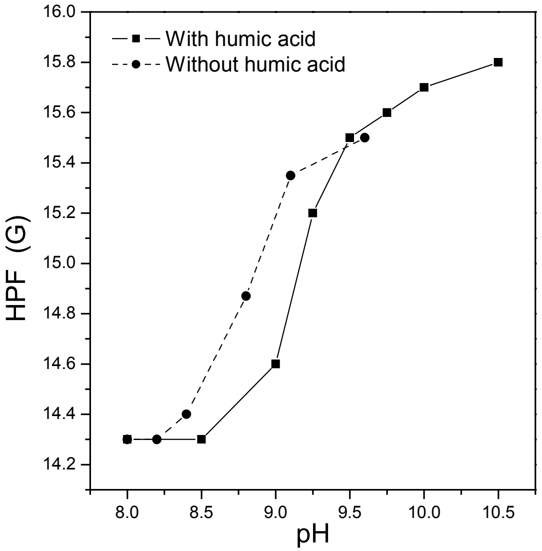

Figure 1. pH titration of a spin probe in the absence (pKa = 8.8, □ dashed line) and in the presence of humic acid (pKa = 9.2, □ solid line).

This is the first demonstration of the applicability of the pH-sensitive spin probe method for this type of macromolecules.

In each experiment, the intensity of the EPR signal of the spin probe was monitored as a function of time. In the presence of humic acid the time dependent increase of the anisotropy of the spin probe spectrum was observed. This trend was not observed in the absence of humic acid. This increase may be attributed to the penetration of the spin probe to the hydrophobic regions of the macromolecule.

The time dependent decrease of the intensity of the spin probe's EPR signal was also monitored. This could be explained by the reduction of nitroxyl radical to EPR-silent hydroxylamine. This reduction is most likely induced by the phenol-type indigenous free radicals presented in humic acids (3).

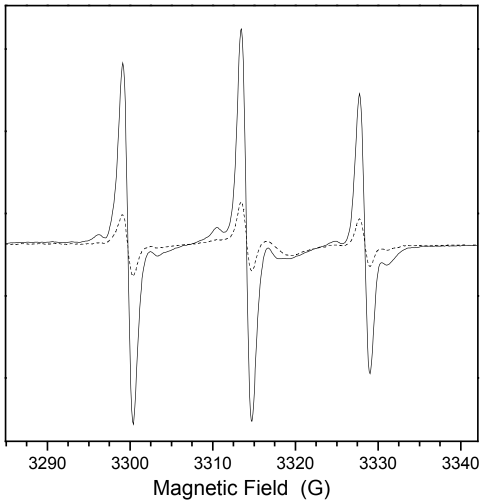

Figure 2. Reduction of the spin probe by humic acid as a function of time. Incubation time: solid line t = 10 min, dashed line t = 60 min.

4. CONCLUSIONS

A systematic study of the local pKa and redox characteristics can be performed by using pH-sensitive-hydrophobic spin probes and EPR spectroscopy. This protocol can provide accurate information on the physicochemical properties of the hydrophobic domains of HA not accessible by water nor by water soluble probes i.e. metals, H^+. In addition, the acknowledged sensitivity of spin-probes can be exploited to map colloidal properties and aggregation phenomena in HA.

ACKNOWLEDGEMENTS

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Study on Self-Assembling Mechanism of IHSS Humic and Fulvic Standards

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Keywords: IHHS humic and fulvic standards, aggregation mechanisms, HRUS

1. INTRODUCTION

Humic substances (HS) are ubiquitous and also the most occurring organic matter on the Earth. They can be generally characterized as yellow to black mixture of compounds rich in oxygen-containing functional groups (carboxyl group -COOH; phenolic and alcoholic -OH group; ketonic and quinoic -C=O group) which are bound on both aromatic and aliphatic carbon skeleton. Due to their high heterogeneity and chemical character they tend to self-aggregate forming various molecular organisations. The knowledge on the aggregates mass and nature is of a great importance since the mass of aggregates influences the function of HS in nature (e.g. in case of higher plants low molecular weight components of HS were shown to be particularly active (1) while high molecular mass components have only residual activity (2)).

Despite an intensive research on humic substances, the mechanism and way of aggregation is still not precisely known. There are several views and works claiming that humic substances exhibit the critical micelle concentration (CMC). This view supposes that amphiphilic molecules exist solely as single unit species at concentrations lower than the CMC, whereas at higher concentration ordered aggregates or micelles are formed. Wershaw (3) proposed that interactions playing role in aggregation are mainly hydrophobic (π–π and charge transfer) and H bonds. Character of prevailing interactions strongly depends on pH and ionic strength of solution (4). Von Wandruszka (5) demonstrated the existence of submicellar aggregates at concentration under supposed CMC (i.e. under 2 g L^{-1}). These conclusions were confirmed by Kučerík et al. (4) who observed aggregation of lignite humic acids even at concentration approximately 0.001 g L^{-1}. Similar results have surprisingly been obtained also at elevated pH and at high ionic strength although such conditions are not supposed to be supportive for self-aggregation of amphiphilic molecules (4).

The aim of this work was to bring new insights into aggregation mechanisms of several HS standards obtained from the International Humic Substance Society (IHSS) employing the high resolution ultrasonic spectroscopy (HRUS). The behaviour of sodium salts of humic and fulvic acids (NaHA, NaFA) was investigated from very low concentration (0.001 g L^{-1}) up to 3.5 g L^{-1}.

2. MATERIALS AND METHODS

HS standards (Table 1) were purchased from IHSS, titrated to pH 7.5 with 0.1 M NaOH in automatic titrator (Schott, TitroLine Alpha Plus). After a constant value was reached for 60 min, the sodium humate solution was filtered and freeze-dried. The product was milled in an agate mortar and stored at room temperature in a sealed container.

Table 1. Examined IHSS humic and fulvic standards

1S104H-5	2S102F	2S101H	1S103H
Leonardite Humic Acid Standard	Elliott Soil Fulvic Acid Standard II	Suwannee River Humic Acid Standard II	Florida Peat Humic Acid Standard

For monitoring ultrasonic velocity and attenuation a HRUS102 device (Ultrasonic-Scientific) was employed. It consists of two independent quartz cells tempered by a water bath; cell 1 serves as sample cell and cell 2 as reference. All experiments were carried out at 25.00 ± 0.02°C, under constant stirring (600 rpm) and at 8 different frequencies. The resolution of the spectrometer is down to 10^{-5}% for ultrasonic velocity and 0.2% for attenuation measurement. NaHA and NaFA were dissolved in deionised water to obtain concentrations of 1, 5 and 10 g L^{-1}, respectively. In order to assess mechanism of aggregation, 1 mL of deionised water was added to each cell. The solution in cell 1 was added stepwise every 10 min (i.e. when constant ultrasound velocity values were achieved) with prepared NaHA or NaFA solutions.

The concentration increment in the ultrasonic velocity A was calculated from the equation (6):

$$A \cong \frac{U_1 - U_2}{U_2 \cdot c \cdot \rho_0}$$

where U_1 and U_2 are the values of ultrasonic velocity in solution and solvent, respectively, c is the weight concentration of the solute and ρ_0 is the density of the solvent. A is determined by the compressibility of water in the hydration shell of the solute molecules and, in the case of molecular aggregates, by their intrinsic compressibility (7).

3. RESULTS AND DISCUSSION

HRUS has become a powerful tool in colloidal chemistry due to its sensitivity to monitor any conformational change in a solution under study. Figure 1 reports the dependence of *A* on concentration of NaHA and NaFA. Constant value of *A* can be observed when molecules are freely dissolved in the solution (i.e. do not form aggregates). When CMC is reached, change of the concentration increment in the ultrasonic velocity *A* occurs, which indicates interaction among molecules (i.e. aggregates formation) (7). It is in line with our previous observation that humic molecules tend to aggregate also in diluted solutions at concentrations significantly lower than concentrations reported as critical micelle concentration (typically in range 2-10 g L^{-1}) (4).

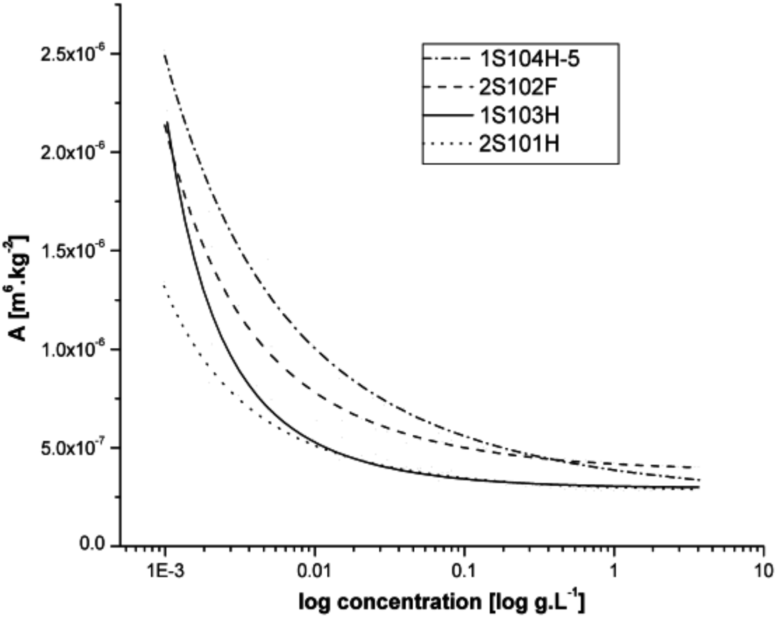

Figure 1. Dependence of ultrasonic velocity increment on concentration.

Extrapolation of obtained dependences to infinite dilution has been published as a measure of affinity of molecules to stuck to each other (7). As it can be seen, the highest increase at infinite dilution gave the lignite humic acid sample (1S104H-5). On the other hand, the lowest affinity showed sample Suwannee River (2S101H). It is in accordance with the chemical character of that sample, i.e. ratio of aliphatic/aromatic C distribution (cf. web page IHSS). In fact, our data are in line with the E4/E6 ratio traditionally interpreted as a degree of humification. It is noteworthy that results obtained from ultrasonic measurement, which is based on completely different principle, are in compliance with optical methods.

Furthermore, ultrasound velocity was measured at 8 different frequencies and at all frequencies did not differ, which implies that although humic molecules aggregate, the compressibility of resulted organizations do not decrease the ultrasonic velocity. Ultrasonic waving is mechanical in nature and causes compression and decompression of present aggregates. Therefore if no decrease in ultrasonic velocity was observed, formation of spherical, i.e. highly compressible micelles, or micelle-like aggregates can be excluded.

4. CONCLUSIONS

High resolution ultrasonic spectroscopy can be used to study assembling mechanism of several systems including humic substances solutions. Sodium salts of humic and fulvic acids self-aggregate from low concentrations but form only small associates and no micelle-like structures occur.

ACKNOWLEDGEMENTS

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Multiple Charged Constituents in Suwannee River Natural Organic Matter

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Keywords: organic matter, FTICR/MS, Ion mobility MS, charge state, molecular formulas

1. INTRODUCTION

Natural organic matters are complex biogeochemical non-repetitive materials existing in such a vast amount, that their quantity easily exceed the amount of functional biomolecules (1, 2). And furthermore their continuous reformation, degradation and diagenesis, which are governed by the rather fundamental restrains of thermodynamics and kinetics, result their extreme intricacy. Therefore the molecular level understanding of such supermixtures is extremely difficult. However the proper characterization of such a vast system is feasible only if minimal–invasive sampling techniques (3,4) and afterwards several high-end analytical techniques are combined, since generally applied methods, for instance elemental analysis, IR spectroscopy describe them only with an averaged characteristic (5). To verify this, surplus information were presented here, by utilizing FT-ICR/MS and Q-TOF-IM/MS and integrated mathematical data analysis. FT-ICR measurements give sufficient mass accuracy and resolution even in broadband mode (6), enabling postulate exact molecular formulae and determine the charge-state (a singly (or doubly) charged $^{13}C^{12}C_{n-1}$ ion will be higher in m/z by 1 (or 0.5) than the corresponding $^{12}C_n$ nuclide). Therefore application of such a method to resolve and describe individual humic constituents is preferable. Despite of ultra-high resolution, non-adequate settings of any kind of experimental settings might mislead the general result of the characterization (7-9). Since the occurrences of poly-carboxylic acids and -hydroxyl groups, and their relative high abundances had been shown in these type of mixtures (10-12), the formation of multiply charged negative ions in the ESI experiments would have been expected. Though it should be noted, that the denoted properties of large molecules might change during their transformation from polyelectrolyte state in solution to the detected ions in the gas phase. On the other hand, till now, the presence of probable multiple charged constituents were only assumed, moreover sometimes depicted as “bumps”, but without further details (9, 13).

2. MATERIALS AND METHODS

The sample, SRDOM was dissolved in methanol directly before the analysis. Ultra-high resolution mass spectra of the sample, was acquired at the Helmholtz Zentrum München with a Bruker APEX Qe Fourier transform mass spectrometer (FTICR-MS) equipped with a 12 Tesla superconducting magnet and an Apollo II electrospray source. The sample was injected with direct infusion with the flow rate of 2 µL/min. The spectrum was obtained in negative mode with the number of data points per measurements of 4 MW (time domain size) with the typical resolution of $5*10^5$. The number of scans was 11000 for 1 µg/mL sample concentration. The spectrum was externally calibrated on arginin clusters and then internally recalibrated with fatty acids. Once the exact masses of the molecules had been determined, their molecular formulae were batch-calculated by a software tool, written in-house and provided by E.V. Kunenkov. Ion mobility (IM) measurements were carried out in negative modus, on a Waters Synapt High Definition MS system in the Waters Application Laboratory (Manchester).

3. RESULTS AND DISCUSSION

Herein, the obtained high resolution mass spectra of SRDOM (Suwannee river dissolved organic matter) and SRFA (Suwannee river fulvic acid) revealed beside the usually characterized single charged constituents further multiple charged species, and were described for the first time. In the spectrum of 1µg/mL SRDOM, beside the regular pattern of singly charged nominal masses, a second series with 0.5 Da mass width, between 250-560 m/z mass range were visible. Since ESI negative ion 12 Tesla FTICR mass spectra of SRDOM resolved over 120.000 peaks, with the average resolution of ≥ 300.000 molecular formula assignment with a software tool, written in-house, with less than 0.5 ppm was possible, resulting over 6000 assigned molecular formulae. The most significant mass differences between $C_cH_hO_oN_nS_s$ and $^{13}CC_{c-1}H_hO_oN_nS_s$ were Δm = 1 Da (singly charged) and 0.5 Da (doubly charged). Afterwards, the charge sorted constituents were plotted based on their H/C and O/C elemental ratios in van Krevelen diagrams.

The peculiar presentation of assigned molecular formulas, enables to individually describe certain structural properties of the observed constituents within this supermixtures. Although we have to note that, in several cases, differentiation between charge states were not possible since constituents with $C_xH_yO_z^-$ and $C_{2x}H_{2y}O_{2z}^{2-}$ elemental composition, denote the same position in the van Krevelen diagram. But still, one of the most remarkable difference between charge states is that, molecules assigned as doubly charged, are densely situated in higher O/C field than the single charged ones.

Beside FTICR measurements, ion mobility experiments were also conducted in order to support the observed results (14). SR DOM sample were injected without preliminary separation in different concentration (100-0.5 µg/mL) resulted similar results independent of the applied concentration. ESI negative ion TOF-Ion Mobility mass spectra and drift time vs. m/z distribution of SRDOM, obtained on Synapt HDMS system, confirmed also the existence of multiple-charged constituents through visible separation of molecules, based on their charge-states. In the drift map, at least two distribution of ions were presented: group of doubly charged constituents with shorter drift time and a group of singly charged molecules with longer drift time.

The efficiency of an automated formula assignment, developed by E. V. Kunenkov, was also tested successfully. For this purpose, beside the complete FTICR mass spectra, an expanded m/z region of 348.8-350.6 was selected, and the possible molecular formulas were individually assigned (51 constituents, belonged to 10 different series), with built in software of Bruker Data Analysis 3.4). Mass accuracy for all assignments were ≤ 90 ppb.

4. CONCLUSIONS

Overall, our results show for the first time that expanded and carefully selected experimental (measurement and data analysis) settings and combined high-end analytical tools can eventuate the extension of the existing knowledge on molecular level understanding of such a supermixtures.

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Depicting Molecular Dissimilarity in Complex Materials

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Keywords: NOM, compositional space, intrinsic averaging, isomers, complexity

1. INTRODUCTION

Natural complex organic materials if classified by their means of synthesis and function divide into discrete molecules which often derive from genetic codes that can be fractionated into unambiguously defined molecular fractions within the scope of current high-performance separation technology, and complex non-repetitive materials which cannot be purified in the conventional meaning of purity because of even higher intricacy.

The fate of this vast group of molecules in the bio- and geosphere is governed according to the rather fundamental restraints of thermodynamics and kinetics. In these intricate materials, the “classical” signatures of the (often ultimately biotic) precursor molecules, like lipids, glycans, proteins have been attenuated, often beyond recognition, during a succession of biotic and abiotic (e.g. photo- and redox chemistry) reactions (1). Because of this loss of biochemical signature, these materials can be designated *non-repetitive complex systems.* Examples include freshwater, marine, and soil organic matter, kerogens and aerosols, among others.

2. MATERIALS AND METHODS

The findings mentioned here have been derived over several years from multinuclear high-field (11.7 T) NMR spectra and high-field (9.4 and 12 T) FTICR mass spectra of natural organic matter (NOM) samples of terrestrial, freshwater and marine origin.

3. RESULTS AND DISCUSSION

The current perception of natural organic matter (NOM) molecular structure is defined by the available analytical methods, which typically depict data-reduced projections of the chemical structure space (i. e., the complement of all chemical structures present in NOM). Related to the true molecular diversity of NOM, the extent of data reduction encountered in any spectra or chromatograms always ranges in the extremes. Accordingly, severe intrinsic averaging, caused by a non-avoidable, massive signal overlap masks molecular-level alterations of NOM in any standard analytical technique. For similar reasons, bulk properties of NOM, like elemental ratios and acidity, regularly fall within remarkably narrow ranges (Figure 1).

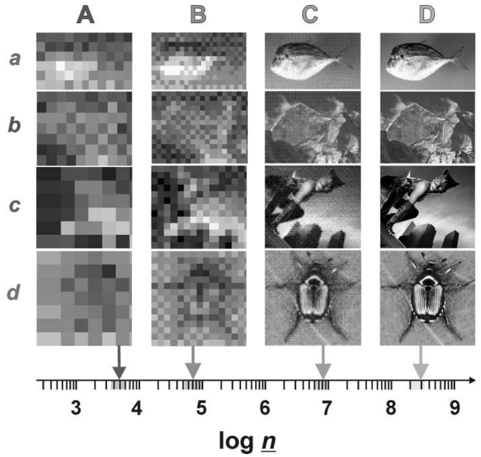

Figure 1. Information transfer in organic structural spectroscopy.

The images depicted here define a three dimensional space composed of the area of the image (pixel resolution in x- and y-axes) and the depth of the color space. This three-dimensional space offers n options to depict dissimilarity (n = x * y * color-depth). Only at considerable extent of information, significant resolution is attained to enable meaningful assessment of data; otherwise, intrinsic averaging precludes meaningful interpretation. See reference 1 for color image and relationships to resolution in separation and spectroscopic methods.

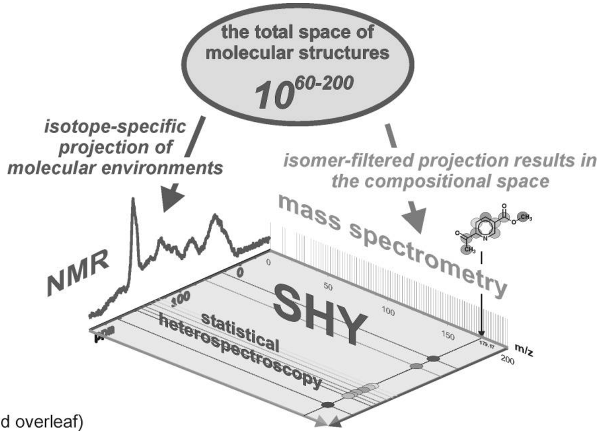

Figure 2. (legend overleaf)

Figure 2.

Molecular level precision analytical data represent projections of the vast total structure space of molecules, for which count estimates range from 10^{60} to 10^{200}. Mass spectra reflect the isomer-filtered complement of the entire space of these molecular structures. The *compositional space* of molecules can be probed with current ultrahigh-resolution FTICR mass spectroscopy, resulting in *molecular signatures* (i.e. single peaks for molecules in absence of fragmentation). NMR spectra represent site- and isotope-specific projections within molecular environments. These *atomic signatures* provide unsurpassed detail about short range molecular order. *Molecular signatures* (arrangement of chemical bonds including connectivities, stereochemistry and spatial proximity) can be assembled from NMR data by use of complementary multinuclear 1,2,3D NMR experiments, obtainable from a single sample. Reconstruction of chemical structures from NMR and MS data is available by back-projection, which in case of mixture analysis is commonly preceded by separation (Figure 3).

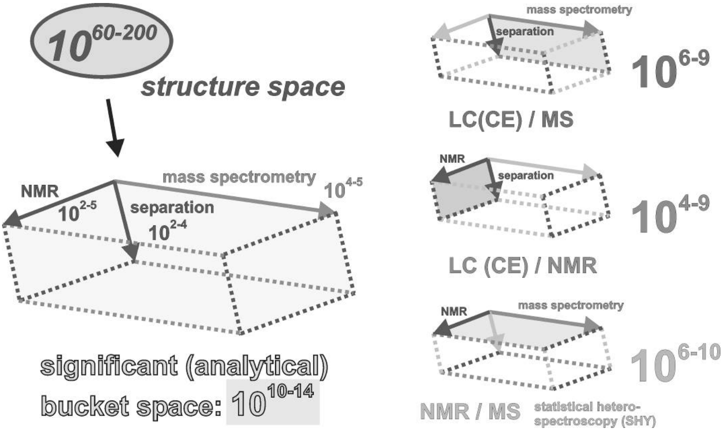

Figure 3.

The currently accessible discrete and quantized analytical voxel space to describe complex systems in the range of 10^{8-14} voxels is defined by the significant resolution of the complementary techniques NMR (depicting short range order within and among molecules), FTICR mass spectrometry (depicting molecular mass and formulae of gas-phase ions) and high-performance separation (depict capacity to investigate both ions and molecules) and therefore capable to provide the necessary detail for a meaningful molecular level analysis of very complex mixtures. The various projections of this bucket space, like separation / MS, separation / NMR and NMR / MS can be realized in the form of direct hyphenation and via mathematical analysis of (these correlated) data sets.

The availability of high-resolution organic structural spectroscopy has the capacity to advance crucial paradigm shifts in the molecular-level analysis of NOM. The combined use of NMR spectroscopy and FTICR mass spectrometry has enabled the identification of CRAM (carboxyl-rich alicyclic molecules) as major constituents of marine organic matter (2). The occurrence of CRAM in freshwater and terrestrial environments seems likely, considering the global distribution of biomolecules and the similarities of biogeochemical processes among environments (3). Another recent study has revealed a near complete turnover of groundwater fulvic acids within decades in a karst aquifer (4), even in absence of the two key abiotic degradation pathways, photo- and redox chemistry. Again, these extraordinary molecular-level structural alterations were only revealed by high-performance non-target organic structural spectroscopy. Even fairly advanced analytical tools at the level of standard 13C-CP/MAS solid-state spectroscopy and unit-resolution mass spectrometry would have failed to detect these extensive changes because of intrinsic averaging (5).

Based on this novel perspective, molecular level alterations of such magnitude and swiftness must be considered as commonplace in the processing of “refractory” NOM, which then has to be perceived, in contrast to conventional wisdom, as a rather more active participant in the global carbon cycle.

4. CONCLUSIONS

High-resolution organic structural spectroscopy has the capacity to advance crucial paradigm shifts in the molecular-level analysis of NOM, which then has to be perceived, in contrast to conventional wisdom, as a rather more active participant in the global carbon cycle.

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Isolation and Characterization of Humic and Fulvic Acids from Thermal Waters as an Unexplored Biogeosystem

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Keywords: thermal water, humic fractions, FTICR/MS

1. INTRODUCTION

The recent interest in groundwater from deep aquifers seems to be increasing. The term thermal water is used, if the temperature of tapped groundwater is above 30ºC. The development of thermal spas for balneotherapy and the exploitation of geothermal energy have been in the focus of the use of this natural resource recently (1).

Hungary is one of the countries of favourable geothermal conditions. In this area, hot water could be exploited, of which temperature is approximately 100ºC, from wells tapped from a depth of 2000 m. These waters got to present position long time ago, and their chemical composition altered profoundly during geological range of time.

The hot waters contain several dissolved mineral and organic components. Their chemical composition depends on the geological location of well and changes instantly during production. Degassing, evaporation of volatile organics, precipitation of salts and minerals take place due to decreasing pressure and temperature. In addition, oxidation processes producing new compounds rapidly occur when hot water in reductive condition reaches up to ground level and comes into contact with atmospheric oxygen. The dissolved organic matter (DOM), such as humic substances (HS) attracted less attention so far, although these organic macromolecules are always present in all kind of groundwater. In this work the isolation of humic substances from thermal water at a depth of about 1000 m and molecular characterization of fulvic and humic acids (FA and HA) will be shown.

2. MATERIALS AND METHODS

Groundwater sample of temperature of 46ºC was collected from well tapped from 993 m from the region of Makó (Southeast Hungary) in February 2006. This hot water is alkali-hydrocarbonated with pH value of 8.2 and more than 1000 mg L^{-1} total dissolved solid content. The samples were stored in plastic containers below 5ºC until processing. After sampling of hot water, it could be observed significant change in appearing yellowish water color in a day or two. This phenomenon was followed up with UV-vis spectroscopy.

Isolation of organic matter was according to the procedure of International Humic Substance Society "Method for Preparation of IHSS Aquatic Humic and Fulvic Acids" (2). Humic acid (HA) and fulvic acid (FA) concentrations in thermal water sample were 1.92 and 1.24 mg L^{-1}, respectively, on the basis of the amounts of isolated material in the reference to the volume 100 L of the water sample.

The isolated HA and FA were characterized by elemental analysis, potentiometric acid-base titration and FTIR spectroscopy. In addition, high-resolution mass spectra of HA and FA for molecular formula assignment were acquired on a Bruker APEX Qe Fourier transform ion cyclotron resonance mass spectrometer (FTICR/MS) equipped with a 12 Tesla superconducting magnet and an APOLLO II ESI source. The HA and FA stock solutions were prepared by dissolving 1-1 mg of samples in trace amount of NH_4OH and Milli-Q purified water, and only in Milli-Q water, respectively. The working solutions were diluted from the stock solutions with methanol such that the concentration of organic matter was about 10 ppm. The measurements were carried out in negative ionization mode. Mass spectra obtained were calibrated with respect to fatty acids. Then formula calculator software was used to calculate all possible formulas based on the requirement that the mass calculated for a given chemical formula agrees with the measured mass within ± 0.001 Da.

3. RESULTS AND DISCUSSION

Table 1. Elemental composition and calculated atomic ratios of HA and FA samples

Sample	Weight percent, %					Atomic ratio	
	C	H	N	S	O	O/C	H/C
HA	57.68	4.26	2.16	1.69	34.21	0.44	0.89
FA	53.19	4.13	1.28	1.70	39.70	0.55	0.97

FA exhibits lower C, higher O contents and higher O/C ratio than HA (Table 1). These results are in agreement with the general finding for HAs and FAs from different sources (soil, freshwater, marine water, peat) (3). However, the values of O/C and especially H/C ratios of hot water HA and FA samples are consistently lower than those from other sources.

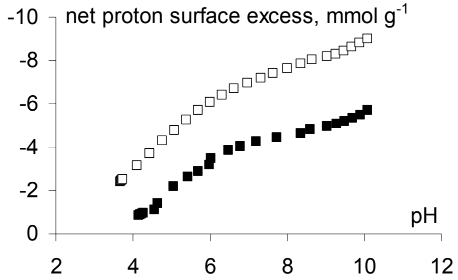

Figure 1. The pH-dependent dissociation of acidic groups on HA (■) and FA (□) in 0.01 M NaCl.

The absolute value of net proton surface excess is equivalent to the amount of negatively charged, i.e. dissociated acidic groups of HA and FA.

The shape of proton binding curves (Fig. 1) is characteristic of HSs, and the amounts of acidic groups are in the range of standard and reference HA and FA samples of IHSS The pH-based estimates of the carboxyl and phenolic functional group contents of HA and FA are 7.8, 4.1 as well as 14.3 and 5.2 mmol g C^{-1}. Carboxyl groups constitute two third and three fourth of the total acidity for HA and FA samples, respectively (4). Although relative amount of phenolic OH content of HA is higher than that of FA, the phenolic compounds are more abundant in FA.

Table 2. IR absorbance values and ratios of those in case of HA (■) and FA (□) at different wavenumbers, assigned to K1: aliphatic/carbonyl, K2: aliphatic/aromatic and K3: carbonyl/aromatic ratios

Sample	Measured absorbance			Calculated ratio		
	aliphatic	carbonyl	aromatic	K1	K2	K3
HA	0.086	0.403	0.277	0.21	0.31	1.45
FA	0.143	1.324	0.494	0.11	0.29	2.68

Table 2 shows that aliphatic, carbonyl and aromatic content is higher in case of FA correlating with the higher O/C and H/C ratios from elemental analysis and greater carboxyl and phenolic functional group contents from acid-base titrations. In addition, the aromatic contents are more dominant than aliphatic ones in both HA and FA samples, the proportions of those are very similar as reflecting by the almost same K2 ratios. The maximum of absorbances appear at wavenumber belonging carbonyl groups. pH-based estimates of the carboxyl functional group contents correspond with the K3 ratios.

Molecular formulae obtained from high-resolution mass spectra by using formula calculator software were sorted on the basis of heteroatomic composition to establish different compound classes, i.e. formulae with the same constitution of heteroatoms (O, N and S). The relative abundances of formulae as showed for oxygen containing compounds in Fig. 2 were calculated by dividing with the summed intensity for all species identified (5). It also comes up that FA is more oxygenated shifting the maximum of relative abundances toward molecules with higher O content (Fig. 2). Moreover detailed insight could be gained since the most abundant compounds of HA and FA with 7 and 10 O atoms, respectively.

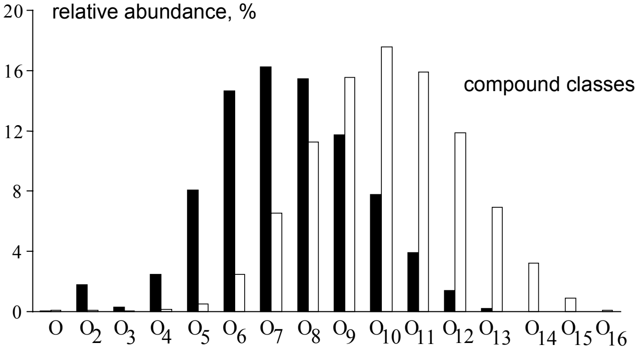

Figure 2. Only oxygen containing compound classes identified in HA (■) and FA (□).

4. CONCLUSIONS

Novel results on molecular characterization of HA and FA samples isolated by IHSS method from an unexplored biogeosystem were showed. Their properties are in the region of natural organic matter especially characteristic of humic substances. The significant darkening of water stored even at low temperature after sampling is interesting and needs detailed investigation in future. The HA and FA samples isolated will be the subjects of detailed organic geochemical investigations to reveal their origin and biogenesis.

ACKNOWLEDGEMENTS

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Hydrous Pyrolysis of Natural Organic Matter from a Highly Coloured Surface Water

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Keywords: NOM, pyrolysis, hydrous pyrolysis

1. INTRODUCTION

Analytical pyrolysis has been extensively applied for the structural characterisation of natural organic matter (NOM). The attraction of the technique lies in its ability to reduce complex macromolecular material to lower molecular weight (MW) compounds more amenable to analysis by gas chromatography-mass spectrometry (GC-MS). Heating of the sample in an inert environment over a time period typically less than 30 seconds to temperatures in excess of 500°C (flash pyrolysis) results in fragmentation and partial defunctionalisation of macromolecules as a result of the large amounts of thermal energy acquired by the sample (1). However, a significant portion of flash pyrolysis products is often highly polar, precluding their analysis by GC-MS (2). Reducing the polarity of products, particularly if controlled such that enough of the hydrocarbon structure is preserved to enable recognition of its primary precursor, would be advantageous.

Low temperature pyrolysis can facilitate the artificial maturation of recent NOM on laboratory time-scales in an analogous manner to the natural geological formation of fossil fuels (3). The study of petroleum formation kinetics using low energy pyrolysis has proved popular (4-7), however, studies on characterisation of recent or extant NOM has received little attention (2, 8).

Hydrous pyrolysis is a low energy pyrolysis technique involving the heating of NOM in a sealed vessel in the presence of water at temperatures below its supercritical point (374°C) (4). Koopmans *et al.* (9-12) have shown the potential of this technique by identifying hydrocarbon biomarkers weakly bound *via* S or O linkages to the Kerogen moiety of immature, sulfur rich organic sediments. In this study, we investigate the potential of hydrous pyrolysis as a technique for characterisation of dissolved NOM, using solid samples isolated from a highly coloured surface water as well as NOM still in solution.

2. MATERIALS AND METHODS

A surface water sample from the Quickup River Dam, Denmark, and Western Australia was collected in September 2006. The highly coloured water (1000 L) was collected in a 1000 L polyethylene container and filtered through a 0.45 μm membrane. The filtered sample was concentrated to approximately 20 L by reverse osmosis (RO) and stored at 4°C until required. A small sub-sample (1 L) of the RO retentate was subjected to lyophilisation without further treatment and the solid NOM collected. The NOM isolate was analyzed by hydrous pyrolysis by the procedure outlined below.

NOM (100 mg) and purified water (2 mL) was placed in a stainless steel container (6 mL internal volume) in a nitrogen environment to eliminate the presence of oxygen and sealed at either end with Swagelok® fittings. The stainless steel container was heated to 330°C for 72 hours and allowed to cool to room temperature for 12 hours. Contents of the container were extracted with dichloromethane and concentrated (2 mL) using a Kederna-Danish apparatus, and dried over anhydrous $MgSO_4$ (Sigma-Aldrich, AR grade). The dried extract was analyzed by GC-MS. A sample of the RO permeate (5 mL) was also analyzed using the identical procedure as above, apart from the addition of water to the system.

3. RESULTS AND DISCUSSION

Two hydrous pyrolysis chromatograms from a NOM sample isolated from a highly coloured source water in the south west of Western Australia are presented in Figure 1. Heating of the solid NOM isolate at 330°C over 72 hours resulted in almost 200 identifiable products detected by GC-MS following extraction of the aqueous phase with DCM. An almost identical suite of products was obtained from hydrous pyrolysis of the corresponding RO NOM concentrate. Analysis of the pyrolysis products indicated that the sample contained significant input from polysaccharides, suggested by the abundance of cyclopentenones and furan derivatives. As well, a small number of protein markers were detected and lignin input appeared substantial.

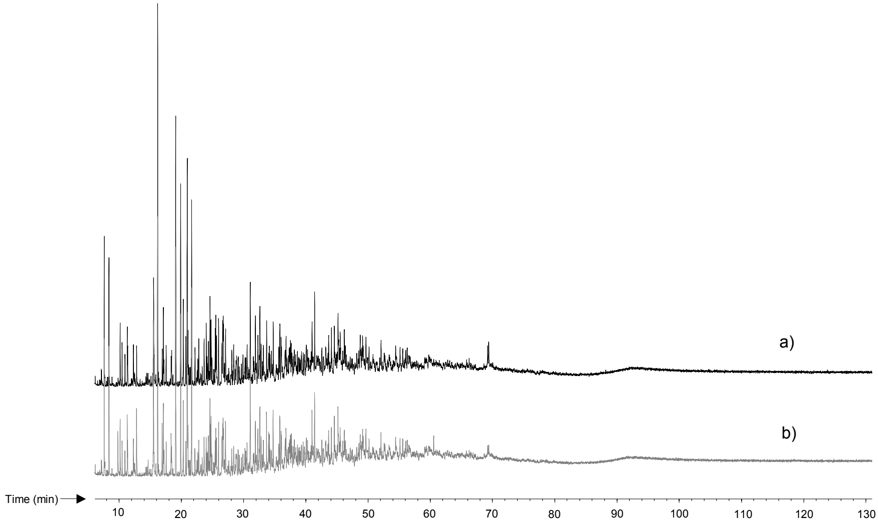

Figure 1. GC-MS analysis of hydrous pyrolysis product extracts;
a) 100 mg of solid NOM + 2 mL of H_2O; b) 5 mL RO NOM concentrate.

Significantly, the almost identical nature of the two pyrograms indicated the potential for hydrous pyrolysis of aqueous NOM samples, eliminating the need for complex isolation procedures. Comparison of hydrous pyrolysis results with a pyrogram obtained from flash pyrolysis of the same solid NOM isolate (results not shown) revealed a number of similar products. The relative abundances of similar compounds from each technique differed; however, hydrous pyrolysis was able to generate a significantly greater number of products identifiable by GC-MS. A particular advantage of hydrous pyrolysis is the ability to analyze gaseous products, collected upon opening of the sealed container, Hydrous pyrolysis also provides the potential to analyze the water phase for pyrolysis products not extractable by DCM. Hydrous pyrolysis should, therefore, enable a far greater percentage of the products of pyrolysis to be identified than is the case using traditional online flash pyrolysis techniques.

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High Resolution and Hyphenated Analytics as Tools for Exploring Chemical Space of HS and NOM from Various Environments

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Keywords: high resolution, analytics, NOM

There is a high need in combining orthogonal analytical tools for the characterization of complex organic mixtures such as natural organic matter (NOM). The presentation focuses on novel tools and methods for a molecular understanding of NOM and humic substances (HS).

NOM consist in a continuum in molecular structures leading to higher levels of complexity from which derives their particular physical-chemical properties. Analyzing the functions of NOM needs the perfect choice of scale-adapted analytical tools when looking at their interactions with natural or anthropogenic chemical or biological systems in the environment (soil, water, aerosols) or looking at chemical transformation processes of humic like compounds (HS, lignins, composts etc.) into added value chemicals at the lab-scale. Bulk analysis may be sufficient or molecular resolution may be needed. All the process from (i) sample preparation to (ii) molecular analysis and (iii) data integration and modelisation needs to be considered and adapted to the natural materials to be analyzed (Fig. 1).

In the emerging field of Metabolomics which was defined by Nicholson *et al* 1999 (3) as “**The quantitative analysis or description of all low molecular weight metabolites in specified cellular, tissue or biofluid compartments”** the need is given to adapt the analytical tools and methods to cover the highest analyzable chemical space (2). The same approach is needed in the field of Humics where techniques are combined and needs to be validated (avoiding artifacts and false assignments). Adequate sampling and preparation (cleanup or concentration of target compounds) is followed with a separation of the analytes based on their structural properties (charge, size, hydrophilicity/hydrophobicity …) and a detection strategy offering best sensitivity or best profiling properties.

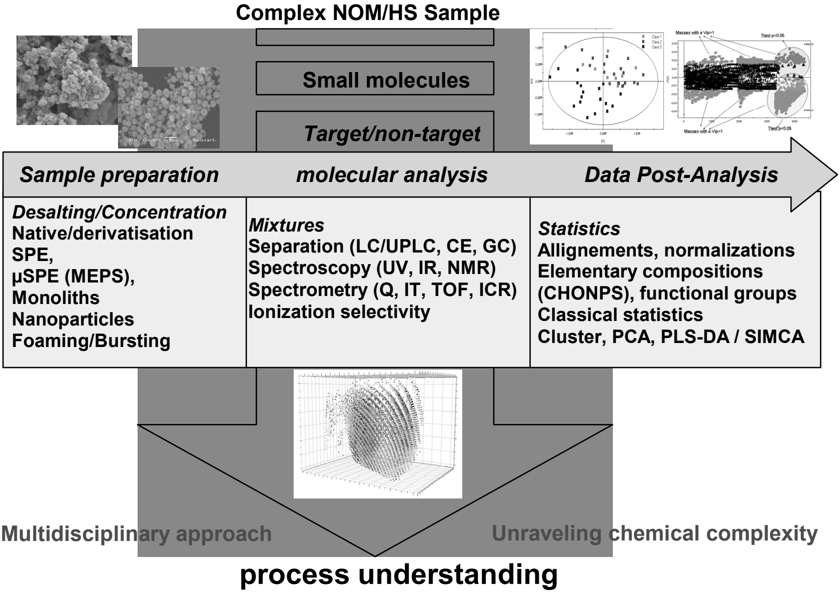

Figure 1. Analytical approaches for complex organic systems from sample preparation, molecular analysis and further chemometrics.

In our humics fields of investigation various levels of the analytical approaches can be distinguished:

- **targeted analysis:** quantitative evaluation of concentrations of chemicals (organic and inorganic, natural and anthropogenic, ambient to trace amounts) from various matrices after precise and adapted sample preparation (cleanup and concentration) (1). When following a hypothesis driven approach specific compounds can be quantified within the humic matrice. Cleanup and sample preconcentration (solid phase (micro)extraction – SPE) are essential and constitute the first steps in the analysis, followed by a electrophoretic or chromatographic separation (capillary electrophoresis, electrochromatography, liquid chromatography, gas chromatography) and an adapted detection (UV-Vis., fluorescence, mass spectrometry).

- **metabolite profiling** – within this approach all classes of metabolites (i.e. lipids, sugars, peptides, proteins etc…) are analyzed in the mixtures. In the corresponding life science investigation field one would consider *proteomics*, *lipidomics*, glycomics.

- **non-targeted analysis** – here new technologies are developed and optimized for a qualitative/semi-quantitative evaluation of the presence of chemical classes in complex mixtures – molecular inventory needed to allow process descriptions or discovery of new biomarkers (4).

High-end technologies such as Nuclear Magnetic Resonance Spectroscopy (NMR) and especially Ion Cyclotron Resonance Fourier Transform Mass Spectrometry (ICR-FT/MS) presented more in details, are ideal tools to get structural information on a large quantity of components within one single sample especially when using high field magnets (Fig. 2).

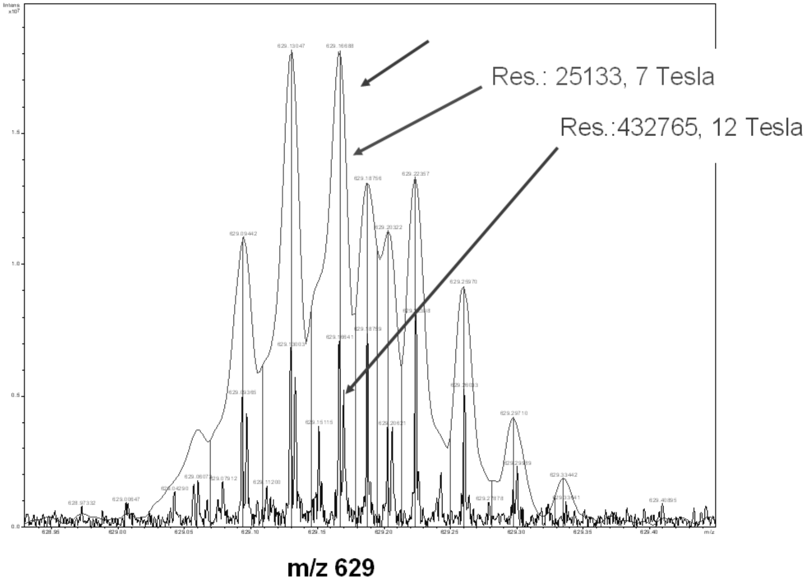

Figure 2. Detail on m/z 628 showing the effect on peak resolution on the limitation in structural information in ultrahigh resolution mass spectrometry (sample is PM 2.5 aerosol particle extract).

The combined use of separation/cleanup technologies and liquid microhandling, with new ionization techniques (APPI, APLI) and ultrahigh resolution mass spectrometry certainly are uniquely suited for identification of known and hitherto unknown metabolites and possible biomarkers in very complex samples and for the analysis of often inseparable mixtures (5).

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X-Ray Spectromicroscopy of Organic Matter in the Environment

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Keywords: X-ray spectromicroscopy, synchrotron radiation, laser plasma X-ray source, organic matter, soil, humic substances, XANES

1. INTRODUCTION

X-ray microscopy achieves a much higher resolution than light microscopy due to the much shorter wavelength of X-rays compared to visible light. The smallest structures that can be seen in an X-ray microscope at present are less than 15 nm in size. Between the K-absorption edge of Carbon and of Oxygen the linear absorption coefficient of water is low compared to of organic or inorganic matter. This gives rise to a natural amplitude contrast even when imaging specimen in aqueous media with X-rays within this energy range. No drying, fixation or staining of a sample is necessary. By choosing the used X-ray energy appropriately, it is possible to perform spectromicroscopy studies. Maps of the distribution of a specific element in a sample can be obtained. It is possible to use near-edge resonances for XANES-studies. As the used X-ray energy is raised to match the absorption edge, resonances reflecting the chemical bonding state of the element can be detected. As a result of the refractive index of matter being close to unity scattered X-ray light will not be reflected from inner surfaces in inhomogeneous media. Clear images without scattering background are obtained even when studying thick and inhomogeneous samples. Therefore, X-ray microscopy images can be used for a tomographic reconstruction of thick samples. Comprising, X-ray spectromicroscopy is a powerful tool for addressing key questions in many scientific areas, e.g. to study structures in the environment showing dimensions on the nanoscale.

2. MATERIALS AND METHODS

Soil colloids have been imaged to visualize the appearance of structures on the nano- and microscale (1). The effect of changing chemical conditions in an aqueous environment on the appearance of these structures has been imaged and evaluated. Morphological effects of biologically induced redox changes of humic substances have been imaged tomographically conveying a detailed three-dimensional presentation of the specimen structure (2). Using the spectromicroscopy potential, the distribution of organic and inorganic components has been studied; spectra have been analyzed for major chemical constituents (3). To achieve reasonable exposure times X-ray microscopes need highly brilliant X-ray sources, e.g. synchrotron radiation from electron storage rings. Several successfully running X-ray microscopes have been installed at these large-scale facilities worldwide. However, only few electron storage rings exist worldwide, hampering the dissemination of X-ray microscopy as a useful technique. Recently, new developments in laser plasma sources for X-rays made first microscopy and spectroscopy experiments possible using a tabletop setup. Images of soil colloids in aqueous suspension have been taken in the first successful experiments with a laboratory size soft X-ray microscope (4). Humic acids, fulvic acids, and organic matter from soils have been characterized and compared by XANES spectroscopy using a tabletop laser plasma X-ray source (5).

3. RESULTS AND DISCUSSION

The two soil samples in fig. 1, show in the colloidal size range clusters of particles with many different appearances. However, particles with similar shapes can be identified quickly. In this size range all samples mainly consist of clay particles making these similarities comprehensible. In addition, microorganisms are very often attached to the particles. They can be found in the images as ellipsoidal or round shapes with a distinct membrane. The images clearly show the desired morphology of clusters of colloids or nanoparticles. A micro zone plate with dr_n = 25 nm has been used as the microscope objective, resulting in a resolution in the images of about the same value. As a result, single particles within the clusters can easily be identified.

A stack of images of a chernozem soil sample have been taken in small energy steps around the K-absorption edge of Carbon. Fig. 2 shows one of these images and three regions marked in light gray. Integrating the transmission signal I_1 of each region as a function of energy and using the region marked dark gray as I_0 the spectra below each image could be obtained as $-\ln(I_1/I_0)$, which yields the optical density. The left image and the appendant spectrum below clearly reveal the L_{II}- and L_{III}-absorption features of potassium, a strong indication for clay particles. The spectrum in the middle reveals the organic origin of the particle marked in the image. Image and spectrum at the left shows the result of the sum of all particles from that flock. The differences in the spectra illustrate clearly the higher information content of spatially resolved data.

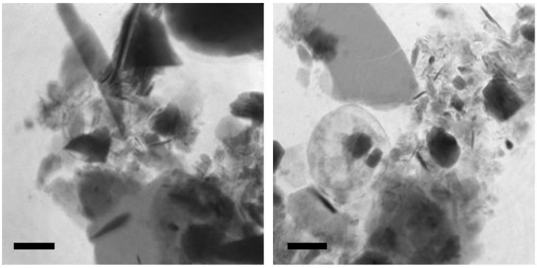

Figure 1. Transmission X-ray microscopy images of two soil types, luvisol (left) and gleysol (right), imaged in aqueous media with the X-ray microscope at BESSY II with a resolution of about 25 nm. The scalebar indicates 2 µm.

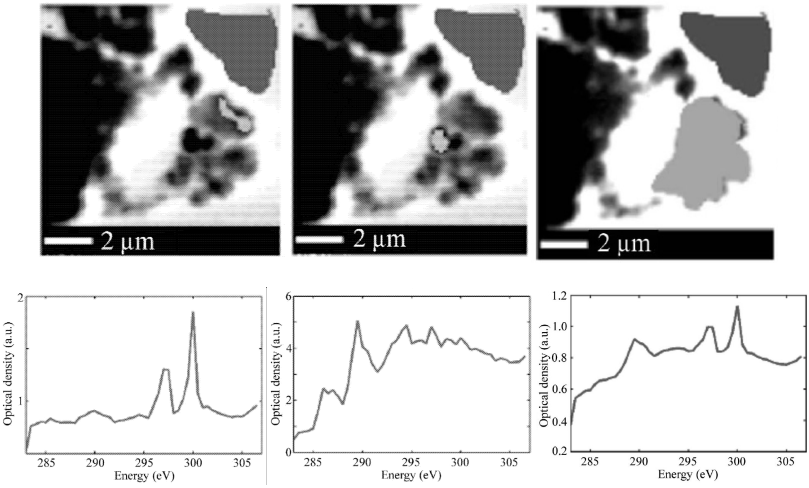

Figure 2. Selected regions marked in light gray from a stack of images and the corresponding spectra at the carbon K-absorption edge. The region used for I_0 is marked dark gray. The scalebar indicates 2 µm.

Imaging experiments with soil colloids in aqueous media using a laboratory size soft X-ray microscope have been performed in collaboration with the Royal Institute of Technology/Albanova, Biomedical and X-Ray Physics, in Stockholm, Sweden. First XANES spectra of organic matter from colloidal samples from the environment have been obtained in collaboration with the Laser Laboratory in Goettingen, Germany. Fig. 3 shows exemplary results from both collaborations.

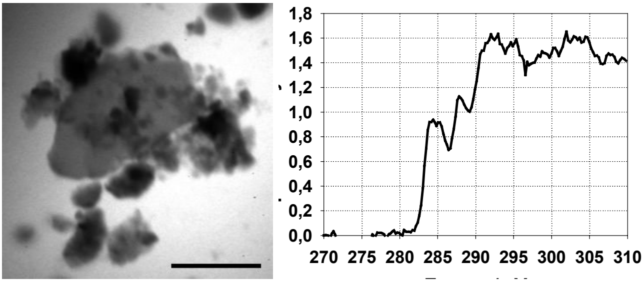

Figure 3. Left: X-ray microscopy image of colloidal particles of a chernozem soil in an aqueous suspension taken with a laboratory size soft X-ray microscope. The scalebar indicates 1 µm. Right: XANES spectrum of a humic substance from a chernozem soil obtained using a spectrometer with a tabletop laser plasma X-ray source.

4. CONCLUSIONS

X-ray spectromicroscopy is be a suitable tool for examining colloidal structures in their original aqueous matrix. Imaging as well as spectroscopy allow the characterization of morphology and chemistry of organic matter in relation to other elements. Laser plasmas as intense X-ray sources will be an alternative light source in the next future for many X-ray spectromicroscopy experiments making this technique more accessible.

ACKNOWLEDGEMENTS

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Effects of pH, Temperature, and Organic and Inorganic Ions on the Dissolution Kinetics of Humic Acid Particles

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Keywords: soil humic acid, humic acid-water interface, dissolution rate

1. INTRODUCTION

Humic acids (HA) represent an important and active fraction of the refractory organic matter in soils, sediments and natural waters. Their ability to become solubilized in water under different conditions may affect greatly the distribution and transport of contaminants in the environment. Information about the dissolution behavior of particulate HA can be assessed by dissolution kinetic experiments, topic that has been rather unexplored in the scientific literature. This kind of studies is needed to properly identify the conditions that promote dissolution of HA, to quantify the dissolution rates under different conditions and to assess information regarding the mobility of HA and the fate of their attached pollutants. Dissolution kinetic studies may also provide mechanistic information on the dissolution process, and fundamental information about the reactivity of the solid HA particle/water interface. This article presents a study of the dissolution kinetics of solid HA particles under different conditions and in the presence of different anionic and cationic species in solution. Data is used to gain insights into factors that promote or prevent dissolution.

2. MATERIALS AND METHODS

The HA was taken from an andisol (A Coruña, Spain). It was obtained according to the procedure recommended by the IHSS. General characteristics of this sample can be found elsewhere (1). Dissolution kinetic experiments were carried out in a cylindrical, water-jacketed reaction vessel under N_2 bubbling. Before starting a kinetic experiment, 100 mL of a 10^{-3} M KCl solution containing also a known concentration of another substance (carboxylic acids, herbicides or inorganic cations) were placed in the reaction vessel. The pH of the solution was then adjusted to the desired value with KOH and/or HCl solutions. The dissolution experiment was started by adding a known weight (between 15 and 20 mg) of solid HA to the mentioned solution. At different reaction times, a 5 mL aliquot was withdrawn, the particles were separated from the supernatant by sedimentation or centrifugation and the supernatant was immediately analysed to quantify the concentration of dissolved HA by UV-VIS spectroscopy. After the quantification, that took around 30 seconds, the supernatant and the HA particles were reintroduced into the reaction vessel. This procedure was repeated during several hours in order to achieve complete dissolution of the sample or to gather enough data points. Special care was taken in keeping constant pH (± 0.1 units) during the whole dissolution run by adding minute amounts of KOH and/or HCl.

3. RESULTS AND DISCUSSION

The dissolution kinetics of the studied HA can be evaluated by plotting the degree of progress of the dissolution reaction, α, as a function of time, *t*. α=0 means that 0% of the solid HA has been dissolved, whereas α=1 means that 100% of the HA has been dissolved. Fig. 1a shows different dissolution curves (α *vs. t)* for the studied HA at different pH. From data such as those plotted in Figure 1a the dissolution rate, *R*, which is defined as the slope of the dissolution curve, can be also plotted (Fig 1b). The results shown in Fig. 1a and 1b reveal that *R* is strongly dependent on pH: there is a 400-fold increase in *R* when the pH is changed from 4 to 11. The dissolution mechanism is believed to be related to the deprotonation of carboxylic and phenolic functional groups of the HA molecules, which results in the breaking of some hydrogen bonds that hold the HA molecules together in the solid, leaving the molecules more susceptible for detachment (2). This deprotonation also results in an increased electrostatic repulsion among surface molecules, which may contribute to increase their rate of detachment from the surface.

The dissolution rate is also increased by increasing temperature (Fig. 1c) and stirring rate (not shown). The apparent activation energy of the process (35 kJ mol^{-1}) and the effects of stirring rate indicate that there is a mixed-control (surface and transport) of the dissolution kinetics under our experimental conditions. The dissolution rate at constant pH is also strongly dependent on Ca^{2+} concentration (Fig. 1d), decreasing as Ca^{2+} concentration increases. Very similar results were obtained with others cations (Mg^{2+}, Sr^{2+}, and Ba^{2+}). These ions are believed to rapidly adsorb at the particle surface decreasing the electrostatic repulsion among HA molecules. Cations could also act as bridges between functional groups of two adjacent molecules increasing the attractive forces between them, contributing to decrease the dissolution rate.

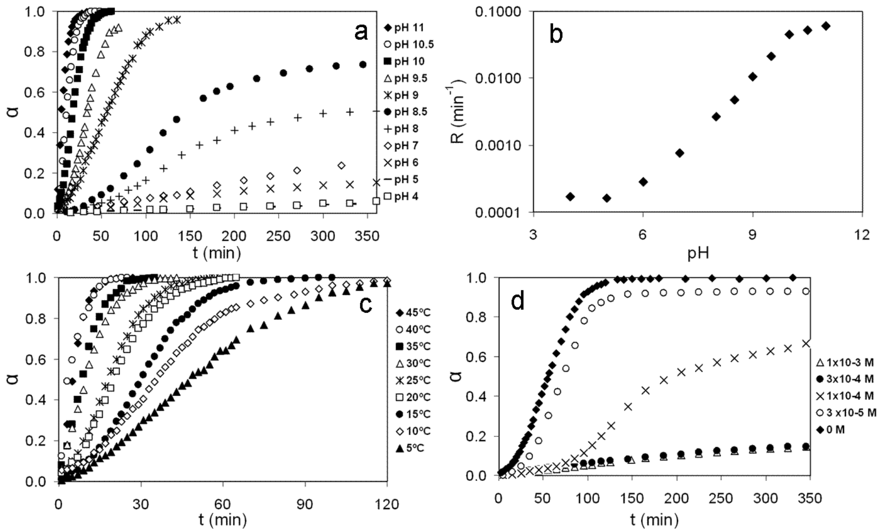

Figure 1. a) Dissolution curves of the studied HA at different pH in 10^{-3}M KCl without the addition of other substance. b) Effect of pH on *R*. c) Dissolution curves at different temperatures and pH=10. d) Effect of Ca^{2+} concentration (values in the figure) on the dissolution rate at pH 9.

The effects of monocarboxylic acid (formic, acetic, and propionic) and herbicides (glyphosate, 2,4-D, and paraquat, PQ) on the dissolution behavior of HA particles are shown in Fig. 2. At any pH the presence of monocarboxylic acids produces an increase in the *R* as compared to the behavior observed in KCl solutions (Fig 2a). The dissolution rate increases in the order propionic>acetic>formic, suggesting that monocarboxylic acids are able to adsorb, disrupting the interactions among HA molecules in the solid phase and promoting the dissolution. The larger the aliphatic chain of the acid is, the bigger the interaction becomes, and the faster the dissolution. This interacting ability of monocarboxylic acids was already demonstrated by Piccolo et al. (3) for humic acid aggregates in solution.

The dissolution rate also significantly increases at all investigated pH when anionic herbicides (glyphosate and 2,4-D) are present in aqueous solution (Fig. 2b). Nevertheless, the cationic herbicide PQ presents a dual effect. It decreases the dissolution rate at pH>7 and increases the rate at pH<7. At pH>7, where the HA has a relatively high negative charge, PQ seems to act as other cations (Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}) do, increasing the bonding among HA molecules in the solid phase. At pH<7, where the negative charge of

HA is relatively low, PQ appears to act as carboxylic acids, disrupting interactions among HA molecules.

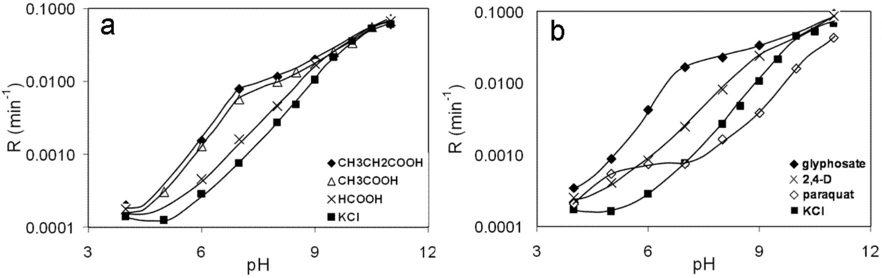

Figure 2. a) Effect of monocarboxylic acids on the dissolution rate of the studied HA at different pH. b) Effect of herbicides on the dissolution rate of the studied HA at different pH.

4. CONCLUSIONS

The results of this work show that the dissolution kinetics of solid HA particles is strongly dependent on pH and temperature. It is also highly dependent on the concentration of dissolved species in aqueous media. In general, anionic species tend to accelerate the dissolution rate, whereas cationic species tend to slow down the rate. These effects may have a profound impact on the mobility of HA and attached pollutants in aqueous media.

ACKNOWLEDGMENTS

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Spectroscopic Evaluation of Charcoal Derived Humic-Like Acid

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Keywords: humic-like, charcoal, spectroscopic, fluorescence, UV-Vis

1. INTRODUCTION

The aim of this work is to study the suitability of using different charcoals in order to produce humic-like acid. Fluorescence and UV-visible spectroscopy were applied to evaluate the humidification degree by using different index: E_4/E_6 (2, 8), A_{254}/A_{465} (8) (UV-Vis spectroscopic); I_{400}/I_{360} (4) and I_{470}/I_{360} (4) (fluorescence spectroscopy in synchronous-scan mode); A_{440} (5) and A_4/A_1 (9) (fluorescence spectroscopic in emission scan).

2. MATERIALS AND METHODS

2.1. Organic Material

It has been used different types of charcoal from: sugar cane bagasse; orange bagasse; sugar cane straw; Elephant grass (*Pennisetum purpureum)* and *Eucalyptus grandis*. Humic acid was also extracted from humus samples from compost of leaves grass and commercial compost by earthworm.

2.2. Oxidation and extraction

Approximately 5.0 g of dry charcoal was mixed with 200 mL of 25% of nitric acid. The mixture was heated at 80°C in reflux condition for 4 h. After this time, the samples were kept at room temperature for 12 h (6). After oxidation, the samples were filtered using a 0.45 µm cellulose membrane. The filtrate separate was considered fulvic acid and the solid material was dissolved in 0.1M KOH (50 mL solution/g solid) and shaked for 12 h in sealed flask with N_2 atmosphere. Humics substances have been separated applying the method recommended by the International Humic Substances Society (IHSS). Total carbon concentration was determined by oxidation with sodium dichromate by photometric methods.

2.3. Spectrophotometric measurements

2.3.1. UV-visible spectroscopy

Humic acid of the charcoal from sugar cane bagasse (BC), charcoal from orange bagasse (BL), charcoal from straw of sugar cane (PC), charcoal from elephant grass (CE), charcoal from *E. grandis* (CV), compost of leaves grass (C) and commercial compost by earthworm (HM) were dissolved at 20 mg/L in de $NaHCO_3$ 0.05 mol/L and pH was kept in 8.5. Ultraviolet and visible spectrophotometric measurements have been made between 190 and 900nm with a Cary-100 UV-VIS Varian^{inc} spectrophotometer using quartz cells (1 × 1 cm). E_4/E_6 (2, 8) and A_{254}/A_{436} (8) ratios were calculated by measured absorbances.

2.3.2. Fluorescence Spectroscopy

Each humic acid sample was also dissolved at 20 mg/L in $NaHCO_3$ 0.05 mol/L and pH was kept in 8.5 (5). Fluorescence spectra has been collected using a fluorescence spectrophotometer from Photon Technology International® equipped with both excitation and emission monochromators. Emission scan were collected between 260-660nm with excitation wavelength of 240 nm and these spectra were divided into four regions. Than rations between the last quarter (A_4) (580-650nm) (9) and the first quarter (A_1) (350-420 nm) (9) were calculated. Emission scan spectra was also collected with excitation wavelength of 440 nm and the total area under the spectra (A_{440}) (5) was calculated. Synchronous spectra were recorded with excitation wavelength range 300-475 nm and a $\Delta\lambda$ of 55 nm. The fluorescence intensity in 360, 400 and 470 nm was determined and the ratios between these intensities were calculated I_{400}/I_{360} (4) and I_{470}/I_{360} (4).

3. RESULTS AND DISCUSSION

3.1. UV-visible spectroscopy analysis

The UV-Vis spectra of different samples have showed a high absorption in the 210-240 nm range and a decrease in absorption with the wavelength increase. It is a typical result for humic acid. Characterization of the samples by different index is showed in Table 1. It can be seen low ratio E_4/E_6 for samples C and HM and high value for samples BL, PC, BC, CE and CV. Ratio A_{254}/A_{436} shows greater value for HM, BL and C. The low values for E_4/E_6 or A_{254}/A_{436} ratio can be attributed to absorption by aromatic C=C functional groups as well as to the high degree of condensation of the aromatic rings.

3.2. Fluorescence spectroscopy analysis

Fluorescence spectra of humic-like acid obtained by acid oxidation exhibits a broad feature emission band with a maximum at ~510 nm. This band is consistent with data published by IHSS. In the other hand, samples C and HM showed a different behavior, as the intensity decreases by increasing the wavelength.

Table 1. Ratios of the measured UV-Vis absorbencies

	HM	CV	BL	C	PC	BC	CE
E_4/E_6	3.65	4.25	7.60	3.52	6.42	6.20	5.89
A_{254}/A_{436}	5.338	4.151	5.766	5.161	4.466	4.255	4.490

Fluorescence synchronous spectra are presented in Figure 1. Most of the samples (BL, CE, PC, BC and CV) show a similar behavior with a more intense peak at 350 nm and a shoulder between 410-470 nm, where the intensity decreases continuously. The sample HM showed a slightly difference with more intense peak at ~375 nm. Sample C showed a different behavior with an intense decrease of intensity until 330 nm.

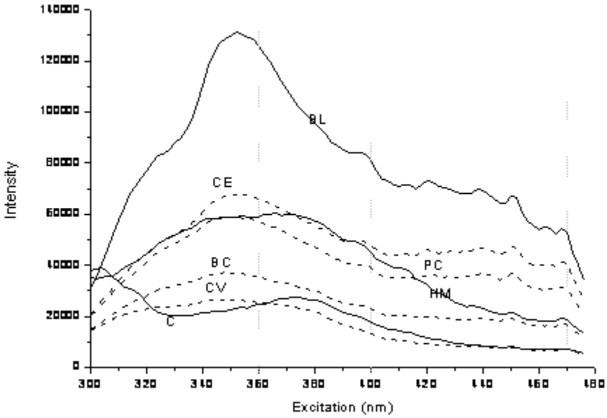

Figure 1. Fluorescence synchronous-scan excitation spectra (Δλ = 55 nm).

The index obtained with fluorescence spectroscopic (Table 2) reinforces the conflicting types of evaluation. Index I_{400}/I_{360} indicates that HM, CE and C as the most humificated. I_{470}/I_{360} indicates as being the samples CE, PC, BC. The index A_{440} indicates BL, CE and PC while the A_4/A_1 indicates BC, PC and BL, as the most humificated, respectively. Therefore, it seems that each group of a specific chemical species should be evaluated by a specific index.

Table 2. Ratios of the measured fluorescence spectroscopic parameters

Sample	Fluorescence Parameter			
	I_{400}/I_{360}	I_{470}/I_{360}	$A_{440} \cdot 10^6$	A_4/A_1
HM	0.77	0.31	1.75	0.08
CV	0.52	0.27	0.65	0.15
BL	0.64	0.42	4.59	0.16
C	0.71	0.29	0.70	0.15
PC	0.69	0.54	2.71	0.19
BC	0.65	0.47	1.48	0.19
CE	0.73	0.61	3.47	0.15

4. CONCLUSIONS

UV-Vis spectra show a typical humic acid behavior for all samples. The ratio E_4/E_5 for samples HM and C shows lower value indicating high degree humification. The same information was also taken from A_4/A_1 and I_{470}/I_{360} Fluorescence index. All these values are near of those given in some references.

Synchronous spectra of sample C showed a similar profile of compost materials (4). Also, the same spectra of sample HM showed a similar profile with those from compost of soil mill olive waste; commercial humic acid by Aldrich Chemicals; compost of a mixture of animal manures and non-composted animal manure (4). All others samples showed similar profiles of the soil humic substances from China and Czech Republic (4).

All index values and the profile of UV-Vis and Fluorescence obtained scan graphics are in accordance with reported references showing the suitability of using different charcoals as starting material to produce humic-like acid.

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Properties of Hydration Shell of IHSS Humic Standards

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Keywords: humic substances, DSC, hydration

1. INTRODUCTION

Humic substances have a profound effect in many processes including cell biology of living organisms. As they are both hydrophobic and hydrophilic in nature, their function is closely related to the properties of water shell intimately bound on humic molecules and consequently on humic aggregates. A strong affinity of water molecules to stick to each other via H-bonds and formation of specific clusters is a driving force assembling humic molecules into complicated organizations. Water is involved in these processes in a variety of ways, ranging from direct bridging to collective effects (such as hydrophobic effects) (1). The enumeration of water molecules is crucial in order to understand how biomolecular processes work.

Phase transition behaviour of sorbed water in HS-water systems was investigated as a function of water content (W_c) using differential scanning calorimetry (DSC). Basically, three types of water can be distinguished by phase transition of sorbed water (2): free water (W_f) whose melting/crystallization temperature and enthalpy are not significantly different from those of normal (bulk) water; those water species exhibiting large differences in transition enthalpies and temperatures, or those for which no phase transition can be observed calorimetrically, known as freezing-bound water (W_{fb}) (2, 3); and non-freezing water (W_{nf}) which is strongly associated with the molecule and shows neither crystallization exotherms nor melting endotherms on DSC curves (2, 3).

2. MATERIALS AND METHODS

Material

IHSS Standard samples were measured: Suwannee River II HA 2S101H (SRHA), Suwannee River II FA 2S101F (SRFA), Elliot Soil HA 1S102H (ESHA), Elliot Soil II FA 2S102F (ESFA), Pahokee Peat HA 1S103H (PPHA), Pahokee Peat II FA 2S103F (PPFA).

DSC Measurements

The IHSS Standard samples were placed in aluminum pans (~5 mg) and excess water was added. Surplus water was allowed to evaporate slowly at room temperature until the desired water content was obtained. Subsequently, the pans were hermetically sealed and left to equilibrate at room temperature overnight. Differential scanning calorimetry (DSC) was performed by using the TA Instruments Q200, equipped with a nitrogen gas-cooling unit, to measure phase transition of sorbed water. The measurements were conducted ranging from 40 to –90°C and then from –90 to 30°C at 3°C min^{-1} under the flow of nitrogen (50 mL min^{-1}). The temperature scale and heat of transition were calibrated using distilled water and indium as standards, respectively.

Water content determination

The thermogravimetry analysis (TGA TA Instruments Q5000) was used to measure water content of original IHSS Standard samples so that the real concentration of water (W_c) in the samples could be recalculated. The W_c was determined:

$$W_c\ (\text{g/g}) = \text{weight of water (g) in HS / weight of dry HS (g).}$$

Assuming both melting enthalpies for freezing-bound water (W_{fb}) and free water (W_f) to be 334 J g^{-1} water, weights of W_{fb} and W_f (g water/ g HS) could be calculated from the endothermic heating transitions. The weight of non-freezing water (W_{nf}) was obtained using the expression:

$$W_{nf} = W_c - (W_{fb} + W_f).$$

3. RESULTS AND DISCUSSION

IHSS standard samples with water content from 10 to 150% were measured. At the highest water content a large heating endotherm could be observed in the region of 0°C whose enthalpy of transition was close to that of bulk water (333 J g^{-1}). This endotherm was ascribed to the melting of the freezing water in the hydrated HS-water system. At lower water content an endothermic transitions appeared below 0°C when the system was heated. This peak indicated the presence of freezing-bound water in a humic sample, i.e. water which is already affected by the interaction with humic molecules. Its clusters structure differs from those of bulk water and is physically recognizable by shifted temperature of melting.

Representative DSC heating curve of ESFA with water content W_c 0.408 is given in Fig. 1 where various endothermic transitions at different temperatures, attributed to the melting of different types of ice, can be observed. These endothermic peaks, or better shoulders, marked with arrows, indicate that there are more than three types of freezing-bound water in the ESFA-water system. In fact, the enthalpy of melting differs from that ice which was attributed to the freezing water. Due to the different structure of ice such enthalpy can drop down to 312 J g^{-1} (4).

Measurement of sample from various sources showed in some cases different results. For instance, sample SRFA did not gave the peak melting around –30°C which indicates i) less complicated and heterogeneous molecular structure than corresponding humic acid SRHA ii) lower hydrophobicity of fulvic acids.

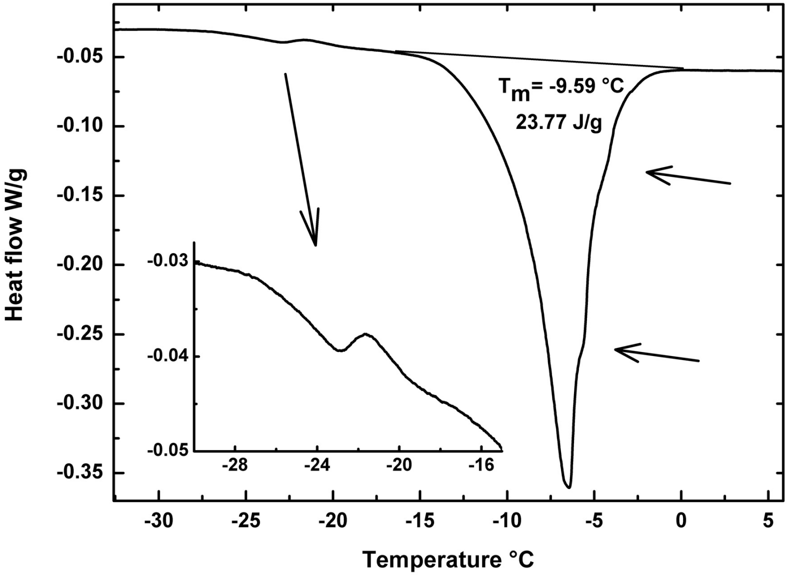

Figure 1. DSC heating curve for ESFA-water system with W_c 0.408.

Obtained results are in line with the theory that there exist more that one type of freezing-bound water and those significantly differ in their physical properties. In case of ES and SR HAs the presence of amorphous “glassy” ice was observed.

Table 1 reports the content of non-freezing water calculated from DSC experiments. All values are determined in the range 0.38 to 0.57 g water per gram of humic substance and for their calculation the enthalpies in the range from 333 to 312 J g^{-1} were used (4). It is noteworthy, that in case of humic substances originated from aqueous system the higher content of non-freezing water was determined for humic instead of fulvic acid while in case of other humic “couples” vice versa. It implies the theory that water is in humic substances bound not only on the surface of aggregate but also into the secondary humic structure.

Table 1. Comparison of non-freezing water content range in IHSS Standard samples with similar water content

IHSS Standard sample	W_{nf}
SRHA	0.556 - 0.536
SRFA	0.380 - 0.397
ESHA	0.503 - 0.511
ESFA	0.560 - 0.569
PPHA	0.484 - 0.495
PPFA	0.516 - 0.524

4. CONCLUSIONS

Differential scanning calorimetry represents a technique which can help to recognize the differences in properties of water surrounding humic matter. Although same DSC technique limitations associated with processes of ice formation should be taken into account (e.g. supercooling effect) that method can bring new information regarding the function of humic substances in biological processes.

ACKNOWLEDGEMENTS

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Insights into the Composition of Humin from an Irish Grassland Soil

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Keywords: humin, humic substances, solid-state ^{13}C NMR spectroscopy

1. INTRODUCTION

Humin is defined as the fraction of the humic substances (HS) that is insoluble in aqueous solution at any pH value (1). Because of its insolubility it has been the least studied of all the humic fractions (2). Humin typically represents more than 50% of the organic carbon (C) in a soil, and is regarded as the residual organic C that remains after extraction of the HAs and FAs (3). Based on data from solid-state ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy, it has been concluded that a repeating aliphatic structural unit, possibly attributable to branched and cross-linked algal or microbial lipids, are common to both sediment and soil humin samples (4). Humin becomes enriched in these aliphatic substances because of their selective preservation during organic diagenesis (2). To investigate the composition of humin, The Clonakilty soil, a well drained Brown Podzolic soil in grassland lysimeter studies at the Teagasc Soils Research Centre, Johnstown Castle, Co. Wexford, Ireland and was used in the present study.

2. MATERIALS AND METHODS

Soil samples were H^+-exchanged and exhaustively extracted with 0.1 M NaOH, at pH 7.0, then with NaOH solution adjusted to pH 10.6, then at pH 12.6, then with 6 M urea in the 0.1 M solution. Extracts were pressure filtered (0.2 µm membrane) at each pH, and sediment on the filter was returned for extraction by the next solvent in the series. The fine clays retained on the filter after extraction with the 6M urea system were collected, dialysed, and freeze dried. To isolate the humin organic C, the residue was treated with 10% HF to dissolve the aluminosilicates. Some samples were further extracted with a DMSO-concentrated H_2SO_4 (94% DMSO: 6% H_2SO_4) mixture to isolate a humin fraction following extraction in aqueous base and in the basic urea medium. Solid-state ^{13}C NMR spectroscopy experiments were carried out at ^{13}C and ^1H frequencies of 100.5 and 400.0 MHz, respectively. Typical cross-polarization times of 1 ms, acquisition times of 13 ms, and recycle delays of 500 ms were used. For Variable Amplitude Cross-Polarization (VACP) experiments a Magic-Angle Spinning (MAS) frequencies of 13 kHz was used. Bloch Decay experiments were carried out while spinning at 13 kHz with a recycle delay of 1 s and a sweep width of 27 kHz.

3. RESULTS AND DISCUSSION

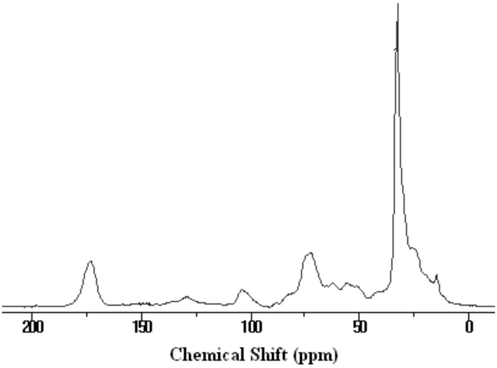

Figure 1. ^{13}C CP-MAS NMR spectrum of Humin isolated from the 0-10 cm depth while spinning at 13 kHz, with a cross-polarization time of 1 ms, acquisition time of 13 ms and a recycle delay of 500 ms.

Fine clay was isolated from soil at the 0-10 cm depth after exhaustive extraction with NaOH followed by NaOH and Urea. The organic matter in the isolated fine clay is classified as humin as it was found to be insoluble in aqueous media at any pH. Figure 1 shows the spectrum for 0-10 cm depth humin. In aerobic soils, the spectra of humin show the presence of polysaccharides and aromatic structures most likely derived from the lignin of vascular plants. However, another major component of humin contains paraffinic carbons and is thought to be derived from algal or microbial sources. A major peak in the humin spectra is that of aliphatic carbons at 30 ppm. Cutin and cutan from plant cuticles are considered to be major contributors to this region. These structures may well be the major contributors to the carboxyl or amide groups (peak at 175 ppm) (2).

Proteinaceous substances would give rise to resonances in the 0-50 ppm region of ^{13}C NMR spectra, but usually the aliphatic signals of protein are centered more closely toward 20, 40, and 50 ppm than the 30 ppm resonance (2). Humin contains significantly less carboxyl than the corresponding HA isolate (not shown). There is little indication for lignin-like structures in the spectra because of the absence of significant peaks at 56 ppm and at 150 ppm for methoxyl and O-aromatic carbons, respectively.

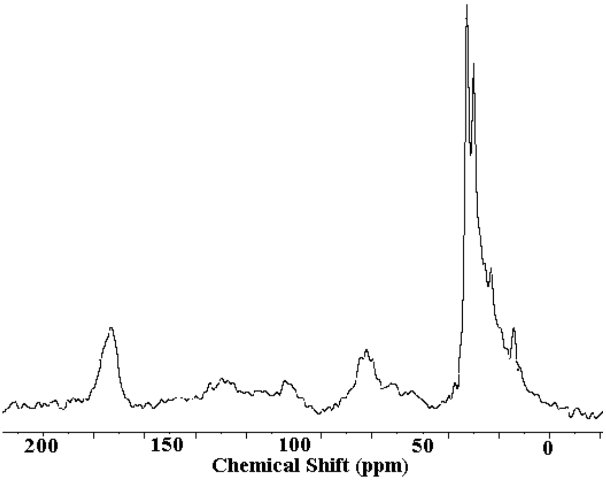

Figure 2. ^{13}C Bloch Decay NMR spectrum of Humin isolated from the 0-10 cm depth while spinning at 13 kHz with a recycle delay of 1 s and a sweep width of 27 kHz.

Figure 2 shows the Bloch decay ^{13}C NMR spectrum for the 0-10 cm depth humin. Again the dominance of aliphatic signals is evident. The spectrum shows an aliphatic region characterized by a small peak at 14 ppm, from CH_3, and a large peak for main-chain CH_2 (30 ppm). It can be seen that the main-chain CH_2 groups are composed of both amorphous chains (29 ppm) and crystalline chains (32 ppm) (5)

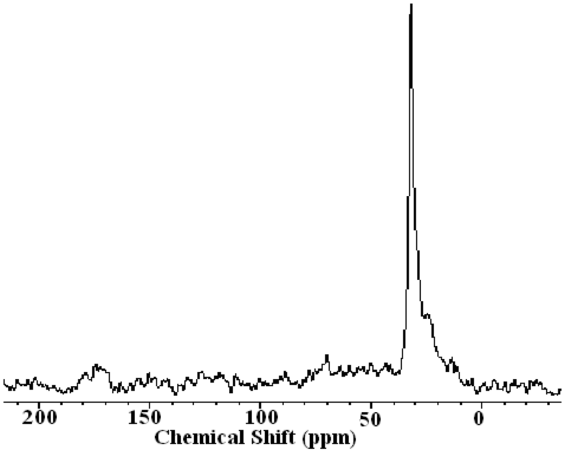

Figure 3. ^{13}C CP-MAS NMR spectrum of the DMSO/H_2SO_4 extract of Humin while spinning at 13 kHz, with a cross-polarization time of 1 ms, acquisition time of 13 ms and a recycle delay of 500 ms.

A DMSO-concentrated H_2SO_4 mixture has been employed to isolate a humin fraction following extraction in aqueous base and in the basic urea medium (6). It was shown that $\geq$ 70% of the traditional humin fraction could be solubilised. The spectrum of the dried extract can be seen in Figure 3. It contains mainly aliphatic signals with some very minor contributions from carbohydrates and carboxyl signals.

4. CONCLUSIONS

The humin samples studied contain contributions from aliphatic, carbohydrate, and little aromatic and carboxyl resonance. The strong aliphatic resonance would support the theory of the “selective preservation” of the more aliphatic compounds. It is necessary to structurally define the paraffinic components of humin and to ascertain whether these are truly biomolecules or are rapidly transformed products of decomposition of vegetal matter (2). Data will be shown to highlight the contribution of cutin/cutan to the humin fraction. Further work needs to be done on this, in addition to analyses of the organic matter in the lower depths, to determine the contribution of biomolecules to humin.

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Characterization of Soil Organic Matter in Different Particle-Size Fractions by CP/MAS ^{13}C NMR Along a Slope of Subtropical Mountain Forest

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Keywords: CP/MAS ^{13}C NMR; forest; particle size

1. INTRODUCTION

Soil organic matter (SOM) represents one of the largest reservoirs of carbon on the global scale and is related to the biological activity in terrestrial ecosystems. Soil along a hillslope usually exhibits remarkable differences in morphology that may be attributed to the influences of moisture status, microclimate and vegetation. Factors affected by topography that differs the status of organic matter in the soil profile. Physical fractionation of soil according to particle size supported by chemical, biological and physical analyses of the fractions obtained has proven to be a useful tool in process-oriented SOM research (3, 4).

This study investigated the characteristics of the soil organic matter (SOM) solid-state CP/MAS ^{13}C NMR spectroscopy at different slope positions in a perhumid mountain forest ecosystem. The sampling site, Yuanyang Lake, is featured with high annual rainfall (>4000 mm) and elevation from 1670 to 2169 m in northeastern Taiwan. The surface soils collected were fractionated into different particle sizes in order to clarify biological significance inside SOM.

2. RESULTS AND DISCUSSION

Fig. 1 shows the functional groups in most of the bulk soils were in the same abundance order despite the generally wide proportion range.

Alkyl-C and carboxyl-C content increased slightly with decreasing particles size as shown in Table 1. This result is consistent with other studies showing that carbonyl/carboxyl content increases with degree of humification (5, 6). The increase in carboxyl-C might come from the lignin side-chain oxidation as well as other chemical reactions. As for aromatic-C, it seems that no consistent trend was observed.

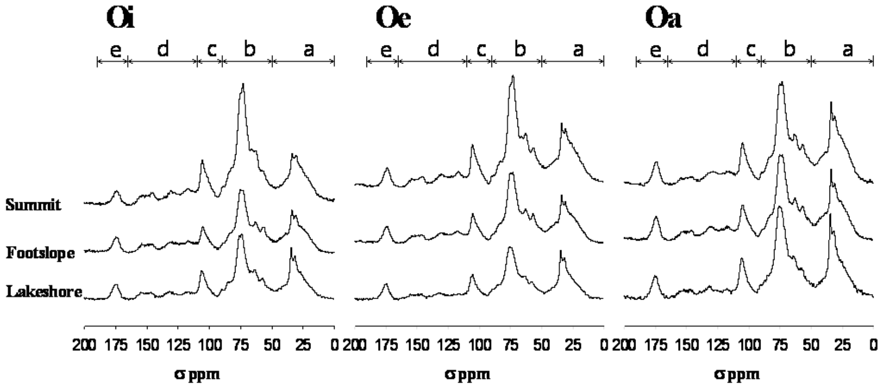

Fig. 1. Topographic change of solid-state CP/MAS ^{13}C NMR spectra for different horizons of per-humid forest soils. (a) alkyl-C, (b) O-alkyl-C, (c) di-O-alkyl-C,(d) aromatic-C, (e) carboxyl-C.

On the other hand, O-alkyl-C and di-O-alkyl-C contents show different trends. The decrease in these two groups of carbons as particle size decreases from coarse to fine indicates that the extent of decomposition was more pronounced for small size fraction than large size fraction. This result strongly suggests that extent of O-alkyl-C decomposition is higher at the summit than that at other sides.

Table 1. Relative intensities of the ^{13}C CP/MAS spectra in different soils

Site	Horizon	Functional group (%)					Alkyl-C/ O-Alkyl-C
		Alkyl-C	O-alkyl-C	Di-O-alkyl-C	Aromatic-C	Carboxyl-C	
Summit	Oi	26.5	45.5	10.2	13.9	3.8	0.48
	Oe	31.7	43.5	8.9	11.8	4.1	0.61
	Oa	32.6	40.1	9.0	13.0	5.2	0.66
Footslope	Oi	27.9	40.4	9.2	16.6	5.8	0.56
	Oe	28.9	41.4	9.1	15.1	5.5	0.57
	Oa	30.3	39.5	9.1	15.0	6.1	0.62
Lakeshore	Oi	29.3	40.4	10.2	14.2	6.0	0.58
	Oe	30.4	38.9	10.3	13.8	6.7	0.62
	Oa	31.0	40.1	9.9	13.1	5.9	0.62

Table 2. The Alkyl-C/O-Alkyl-C ratios of solid-state CP/MAS ^{13}C NMR spectra in each particle size (mm) fraction of different soils

Site	<0.1	0.1~2	2~53	53~250	>250
Summit	1.05	0.85	0.74	0.61	0.32
Footslope	0.92	0.98	0.73	0.62	0.55
Lakeshore	0.62	0.77	0.69	0.52	0.5

As suggested by (1), the ratio of alkyl-C to O-alkyl-C and di-alkyl-C (A/(O-A+di-O-A)) could be used as indicator to assess the degree of decomposition. It is clear that small particle size fraction has higher degree of decomposition as shown in Table 2. The results show that higher extent of decomposition occurred at summit than that at footslope or lakeshore. In general, drainage of soils at lakeshore site was much poorer than those at summit and footslope sites. SOM at lakeshore was the least humified.

Table 3. Total C, N content (mg/g) and C/N ratio at different horizons and sites

Horizon	Summit			Footslope			Lakeshore		
	C	N	C/N	C	N	C/N	C	N	C/N
Oi	352	8.9	39.7	542	12.8	42.3	363	12.6	28.7
Oe	492	17.5	28.2	512	19.1	26.7	187	8.7	21.6
Oa	492	22.1	22.3	477	22.9	20.8	177	7.4	23.9

In general, soil at footslope contained higher C and N content which might be due to downhill erosion and deposition (Table 3). Soils at lakeshore regions have lower C and N content because of flooding (2).

3. CONCLUSIONS

Both the ^{13}C NMR and C/N results suggest that extent of decomposition was higher at summit than those at lakeshore and footslope. Based on a humification index of alkyl/(O-alkyl-C+di-O-alkyl-C), the degree of humification increased with the decrease of the particle size. Combining the physical fractionation with chemical analysis tools could provide valuable information in SOM characterization.

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Characterization of Chernozem Humic Acid SEC-PAGE Fractions Using DRIFT Spectroscopy and Thermal Analysis

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Keywords: soil humic acid, PAGE, SEC, DRIFT, TG-DTA

1. INTRODUCTION

Structural characterization of humic acids (HA) is a focal point to understand and establish a relationship between their structure and biological properties. However, the complexity of HAs seriously limits the application of a single analytical approach. A step towards improving our understanding of HAs role in the environments is to develop methods which can provide a complete characterization of HA structure.

Infrared spectroscopy (FT-IR) is considered a powerful tool in distinguishing individual structural differences in a heterogeneous organic mixture. Particular attention was devoted to diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy as a technique for analysis of humic substances from different origins (1-3). Combining this technique with thermal analysis (thermogravimetric analysis, TG and differential thermal analysis, DTA) or thermogravimetry–differential scanning calorimetry (TG-DSC) important information has been achieved on HA from different origin (3-4) or on humification process in soils and composts (3). TG-DTA analysis involves a continuous and simultaneous measurement of weight loss and energy change during heating of the sample. The mass loss can be used to compare the relative abundance of more or less labile C whilst the position of DTA peaks reflects the structure and chemical composition of the sample.

In the present paper the Chernozem HA and its fractions obtained by combination size exclusion chromatography and polyacrylamide gel electrophoresis (SEC-PAGE set-up) were investigated using DRIFT spectroscopy and TG-DTA analysis.

2. MATERIALS AND METHODS

The soil sample was taken from the A horizon of a Chernozem soil located in Kursk region, Russia. Method for HA extraction and their fractionation by SEC-PAGE set-up on different on molecular size (MS) and electrophoretic mobilities (EM) fractions A, B and C+D are reported elsewhere (5). The MS of fractions decreased with increasing of their EM. Fraction A had the highest MS and fraction C+D - the smallest MS.

The elemental analysis (C and N) and the $^{13/12}C$ isotopic ratio ($\delta^{13}C$) analyses were carried out using an elemental analyser (CHNS-O mod. EA 1110) coupled with an isotope ratio mass spectrometer (IRMS) (Finnigan MAT Mod. Delta Plus) (4).

TG and DTA were carried out simultaneously using a TG-DTA92 instrument (Setaram, France) as described in a previous paper (2).

The infrared spectra were recorded with a Bruker TENSOR series FT-IR Spectrophotometer (Madison, WI) equipped with an apparatus for diffuse reflectance (Spectra-Tech. Inc., Stamford, CT) (1).

3. RESULTS AND DISCUSSION

The elemental analysis results are shown in Table 1. The amount of N generally increased in the fractions and it showed an opposite trend with respect to that of the C. The $\delta^{13}C$ became more negative in the lower fractions. The increased amount of N and the negative deviations of $\delta^{13}C$ in the fractions with respect to HA might be due to urea traces immobilized during fractionation procedures (6).

Table 1. Elemental and isotopic analyses ($\delta^{13}C$) of HA and its fractions A, B and C+D.

Samples	C (%)	N (%)	C/N	$\delta^{13}C$(‰)
HA	54	5.4	10.0	-25.17
A	46	7.6	6.1	-25.81
B	48	9.0	5.3	-26.63
C+D	48	6.8	7.1	-27.22

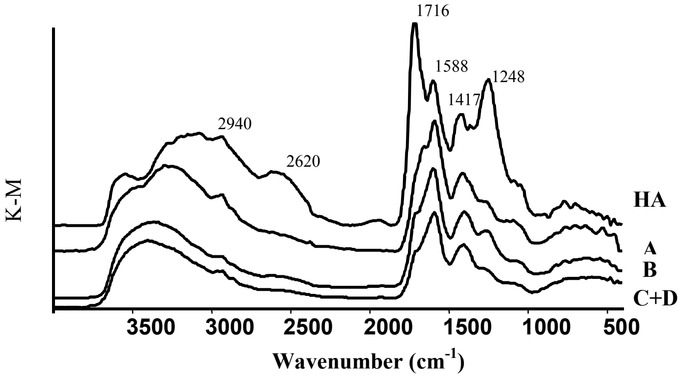

Figure 1. DRIFT spectra of HA and its fractions A, B and C+D.

The DRIFT spectra of humic fractions (Figure 1) indicated significant structural modifications. In particular, in HA appeared two strong bands at 1716 and 1248 cm^{-1} that are characteristic of C=O stretching and C–O stretching motions in protonated carboxyl groups COOH, respectively. In addition, the broad band at around 2600 cm^{-1} suggested the formation of intermolecular H-bonding between COOH groups in acid dimers (2).

In contrast, in all fractions, the relative intensity of bands at 1716 and 1248 cm^{-1} decreased whilst the bands at around 1588 and 1417 cm^{-1}, which possibly correspond to C=O stretching and C–O stretching motions in not protonated carboxyl groups COO^- and/or N-H bending, did not show changes in the relative intensity. Moreover, the disappearing of the band at 2600 cm^{-1} in fractions, suggests that H-bond are mostly broken by the action of urea.

The intensity of the 1040 $cm^{-}1$ band decreases with fraction MS. This band corresponds to C-O vibrations of ethers or alcohols and can be attributed to carbohydrates. Thus one can conclude that their concentration decreases with decreasing of fraction MS. Other characteristic bands appearing at 2940 and 2825 cm^{-1} were assigned to C-H vibrations in methyl and methylene groups in aliphatic components. The relative intensity of these bands noteworthy decreased in lower MS fractions B and C+D.

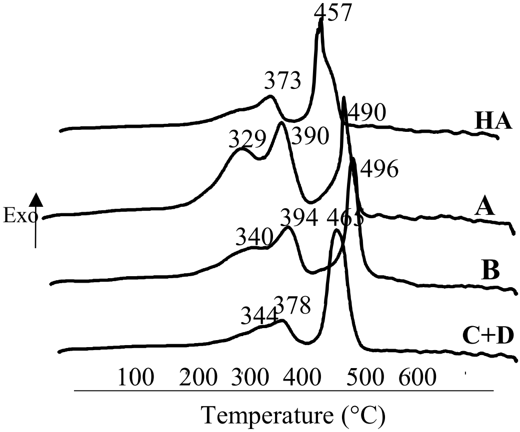

Figure 2. DTA of HA and its fractions A, B and C+D.

The DTA curves of HA and its fractions (Figure 2) were mainly characterized by two exothermic reactions: the first (300–450 °C) corresponded to the decarboxylation reaction of acidic groups, proteins, carbohydrates and fatty acid decomposition, while the second (450–600°C) was attributed to the breakdown of aromatic structures and cleavage of the C-C bond (2, 4). Different thermal profiles and mass losses were measured in fractions A, B, C+D. In high MS fraction A a strong mass loss (about of 63%) was observed for the 1st peak and a mass loss of about 18% for the 2nd peak, indicating that the thermal decomposition mainly involved carboxyl groups and aliphatic components. In fraction B decreased the mass loss of the 1st peak (about 44%) and increased that of the 2nd peak (about 25%). In the lower MS fraction C+D we observed a noteworthy decreased of the 1st exothermic reaction (loss mass about 31%) while thermal reaction of 2nd peak was prevalent. A shift of the 2nd peak towards lower temperature in C+D than those of A and B fractions suggested the presence in C+D of aromatic moieties with a large number of oxygenate substituents.

4. CONCLUSIONS

TG-DTA and DRIFT analysis of Chernozem HA fractions A, B, C+D obtained by SEC-PAGE setup showed important structural modifications with respect to HA unfractionated. This data is likely to have a broad environmental significance because the lower MS humic structures are directly involved in chemical/biological processes at soil-plant interface.

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Autodock Simulation of Interactions Among Humic Substances and Proteins

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Keywords: computer simulation, docking, humic substances, protein, prion.

1. INTRODUCTION

Computer simulation was employed in this work to individuate possibile binding sites of humic substances to proteins and in particolar with segments 121-231 of the murine prion protein and 90-231 of the human prion protein. The simulation was carried out with the aid of the program AutoDock 3.05 (1). Autodock was developed to provide an automated procedure for predicting the interaction of ligands with biomacromolecular targets and was initially tested on a number of protein-substrate complexes characterized by x-ray crystallography. The original procedure used a Monte Carlo simulated annealing technique for configurational exploration using grid-based molecular affinity potentials and has proven to be a powerful approach to the problem of molecular docking. In the later versions, which adopt an AutoGrid procedure, the protein is embedded in a three-dimensional grid and a probe atom is placed at each grid point. The energy of interaction of this single atom with the protein is assigned to the grid point. An affinity grid is calculated for each type of atom in the substrate, typically carbon, oxygen, nitrogen and hydrogen, as well as a grid of electrostatic potential, either using a unit positive point charge as probe, a Poisson-Boltzmann finite difference method. AutoDock version 3.0, utilizes a new, hybrid search technique based on a modified genetic algorithm, with 2-point crossover and random mutation.

2. MATERIALS AND METHODS

The HA initial model structures were generated by means of PRODRG server (2). Geometry optimization was then performed using the PM3 semiempirical method as implemented in Gaussian 03 program. Partial point charges were calculated by means of a *ab-initio* single-point calculation at HF/6-31G(d) level and RESP fitting. The PrP^C model (from V121 to S232) derived from the NMR structure of the prion proteins was obtained from the Protein Data Bank.

The automated docking procedure was performed considering the protein static. The Lamarckian genetic algorithm was used as search method, starting from a population of 1000 individuals with a maximum of 750,000 energy evaluations and 256x256x256 points grid with 0.3 Å spacing. All other parameters were left as the default. As a result, 1000 different complexes were obtained. Electrostatic potentials at the proteins surfaces were calculated by solving the Poisson-Bolzmann equation using the MOLMOL program.

3. RESULTS AND DISCUSSION

The humic model molecules can assume many different conformations and bind to at several different positions on the surface of the proteins without any apparent specificity. In order to cast light on the binding between proteins and HA, we therefore analyzed the docked complexes in order to find which amino acids, are more frequently found in the proximity ($<$5 Å) of the humic model molecules. Results showed that thirteen amino acids are more frequently found at the interface : seven of them are positively charged (R136, H140, R151, R164, H177, K185 and K194), five are polar (S135, N171, N173, N174 and N181) and only one is negatively charged (D178). They mostly correspond to two regions of murine prion protein, approximately identified with helices H1 and H2, respectively. Notably, these regions correspond to zones that have been suggested to be sensible in the process of conversion of PrPC toward the infectious form.

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Structural Characteristics of Soil Humin Fractions in Different Tillage Systems

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Keywords: structural characteristics, humin composition, iron-linked HM (HMi), clay-combined HM (HMc), tillage system

1. INTRODUCTION

Soil humin (HM) is an important fraction of humic substances, accounting for 30% to 50%. HM is of great significance in agricultural production, environmental protection and carbon sequestration (1). Pallo method fractionate HM into iron-linked HM (HMi), clay-combined HM (HMc), inherited HM and highly developed HM (2), Because there are less inherited HM, Dou combined it with highly developed HM into a new one and named insoluble humin residues (HMr) (2).

The information on the effect of tillage systems on humic substances could be found, such as Pérez (4) and Slepetiene (5), but very limited. Especially the report on HM compositional fractions (HMi, HMc and HMr) has not appeared. In order to evaluate the effects of different tillage systems on the different HM fractions, the elemental composition, thermal properties, IR spectra and solid ^{13}C-NMR spectra of HMi and HMc extracting from black soils of different tillage systems were analyzed in this paper.

2. MATERIALS AND METHODS

Black soil samples (Argiudolls in USDA Soil Taxonomy) were collected from the long-term field experiment of Changchun Institute of Geography and Agricultural Ecology, Chinese Academy of Sciences that was established at Dehui county (N44°21′, E148°150′), Changchun city, Jilin province, China, in 2001. The climate is temperate continent monsoon climate with a yearly average temperature of 4.4°C. The tillage treatments were ridge tillage (crop residues on the ridge of the harvest were not cleared until the next sowing, and no disturbance between the harvest and the next sowing, RT), no tillage (using the crop residues of harvest to cover soil and no moving when next sowing, NT) and autumn plough (the crop residues of the harvest were directly ploughed into soil in the autumn, AP). The tillage plots (RT, NT and AP) were 5.2m×20m with successive maize-bean rotation for 5 years and arranged 4 replications. The above samples are all from

0~20cm topsoil. With the Pallo method, fractionated soil organic matter into humic acid (HA), fulvic acid (FA) and HM, and then fractionate HM into HMi, HMc and HMr. The HMi and HMc were purified and freeze dried. Elemental composition was determined by Vario-EL III (Germany). Differential thermal analysis (DTA) was conducted with Shimazu TG-60 (Japan). The IR spectra were measured with KBr pellet method by a Nicolet AVATAR 360 instrument (USA) and semi-quantitative treating was carried out. The E_4/E_6 ratios and LogK values were calculated as the method of Dou (3). Solid ^{13}CP/MAS NMR were recorded on Bruker AV400 (Switzerland). The other measurements were proceeded by conventional methods.

3. RESULTS AND DISCUSSION

3.1. Humus composition

The soil total carbon contents of RT, NT and AP were 18.58 g kg^{-1}, 18.09 g kg^{-1}, and 18.15 g kg^{-1}, respectively. The carbon proportion ranges of HA and FA were 26.8~34.71% and 7.27~14.37%, and the carbon proportions of HMs for RT, NT and AP were 32.73%, 32.88% and 33.49%, respectively.

3.2. Elemental compositions of HMi and HMc

The C, H, N, and O+S contents of the extracted HMi and HMc under 3 tillage treatments were showed in Table 1. The element compositions of HMi and HMc were different in 3 tillage systems. The average C/H ratio of HMc was higher than HMi. This indicated that the molecular condensation degree of HMc was higher than HMi. Under different tillage systems, The HMi of NT had the highest C/H (1.194) ratios. For HMc the sequence of C/H ratio was RT>NT>AP, and NT was quite close to RT. It indicated that NT was the highest one for the molecular condensation degrees of HMi and HMc.

Table1. Elemental composition of HMi and HMc under different tillage systems

Samples	C	H	N	O+S	C/H	C/N	O/C
	(g kg^{-1})						
NT-HMi	540.0	37.68	25.54	396.8	1.194	24.67	0.551
AP-HMi	510.7	37.77	25.62	425.9	1.127	23.26	0.626
RT-HMi	485.9	44.98	25.66	443.4	0.900	22.09	0.684
Average-HMi	512.2	40.14	25.61	422.1	1.074	23.34	0.620
NT-HMc	551.6	30.19	33.16	385.1	1.522	19.40	0.524
AP-HMc	519.7	29.56	32.26	418.4	1.465	18.79	0.604
RT-HMc	534.2	29.07	32.57	404.2	1.531	19.13	0.567
Average-HMc	535.2	29.61	32.67	402.6	1.506	19.11	0.565

Note: RT, NT and AP represent ridge tillage, no tillage and autumn plough, respectively. The notes for the treatments in the following figures were same.

3.3. Thermal properties of HMi and HMc

As we can see from Figure 1, the HMi and HMc had exothermic peaks in moderate (250~301°C) and high (401~446°C) temperature regions. The average heat ratio of the high to moderate temperature exothermic regions (H_3/H_2 ratio) of HMi was higher than HMc. The heat of total reactions (H_t) and the H_3/H_2 ratio of HMc in the NT treatment were the highest in the 3 tillage systems and were 14.7 KJ g^{-1} and 97.0, respectively. This showed that HMc had lower aromatic/aliphatic ratio compared with HMi and that aromatic/aliphatic ratio for HMc of NT were the highest in the 3 tillage systems.

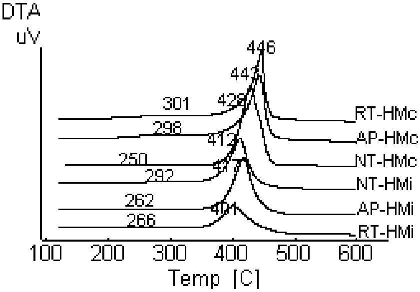

Figure 1. The DTA of HMi and HMc under different tillage systems.

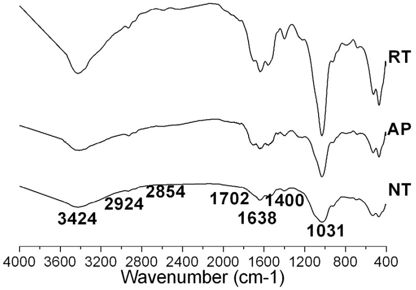

Figure 2. The IR spectra of HMi under different tillage systems.

3.4. IR spectra of HMi and HMc

It can be seen from Figure 2 and Figure 3 that the aliphatic C-H vibration (2920cm^{-1}, 2850 cm^{-1}) and the 2920/1620 ratios of HMc were stronger than HMi. Comparing IR spectra of different tillage treatments, the sequences of the 2920/1620 ratios for HMi and HMc were AP(0.362)>RT(0.262)>NT(0.069) and RT(0.837)>AP(0.598)>NT(0.179), respectively. This showed that HMc was more aliphatic than HMi.

3.5. Solid 13CP/MAS NMR spectra of HMi and HMc

The signals of HMc at 173ppm, 72ppm, 55ppm and 29ppm were stronger than HMi (Figure 4). The average relative carbon contents of alkyl were HMi(33.3%)>HMc(30.9%), but it was opposite for the contents of O-alkyl and carboxyl. The HMi and HMc had more aromatic C (18.3-23.2%)and less aliphatic C (44.7-44.8%) compared with the AP(16.3-18.7% for aromatic C and 47.3-50.3% for aliphatic C). This indicated that NT was more favorable to increasing the aromatic structure and decreasing the aliphatic structure, which was consistent with the results of the E_4/E_6 ratios and the LogK values.

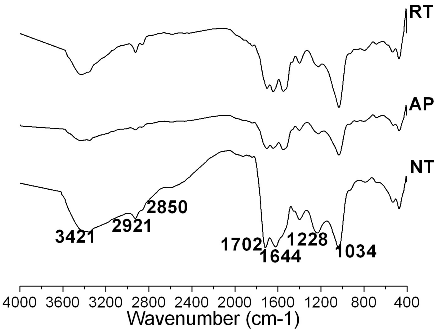

Figure 3. The IR spectra of HMc under different tillage systems.

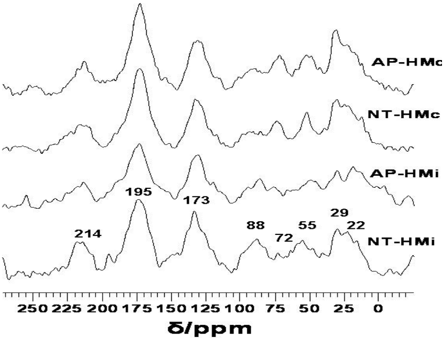

Figure 4. The ^{13}C-NMR spectra of HMi under different tillage systems.

4. CONCLUSIONS

The molecular condensation degree, complex degrees and the amount of aliphatic groups were HMc>HMi. Under different tillage systems, NT was highly favorable to increasing the condensation and complex degrees of HMi and HMc.

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A Water Soluble Polymer as a Working Structural Model for Humic Acids: H-binding and Spectroscopic Properties

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Keywords: humic, model, polymer

1. INTRODUCTION

HA occur ubiquitously in terrestrial and aquatic environments, indicating that many precursors and several formation pathways exist. Distinguishing among these pathways has been difficult because individual scientists have tended to concentrate their efforts on specific types of samples (e.g. soil, sediment or water) for which different isolation or fractionation methods are often used (1). Humic acids ultimately result from abiotic and biotic polymerisation and polycondensation reactions involving such small precursors (1). The relative importance of abiotic and biotic processes in humification in soils and sediments still remains obscure even though much research has been devoted to them (2). For example the oxidative polymerization of phenols, which is an important precursor reaction in the formation of HA, is catalysed both by enzymes and abiotic (inorganic) catalysts (2, 3, 4) Polyphenols have been shown to be important precursors in the abiotic formation of HA (4). More particularly, the oxidative polymerization of polyphenols in soils is thought to be one of the major processes of formation of humic substances (5). According to the polyphenolic theory (6) , humic acids are formed from simple phenols and phenolic acids via the formation of a semiquinone radical (7). Coupling of semiquinones originating from diphenols can lead to the formation of stable humic polymers under the catalytic action of Mn (IV) oxides (2). In the present case, we have focused our work on molecules bearing both carboxy and phenolic moieties as well as radical activity. In this model, protocatechuic acid (3,4-dihydroxybenzoic acid), and gallic acid (3,4,5-trihydroxybenzoic acid) are among the main low molecular weight aromatic acids formed after lignin degradation (7). As we have reported recently (7, 8) polymerization of the mono-, di-, and trihydroxyphenolic compounds may occur in alkaline media i.e. under ambient O_2. i.e. under conditions where radicals are formed.

In the present work, we have investigated the oxidative co-polymerization of some simple hydrobenzoic molecules which have been shown to be good models certain physicochemical properties of humic acids. As we show by controlling the pH, redox potential and the ratio of the polyphenolics, a water soluble polymer is produced that mimics certain key- physicochemical and spectroscopic properties of humic acids.

3. RESULTS AND DISCUSSION

The UV-Vis spectrum for the water soluble polymer derived by copolymerisation of gallic acid and protocatachuic acid is shown in Figure 1. The spectrum bears strong resemblance with the UV-Vis spectrum for HA (6).

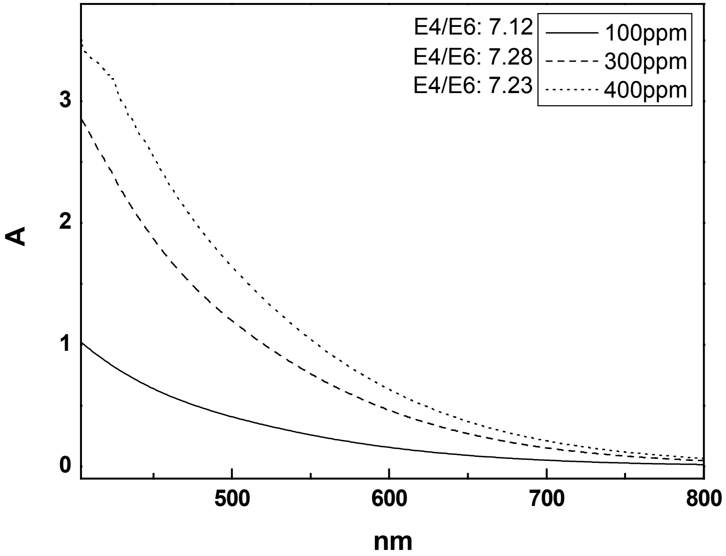

Figure 1. UV-Vis spectra (pH 8, 0.05M NaHCO3) for the synthetic HA at various concentration. The calculated E4/E6 ratios are shown for each concentration.

FT-IR Spectroscopy:

The FT-IR spectra for the synthetic polymer, Figure 2, exhibit the typical FT-IR bands of humic substances in the regions of 3410 cm^{-1} (H-bonded OH groups), 2920 cm^{-1} (aliphatic C-H stretching), 1720 cm^{-1} (C=O stretching of COOH and ketones), 1620 cm^{-1} (aromatic C=C and H-bonded C=O), 1400 cm^{-1} (O-H deformation, CH_3 bending, C-O stretching of phenolic OH, and COO^- antisymetric stretching of aryl esters), 1250 cm^{-1} (C-O stretching and OH deformation of COOH, C-O stretching of aryl esters), and 1050 (C-O stretching of polysaccharide or polysaccharide-like substances) (6).

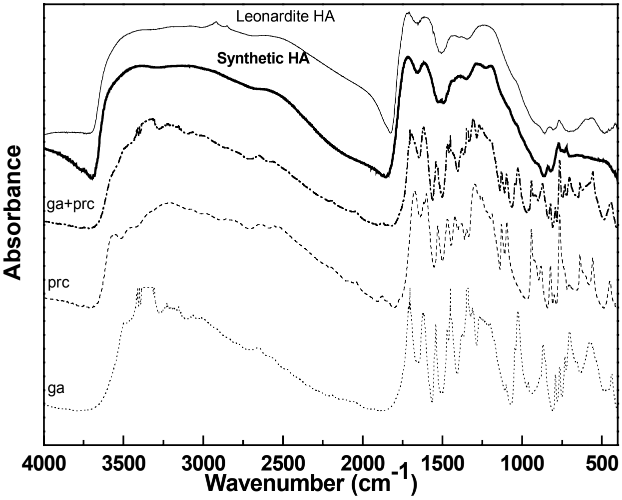

Figure 2. FT-IR Spectra of gallic acid, protocatechuic acid and both of them as monomers versus the polymer form of synthetic HA and showing the similarity to Leonardite HA.

H-Binding: The pH-dependence of the charge of the synthetic polymer determined from the H-binding data is plotted in Figure 3. The charging curve in Figure 3 show that in the pH range 4 to 10, soil HA show a charge variation ~ 4.5 [equiv. Kg^{-1}].

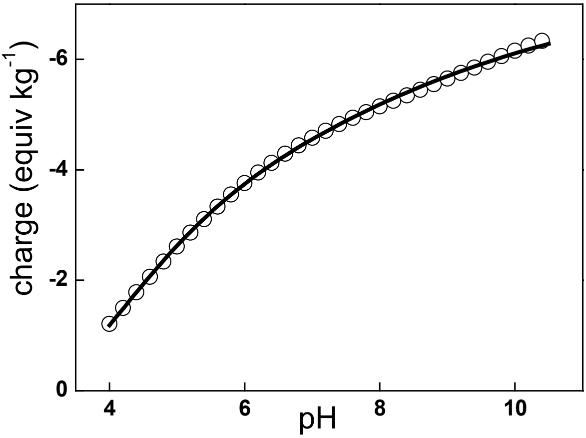

Figure 3. Charge vs. pH curves for the synthetic polymer. Solid lines is NICA-Donnan fit to the data by using the parameters listed in Table 1.

The H-binding data can be fitted by using the NICA-Donnan model which invokes two sets of distributed pKa values in the range 4-6 corresponding to carboxy groups, and pKa =7-9 corresponding to phenolic groups, similar to that typically observed in natural humic acids (6). The H-binding curve for the synthetic polymer bears strong resemblance with the H-binding curves usually reported for natural humic acids (6). Moreover the H-binding data can be quantitatively fitted, by using the NICA-Donnan parameters listed in Table 1

Table 1. Otimal Parameter Values for the Proton Binding Data for the NICA-Donnan Model

Sample	R^2	b	Q_1	Q_2	Q_1%	Q_2%	m_1	m_2	$\log K_1$	$\log K_2$
Synthetic	0.9995	0.68	4.05	5.39	42.9	57.1	0.47	0.18	4	6.7

4. CONCLUSIONS

A polymer derived from copolymerization of gallic acid and protocatachuic acid can be precipitated from aqueous solution at pH<2. The polymer (a) has two sets of distributed pK values in the range 4-6 corresponding to carboxy groups, and 7-9 corresponding to phenolic groups, similar to that typically observed in natural humic acids. (b) the UV-Vis spectrum and FT-IR spectra of the polymer bear strong resemblance to the corresponding spectra of natural humic acids. We suggest that this material as a working model for structural and spectroscopic properties of natural humic substances.

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Characterization of Humic Acids from Peat Column Using Pyrolysis-GC/MS

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Keywords: peat, humic acid, pyrolysis-GC/MS

1. INTRODUCTION

The importance of humic acids (HA) in nature is invaluable. Despite HA are involved in many processes in environment (carbon and nutrient cycles, buffering capacity of soils and water, interactions with mineral particles and organic pollutants) the understanding of their structural chemistry is still insufficient. One approach for investigation of complicated structure of humic substances is use of degradation methods of well characterized samples, following with analyses of degradation products. In this study the pyrolitical degradation of humic acids were performed and product analysis with GC/MS were done. The aim of this study was to find out basic structural similarities/ dissimilarities between various humic acid samples isolated from deferent depths of peat column.

2. MATERIALS AND METHODS

Peat samples were collected from high type bog Ploce, Mazais Veikenieks and Dizais Veikenieks, Western part of Latvia. 5 profiles of peat column were separated: 0-25, 25-52, 52-75, 75-98, 98-118 cm and extraction of humic acids were performed using 0.1 M NaOH. The age of the peat samples were detected using carbon dating. The chemical nature and structural composition of those samples of humic substances were analyzed with an off-line tetramethylammonium hydroxide (TMAH) treatment procedure followed up with Py-GC/MS (1). In the same samples their element composition and spectral characterization (UV, fluorescence, IR, ^{1}H NMR, ^{13}C NMR) has been determined.

3. RESULTS AND DISCUSSION

The macromolecules of humic substances in the process of pyrolysis decompose to 80-100 various low molecular compounds. The main degradation products obtained by Py-GC/MS (Fig. 1) were the alkanes, alkenes, methyl derivatives of phenols and alkylphenols, phenolic and aliphatic acids. These findings allow to compare different humic substances according to their structural composition.

Table 1. The characteristic of peat, used for isolation of humic acids

Peat sample	Depth, cm	Peat age, years	Peat type
Ploce 110 -130	110-130	1670	High type cotton-grass – sphagnum peat
Mazais Veikenieks 30-60	30-60	650	High type Magellan's sphagnum peat
Mazais Veikenieks 90-125	90-125	1180	Low type tree – grass peat
Dizais Veikenieks 0-25	0-25	520	High type fuscum peat
Dizais Veikenieks 25-52	25-52	750	High type fuscum peat
Dizais Veikenieks 52-75	52-75	980	High type fuscum peat
Dizais Veikenieks 75-98	75-98	1165	High type fuscum peat
Dizais Veikenieks 98-118	98-118	1350	High type fuscum peat

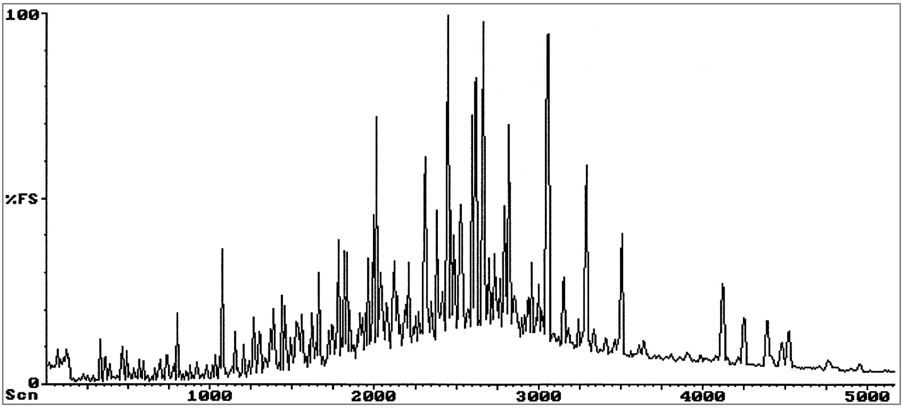

Figure 1. GC/MS chromatography of humic acid isolated from Dizais Veikenieks 25-52.

4. CONCLUSIONS

Despite studied peat samples very much differ in their origin and botanical composition, the properties of their degradation products and other characteristic indicate high degree of similarity.

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Random Generation of 3D Structures of Humic Substances

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Keywords: humic, fulvic, 3D structures, random generation

The impact of humic substances on key environmental and biogeochemical processes cannot be overstated. Yet, despite decades of investigations, the fundamental question of the 3D structures of humic substances remains unresolved. This is the underlying reason behind the ongoing debate about the molecular and supramolecular structure of humic substances in aqueous solutions, in soils and at mineral-water interfaces (i.e., true macromolecular entity *versus* supramolecular assemblage of relatively small molecules). In addition, the application of computational modeling -widely used in many areas of modern chemical, biological and geological sciences- to the investigation of humics and their interactions is jeopardized by the lack of reliable 3D structrures because it requires the input of the detailed atomic structure of the substances studied.

Patrick MacCarthy, in an extremely clarifying book chapter published in 2001, stated the following "First Principle of humic substances" (1): "*Humic substances comprise an extraordinary complex, amorphous mixture of highly heterogeneous, chemically reactive yet refractory molecules, produced during early diagenesis in the decay of biomatter, and formed ubiquitously in the environment via processes involving chemical reactions of species* ***randomly*** *chosen from a pool of diverse molecules and through* ***random*** *chemical alteration of precursor molecules*" (bold ours). It follows from this principle that two different approaches can be applied when trying to solve the problem of the 3D structure of humic substances: (i) to assume a mean structure whose properties reflect the mean properties experimentally measured in humic substances; (ii) to mimic the random nature of humic structures by generating random structures from predefined fragments. Both approaches have been used in the generation of 3D structures of humic substances. Earlier computational studies exploited fixed 'building-block' structures, such as the well-known Steeling and TNB (Temple-Northeastern-Birmingham) structures (2, 3) constructed on the basis of known elemental analysis and distributions of organic functional groups. The generation of 3D structures using an algorithm of computed-assisted structure elucidation

(CASE) to a comprehensive set of spectroscopic and analytical data (4) is an example of the second approach.

We have developed a program (FULVITOR, written in C) for the generation of statistical series of 3D structures from a given collection of elementary chemical functional groups (the fragment database). The gradual assembly of the various fragments present in the database is performed randomly according to an original ‘self-generation’ algorithm. Our conceptual approach extends to 3D structures the stochastic methodology originally developed by Murray and Linder in RANDOM (5-7) for 2D structures. The algorithm, as well as the links between the fragment database composition and the final ensemble of produced structures, have been described in detail in (8). FULVITOR contains two additional modules that test whether the structures obtained during the generation process adjust to some pre-established steric and chemical constraints. A first fragment database has been built to specifically model Suwannee River fulvic acid (SRFA). It is composed of 27 fragments chosen on the basis of a number of published experimental data. The analysis of 5000 structures generated by FULVITOR for SRFA shows that their elementary analysis differs by less than 1% from experimental values. Additionally, the calculated mass distribution overlaps experimental distributions obtained by Fluorescence Correlation Spectroscopy, Field Flow Fractionation and Nuclear Magnetic Resonance.

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Biodegradation of Humic Acid by White Rot Fungi Determined by ^{1}H NMR Spectroscopy and Ultra-High Resolution FTICR Mass Spectrometry

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Keywords: humic substances, white rot fungi, NMR, FTICR-MS

1. INTRODUCTION

Humic acids (HA), the main fraction of humic substances, comprise an extraordinarily complex, amorphous mixture of highly heterogeneous, chemically reactive yet refractory molecules (1). White rots fungi (WRF) are among the most efficient HA degraders due to their nonspecific oxidizing enzymes: manganese peroxidase (MnP), lignin peroxidase (LiP) and laccase. In addition, other enzymes such as hydrolases are probably involved during biodegradation (2), and numerous studies regarding to the enzymatic patterns have been performed (2,3). However, little is known about the chemical changes occurring during the biodegradation. Here, we present in-depth analyses of HA biodegradation by WRF using ^{1}H NMR spectroscopy and Fourier transform cyclotron resonance mass spectrometry (FTICR-MS), an advantageous combination, considering the complex composition of both HA, the fungal medium and its metabolites.

2. MATERIALS AND METHODS

2.1. Organisms: *Trametes* sp. M23 and *Phanerochaete sp*. Y6 were grown on PDA plates (4).

2.2. Media and culture condition: The fungi were grown in shaked Erlenmeyer flasks containing defined medium and 300 mg l^{-1} HA. HA was extracted from compost as described previously (4). The fungi were incubated at 35^{o}C. The degradation products were collected from the incubated cultures on days 3, 5, and 8 by filtration through glass-microfiber filters, followed by 0.22-µm filter. Control treatments were tested without HA and in non-inoculated flasks. For organic structural spectroscopy, HA were isolated from 2 ml acidified samples (20 µl formic acid, 25%) by solid phase extraction on preconditioned C_{18}-

disks (Varian, Inc.; conditioning with pure methanol and with 0.25% formic acid). Elution was done with methanol ("hypergrade" quality, Merk, Darmstadt, Germany).

2.3. Chemical and physical analyses: The UV-VIS spectra were measured using an Ultrospec 2100 pro (Amersham Biosciences) to asses bleaching of the HA.

FTICR mass spectrometry: Negative ion ESI mass spectra were acquired on a Bruker Daltonics (Bremen, Germany) 12 T Apex Qe FTICR mass spectrometer equipped with an Advion (Ithaca, NY, USA) TriVersa Nanomate on-chip nano-electrospray system. Spectra were calibrated internally on clusters of fatty acids in the required mass range. Mass errors of these peaks were always less than 0.1 ppm. Mass spectra were exported to peak lists and elemental formulas were calculated using a program written in-house. Double bond equivalents (DBE) and aromaticity index (AI) were calculated as previously described (5). The following elements and number of atoms were considered in the calculation: ^{12}C (1-100), ^{1}H (1-200), ^{16}O (0-80), ^{14}N (0-5), ^{32}S (0-1), with the following restrictions applied after calculations: $H/C \leq 2.2$, $O/C \leq 1$, $N/C \leq 0.5$, and $DBE \geq 0$.

^{1}H-NMR spectroscopy: spectra were acquired with a Bruker (Rheinstetten, Germany) DMX 500 NMR spectrometer operating at 500.13 MHz proton frequency. SPE derived methanolic solutions were evaporated in vacuum solubilized in CD_3OD, evaporated again and redissolved in 700 µl 0.1 N NaOD prior to NMR acquisition. One-dimensional ^{1}H NMR spectra were recorded using the first increment of the presat-NOESY sequence (90°(^{1}H) = 9.3 µs; acquisition time = 4.7 s, relaxation delay = 10.3 s, mixing time = 1 ms, exponential line broadening = 0.3 Hz). NMR integration was performed manually from printed spectra.

3. RESULTS AND DISCUSSION

3.1. HA bleaching: the extent of bleaching was considered to be an indicator of the HA degradation rate. A comparison of the bleaching abilities of *Trametes* sp. M23 and *Phanerochaete* sp. Y6, presented in Fig 1, shows large bleaching ability for both fungi with *P*. Y6 appearing more active under these conditions. These results are similar to previous HA degradation experiments with fungi grown on perlite as a solid support (4). Although perlite simulates fungal growth under aerobic condition, the use of liquid cultures with defined media allowed us to eliminate the NaOH extraction. Under liquid culture conditions, the samples were more homogenous and the isolation of the HA degradation products was more straightforward.

3.2. ^{1}H-NMR spectroscopy and FTICR mass spectrometry: ^{1}H NMR spectra of the HA extracted from the fungal cultures at days 3, 5, and 8 are shown in Fig 1. Integration of the ^{1}H-NMR spectra according to specific regions of chemical shift provides the amounts of non-exchangeable protons shown in Table 1. About 12% of the non-exchangeable protons of the HA extracted from the non inoculated control were assigned to terminal aliphatic groups, whereas after 8 days of biodegradation this amount decreased to almost half in both treatments (6.3 % for both *P*. Y6 and *T*. M23 respectively). A similar decrease was observed for the NMR resonance with $\delta(^1H)$ = 0.9-1.3 ppm, assigned mainly to CH_2 groups (Fig. 1). Remarkable decrease in aromaticity was also detected during biodegradation: the amount of the non-exchangeable aromatic protons of HA extracted from the control (12.8%) decreased to near half its initial value in day 8 for both fungi (6.1 and 5.8%, respectively, Table 1). The aromatic index (AI), computed from the FTICR-MS data (Table 1) exhibits sizable decrease in case of *P.* Y6 and near constancy in case of *T.* M23. Within the chemical shift region of 2.8-4.4 ppm we noticed an increase in both treatments as compared with the control, indicating an increase of oxygen-containing functional groups in HA during biodegradation. These findings are in agreement with the increase in the O/C elemental ratios observed using the FTICR mass spectral data. (Table 1).

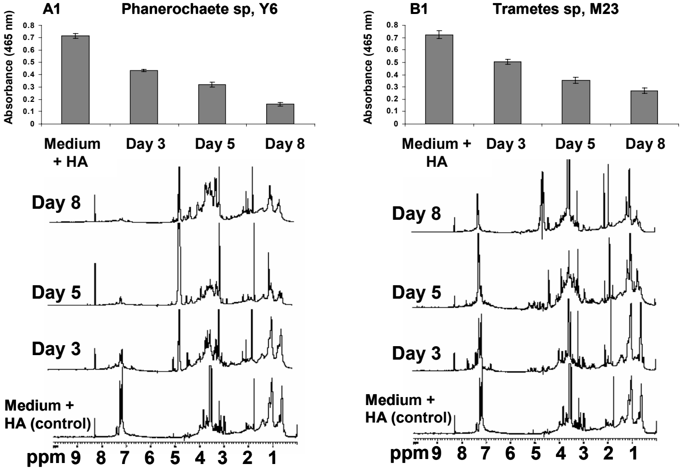

Figure 1. Decolorization of HA by *Phanerochaete* sp. Y6 (A1) and *Trametes* sp. M23 (B1). ^{1}H-NMR spectra (A2 and B2) of HA extracted during biodegradation.

4. CONCLUSIONS

In summary, we observed bleaching of HA in liquid culture. Degradation of HA was verified by physical and chemical changes measured during the process by two different WRF using ^{1}H NMR spectroscopy and FTICR mass spectrometry. During the process we detected oxidation, dealkylation and a decrease in the aromatic content of the HA.

Table 1. (A) 1H-NMR integrals of six major resonance regions of the spectra presented above; (B) Aromaticity index and elemental analyses as detected by FTICR-MS

A		^{1}H NMR section integrals						
Assignment $\delta(^1H)$ ppm		HA extracted from fungal medium	*P.*6 day 3	*P.*Y6 day 5	*P.*Y6 day 8	*T.*M23 day 3	*T.*M23 day 5	*T.*M23 day 8
				% ^{1}H NMR integral				
10.0-6.0	C_{ar}-H	12.8	7.3	6.1	7.2	13.1	16.8	5.8
6.0-4.8	R_nC=CH OCO-CHR	0.6	3.3	0.9	0.7	1.5	1.5	0.9
4.4-2.8	R_nCHOR, R_nNCH	38.9	41.2	52.0	48.9	36.1	33.4	61.8
2.8-1.7	C_{ar}-CH_n R_2NCH_n RC(=O)CHR_n	11.6	14.1	13.9	16.4	9.6	10.4	8.1
1.7-0.9	R_nCH	24.3	21.6	19.8	20.5	26.7	26.6	17.1
0.9-0.5	RCH_3	11.8	12.5	7.3	6.3	13.0	11.3	6.3
B		FTICR mass spectral analyses						
aromaticity index analyses (AI) (%) *		17.6	11.1	11.2	5.3	10.9	13.8	11.0
				% weight				
computed average elemental compositions		C	62.6	59.0	58.3	58.8	60.4	58.7
		H	6.1	6.5	5.9	6.4	6.7	6.4
		N	4.0	3.6	4.7	5.7	2.6	5.1
		O	24.8	27.0	27.1	26.6	27.1	26.9

* % AI was calculated as summing the number of all molecules with AI above 0.67, divided by total number of molecules detected

ACKNOWLEDGEMENTS

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Limitations in High Resolution NMR Analysis of Humic Acids

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Keywords: humic acid, ^{13}C NMR, olefinic and aromatic double bonds

1. INTRODUCTION

According to the wet chemical determinations the structure of humic acids (HA) is not very aromatic. Yields of aromatic degradation products are usually less than 3%. On the other hand, according to the ^{13}C NMR analyses the aromatic structure of HA is claimed to be more than 30%.

The aim of this study was to find out why two chemical methods give contradictory rather than expected complementary results when applied to the structural study of HA.

2. MATERIALS AND METHODS

This study is made deductively, studying the potentials of modern high resolution NMR spectroscopy to resolve reliably olefinic and aromatic double bond carbon and protons in the structure of HA.

3. RESULTS AND DISCUSSION

Olefinic and aromatic protons and carbons in single, well-defined compounds having conjugated carbon-carbon double bonds can be differentiated from each other by high resolution NMR. The number of carbons and protons in the structures of these single compounds are relatively few. So the number of magnetically non-equivalent carbons and protons are also low. NMR spectra contain now only few signals which furthermore are very sharp. In ^{13}C NMR spectrum the signals are practically sharp lines, the chemical shift values of which can be accurately determined.

The spectrum also exhibits a characteristic fine splitting provided by the spin-spin coupling of the adjacent protons or carbons. This method is important in ^{1}H NMR spectroscopy (^{13}C NMR is usually run with the decoupling mode). For structural identification of organic molecules the combined data of chemical shift values and that of spin-spin coupling is required.

HA have a very heterogeneous structure. In ^{13}C NMR or ^{1}H NMR spectroscopy this means that the number of magnetically non-equivalent protons and carbons are very large.

Each of them gives, of course, a sharp peak with low intensity, but with slightly different chemical shift value. Actually, these shifts are so close to each other that the resolution power of the present NMR instruments cannot differentiate them as isolated lines. This is causing broadening of the line widths which is appearing in ^{1}H or in ^{13}C NMR spectra as a single broad peak.

First of all, this means that spin-spin coupling cannot provide any fine splitting and so reliable structural identification thereof is not possible. The structural identification of HA must be based on the chemical shift values alone.

The chemical shift values of olefinic and aromatic protons or carbons are, however, overlapping due to broad peak. This renders the differentiation of olefinic carbons or protons from those of aromatic ones extremely uncertain, practically impossible.

The assignment of a certain ^{13}C NMR or ^{1}H NMR signal of HA as aromatic in terms of chemical shift values is possible only, when it is otherwise proved that the particular HA in question does not contain olefinic double bonds. So far the non-existence of olefinic structures in HA has not proved.

In spite of that, the signals due to the unsaturated carbons of HA are widely assigned solely as aromatic in many HA studies. This assignment is more or less wishful thinking.

In terms of aromaticity the spectral determinations comes closer to the values provided by wet chemical analyses when we accept the fact that the carbon-carbon double bonds in HA are more olefinic than aromatic of nature.

4. CONCLUSIONS

The ^{1}H and ^{13}C NMR spectra of HA are rather reliably telling the amount of carbon-carbon unsaturation, the sum of olefinic and aromatic double bonds. Due to the limitations of the high resolution NMR analysis in the HA research, the interpretation of ^{1}H and ^{13}C NMR signals solely as aromatic on the basis of chemical shift values is inaccurate.

Investigation of Chemical Structure of Natural Organic Matters from Dried Figs

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Keywords: chemical structure; *Ficus carica*; humic substance; organic matter; proteinase inhibitor

1. INTRODUCTION

Humic substances (HS) are polyelectrolytes produced by the abiotic alteration of plant and animal tissue during the early decomposition of natural organic matter and are found in the soils, sediments, and surface waters on the earth (1). They are important components of soil refractory organic matter and play critical roles in sediment processes such as metal reduction and pollutant transport. Recently, Hatano has found that decomposing plant tissues, dried figs contains novel colored materials, P and B samples (2). They were revealed not to adsorb a column of CM-Sephadex under any condition, while they strongly adsorbed a DEAE-Sephadex column. This indicates that these samples have a lot of strong negative charges, which is a representative characteristic of HS. It is worth noting that the UV-vis absorption and IR spectra of the P and B samples resemble those of HS (2), indicative of a kind of HS (2).

Previously, we measured the preliminary spectra of the P and B samples by using matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF-MS). As a result, these samples were revealed to consist of relatively small molecules with molecular masses around 500–1400 Da (2), although the detailed chemical and conformational structure could not be determined. In this presentation, we focus on the sample B due to its high ionization efficiency and chose several MS techniques to obtain insight into the structural regularities as observed in a kind of HS, fulvic acids. As a consequence, the regularities of molecular mass patterns were also observed on the MS spectra of the B sample (3), but the elemental analysis and solid-state nuclear magnetic resonance spectra showed that the sample was not typical humic materials, indicative of a novel organic matter (3). At last, we propose the putative chemical structure of the sample B, based on the above-mentioned data.

2. MATERIALS AND METHODS

2.1. Materials

Dried fig fruit in California (Black Mission) was purchased from a local market, and the P and B samples were purified as described previously (2). Stock solutions of the samples for MS analysis were prepared in Milli-Q water at a concentration of 1 mg mL^{-1}. 2,5-dihydroxybenzoic acid (DHB) was purchased from Sigma–Aldrich (St. Louis, MO).

2.3. Mass spectrometry

Fast atom bombardment (FAB)-MS was carried out on a JEOL JMS-SX102 spectrometer (JEOL, Tokyo, Japan) for the initial characterization. Thioglycerol and 1-N hydrochloric acid were added to the stock solution in a volume ratio of 5:1. The JMS-SX102 has a mass range of 3500 Da at 8-kV accelerating potential, and approximately 0.5–0.7 μL of the thioglycerol matrix containing the sample was applied to a stainless steel sample stage mounted on the end of a high-vacuum push rod.

MALDI-TOF-MS analysis was performed on a KOMPACT MALDI-4 mass spectrometer (Shimadzu/Kratos, Manchester, UK). A DHB matrix-saturated solution was prepared in a 3:7 mixture of acetonitrile and Milli-Q water containing 0.1% trifluoroacetic acid. The dried-droplet technique was used for MALDI-TOF-MS sample preparation. The mass spectra were recorded in both linear and reflection modes, and the mass range was m/z 0–3000.

Tandem MS (MS/MS) experiments were conducted using a Q-TOF-II mass spectrometer (JASCO International/Micromass, Manchester, UK) equipped with a Nanoflow electrospray ionization (ESI) interface. Sample solutions were prepared by tenfold dilution of the stock solutions in methanol/0.28% ammonia (1:1) and introduced into a Nanoflow capillary with a flow rate set to 10–50 nL min^{-1}.

3. RESULTS AND DISCUSSION

3.1. FAB and MALDI-TOF mass spectra

The FAB-MS spectra of the P and B samples in a positive ion mode were very similar to each other, although the signal-to-noise ratio (S/N) was relatively poor (data not shown). The mass-distribution shapes of the sample B indicated a shifted-sinebell function with a periodicity of 44 Da, and the maximum molecular weight of the B sample was estimated approximately 1500 Da. We call the fragment of 44 Da as a quark (Q) module for an explanation of the fragmentation scheme.

The MALDI-TOF mass spectra of the sample B showed obvious regularities in peak appearance (3). There were two peak-appearance groups (higher peaks at m/z 361, 699, 1037, 1375; lower ones at m/z 537, 875, 1213, 1566). The mass difference between the two groups (for example, peaks at m/z 361 and 537) certainly indicated 176 Da; on the other hand, the differences among the peaks within each group (for instance, peaks at m/z 361 and 699) were 338 Da. Here, we respectively designate the fragments of 176 Da and 338 Da as atom modules, A1 and A2. Each peak had some additional peaks, probably the precursor ions $[M–O]^+$ and $[O–M–O]^+$, indicating that each precursor ion has two ether-linkage sites.

In the post-source decay (PSD) spectrum of the B sample, the most intense product ion at m/z 361 would probably be a starting material for the polymerization of the sample B, since sample fragmentations in the source region are strongly induced in the PSD experiments (3). The product ions at m/z 355, 361, and 377 would be the oxygen and sodium adduct of the A2 module, probably $[A2–O]^+$, $[A2–Na]^+$, and $[O–A2–Na]^+$, respectively. Actually, sodium ions were detected as a peak at m/z 23 in the spectrum.

3.2. ESI-Q-TOF tandem mass spectra

In the ESI-Q-TOF mass spectrum, two mass distributions (m/z ranges of 100–400 and 1000–1500) indicated monovalent anions with 1.0-Da inter-peak spacing; in contrast, the distribution in the range of m/z between 600–900 showed divalent anions that were observed at every half-mass (3). We observed three peak clusters at the beginning of m/z 624, 712, and 800 in the enlarged spectrum, each of which would have two binding sites such as $[M–K]^–$ and $[K–M–K]^–$. The mass difference between the three clusters appeared to be 88 Da, which corresponded to singly charged ions at m/z 176.

In the product-ion spectra of m/z 712, 731, and 750, the fragmentation ion at m/z 175 was the most intense among the other peaks and was considered as a representative repeated structure of the parent ions (3). Accordingly, these product ions would be assembled by a number of the A1 modules. We also observed the other important fragments of 113, 131, 193, and 351 Da, indicating elimination of water from three Q modules, three Q modules, the addition of water to the A1 module, and two A1 modules, respectively. Thus, we could explain the constitution of all the product ions by using the Q module, which is consistent with the results of the FAB-MS experiment (data not shown).

3.3. Putative molecular structure

We propose here the fragmentation scheme of the sample B, which allows us to predict the preliminary molecular structure (Q module) (Figure 1A). The sample would be composed of the modules A1 and A2, each of which is made of the Q module. The A1 module would be formed by polymerization of four Q modules (Figure 1B), while the module A2 might be formed by trimerization of the dehydrated polymer from three Q modules (Figure 1C). The modules A1 and A2 might connect with each other, probably by polymerizations, ether linkages, and hydrogen bonding, leading to a mass variety of the B sample (designed as a molecular module) as observed in the MS spectra. For example, the precursor ion of 891 Da, one of the molecular (M) module, might be composed of one A1 module and two A2 modules, one of which is the sodium and oxygen adduct. Thus, we could explain almost all M modules of the sample B by using the modules Q, A1, and A2. However, we must remember that these product ions were observed just by ionization of the MS experiments. In this scheme, we do not address the large amounts of nitrogen observed in the elemental analysis (3). Probably, the nitrogen atoms would constitute a part of the unionized moiety, which might be involved in hydrogen bond like O–H···N. Similarly, the P sample would be composed of the Q module basically, since we also observed the 44-Da inter-peak spacing in the FAB-MS spectrum. The molecular structure of the P sample would be much more complicated than that of the B sample.

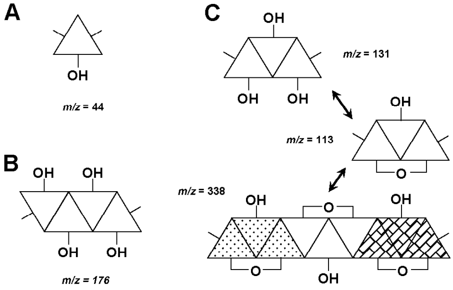

Figure 1. Schematic representation of the Q (A), A1 (B), and A2 modules (C). Triangles indicate the backbone of the Q module, and available double bonds in the Q module are shown as solid lines from the triangles. The module A2 at m/z 338 would be formed by trimerization of the dehydrated tri-Q polymer at m/z 113.

ACKNOWLEDGEMENTS

We wish to thank Dr. Noriko Kagi (JASCO International, Japan) for the use of a Micromass Q-Tof II spectrometer with a Nanoflow ESI interface and for her analytical and technical support. The authors are grateful to Dr. Jun Ashida (Varian Technologies Japan Ltd., Japan) for the use of a Varian Unity INOVA 400WB spectrometer. Drs. Kimihiro Goto (Sumika Chemical Analysis Service) and Koji Nagata (University of Tokyo, Japan) are also appreciated in the elemental analyses and FAB-MS measurements, respectively.

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Spectral Characterization of Plant-Derived Dissolved Organic Matter

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Keywords: dissolved organic matter, Fourier-transform infrared spectroscopy, nuclear magnetic resonance spectroscopy

1. INTRODUCTION

Dissolved organic matter (DOM) derived from fresh or early-stage decomposing soil amendment materials may play an important role in the process of organic matter accumulation (1). The DOM can influence many chemical processes, due to its reactivity with both soil solution components and soil surfaces (1, 2). Previously, DOM extracted from plant biomass representing commonly employed U.S. agricultural amendments was used to investigate P sorption inhibition by DOM (2). The results demonstrated that the extent of inhibition is closely related to the chemical structure of both the DOM and the sorbing surface (2). However, the mechanism by which this inhibition operates remains unresolved (2). Characterization by wet chemistry methods demonstrated that, in contrast to previously-published observations, characteristics of these DOMs were wide ranging, varying greatly within fractions for different sources (1). In this work, therefore, we characterized the plant-derived DOM with Fourier-transform infrared (FT-IR), solution ^{31}P nuclear magnetic resonance (NMR), and solid-state ^{13}C NMR spectroscopies. Spectral information obtained from this study will provide a better understanding of the relationship between DOM composition and behavior in soil environments. For comparison, DOM from dairy manure was also characterized in the same way.

2. MATERIALS AND METHODS

Plant shoot biomass was obtained from field grown Alfalfa (*Medicago sativa* L.), corn (*Zea mays* L.), crimson clover (*Trifolium incarnatum* L.), hairy vetch (*Vicia villosa* L.), lupin (*Lupinus albus* L.), soybean (*Glycine max* L. Merr.), and wheat (*Triticum aestivum* L.), air-dried, and ground to pass through a 1-mm sieve. The dairy cattle (*Bos taurus*) manure sample was acquired locally, air-dried, and passed through a 2-mm sieve. The DOM was extracted with a 40:1 (v:w) water to sample ratio using cold water and refrigerating (4°C) the suspension for 18 h with periodic shaking by hand. The suspensions were then centrifuged (900 × *g*) for 30 min prior to vacuum filtering through 0.4 micron pore size polycarbonate filters (2). The DOM extracts were then freeze-dried and kept at a dessicator at room temperature until use.

Freeze-dried DOM was dissolved in 0.6 mL 10 M NaOH, 1.0 mL D_2O, and 0.6 mL water and was allowed to stand for 30 min with occasional vortexing. Solution ^{31}P NMR spectra were acquired at 242.75 MHz on a Varian INOVA600 MHz spectrometer equipped with a 10-mm broadband probe, using a 90° pulse, 0.68-s acquisition, 4.32-s pulse delay, 82.4-µsec dwell time, 17.9-µsec pulse width and 12-Hz spinning (3).

The Fourier Transform-Infrared Spectra (FT-IR) were obtained in the 450-4,000 cm^{-1} range on a PerkinElmer Spectrum One FT-IR Spectrophotometer from discs containing about 1 mg sample and 100 mg KBr. Each sample was scanned 24 times with a resolution of 2 cm^{-1}. All spectra were normalized.

Solid-state ^{13}C NMR spectroscopic analysis was performed in a Bruker DSX400 spectrometer at 100 MHz for ^{13}C using 7-mm sample rotors for best sensitivity, in a double-resonance probe head. Three experiments (Fig. 2) were run for each sample (4, 5).

3. RESULTS AND DISCUSSION

The solution ^{31}P NMR spectra of DOM extracted from alfalfa and soybean shoots were relatively simple (Fig. 1) as both contain only orthophosphate and monoester P. The distribution between the two types of P is 86% orthophosphate and 14% monoester P in alfalfa shoot. However, the distribution in soybean shoot was 68% orthophosphate and 32% monoester P. Within the monoester P of soybean shoot DOM, 70% is phytate P. DOM from corn shoot contained less monoester P (8% of total P) and the other five DOM samples contained orthophosphate only (data not shown).

FT-IR spectra of plant-derived DOM were typical for natural organic matter (data not shown). However, there were some differences observed among the eight DOM samples. For example, the bands at 1678 and 1529 cm^{-1} were more obvious in the FT-IR spectrum of lupin DOM, suggesting it contained more olefinic or aromatic compounds. In the spectra of alfalfa and wheat DOM, the bands at 1400 cm^{-1} were broad and strong, suggesting these two DOM sources contain more aliphatic and/or phenolic groups.

The solid-state ^{13}C NMR spectra of the seven plant-derived DOM samples can be classified into three groups (Fig. 2). The spectra of three leguminous plants, alfalfa, hairy vetch, and soybean, are quite similar. The spectral characteristics indicated that these

DOM sources were primarily composed of soluble carbohydrates and certain soluble organic acids, amino acids, peptides, and phenolic compounds. The spectra of the other two leguminous plants, crimson clover and lupin, seem similar but with three sharp peaks at 35, 51, and 176 ppm. The three additional peaks indicate that these two DOM sources could contain significant aspartate. The spectra of wheat and corn are also similar to each other and similar to those of soybean DOM, although certain differences exist. In contrast, the spectra of dairy manure are significantly different. Its spectra are broad and more featureless, indicating humification with significant amounts of nonprotonated carbons and lignin residues.

4. CONCLUSIONS

Dissolved organic matter from seven plant shoots and a dairy cattle manure were characterized by FT-IR, solution ^{31}P and solid-state ^{13}C NMR spectroscopic techniques. The spectral data provided P and C bonding information for these DOM samples. All DOM sources were primarily composed of soluble carbohydrates, as well as some other soluble organic compounds such as organic acids, amino acids, and phenolic compounds. In contrast, dairy manure displayed properties characteristic of humified products. The differential structures of these DOM sources could be useful for understanding their effects on soil nutrient availability to plants.

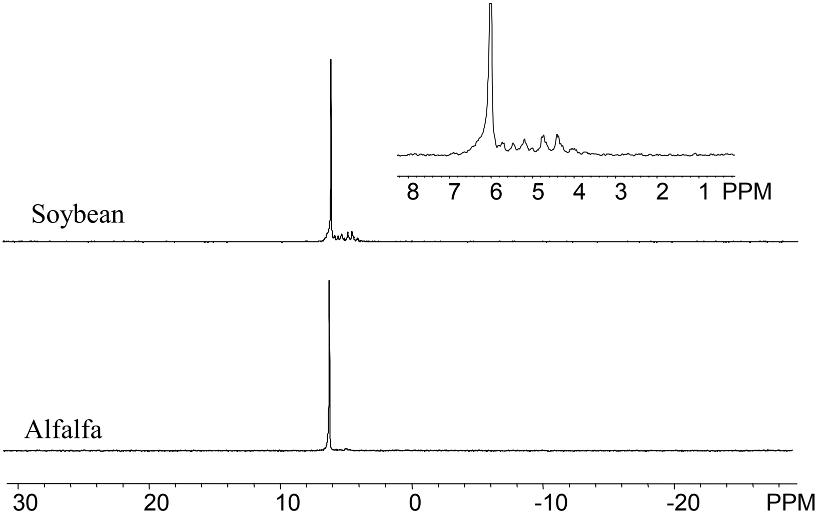

Figure 1. Solution ^{31}P NMR spectra of dissolved organic matters extracted from soybean and alfalfa shoots.

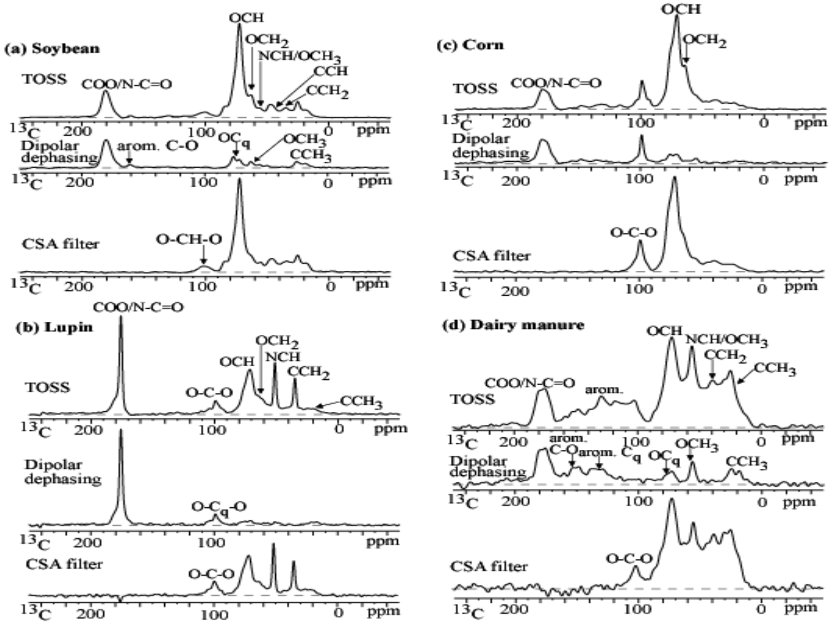

Figure 2. Spectral editing for identification of functional groups in ^{13}C NMR. Three experiments were conducted: ^{13}C CP/TOSS spectra showing qualitative structural information; corresponding dipolar-dephased ^{13}C CP/TOSS spectra showing nonprotonated carbons and mobile segments like CH_3, acquired after a period of 40 μs without decoupling; and selection of sp^3-hybridized carbon signals by a chemical-shift anisotropy filter, which in particular identifies OCO carbons near 100 ppm, which are typical of sugar rings. (a) Soybean, (b) Lupin, (g) Corn, and (d) Dairy manure.

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Capillary Electrophoresis and Fluorescence Excitation-Emission Matrix Characterization of Soil Mobile and Calcium Humates

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Keywords: humic, fulvic, template

1. INTRODUCTION

Capillary electrophoresis (CE) and Excitation-emission matrix (EEM) fluorescence spectroscopy have been used in natural organic matter (NOM) studies. The combination of CE and EEM fluorescence spectroscopy together provided a novel approach for characterizing humic substances. In a previous project, we characterized 13 NOM samples obtained from the International Humic Substances Society (IHSS) using CE; and analyzed these IHSS samples using EEM spectroscopy with parallel factor analysis (PARAFAC) modeling to gain a better understanding of the make-up of these NOM samples (1). The mutual relevance of data collected from each of the two methods provided novel insight into the correlation of complex NOM fluorescence spectra to specific NOM fractions. We applied this approach to explore the characteristics of soil mobile humic acid (MHA) and recalcitrant calcium humate (CaHA) in relation to different cropping management practices.

2. MATERIALS AND METHODS

Soil samples were collected from a private farm in Hamilton County near Phillips, Nebraska, USA on an irrigated field with a continuous corn system. Nutrient management treatments included application of beef cattle feedlot manure, inorganic N fertilizer, and an unfertilized control. The soil is classified as the Hord silt loam (fine-silty, mixed, mesic Cumulic Haplustolls). Surface soil (0-15 cm) was sampled and air-dried in a greenhouse, hand-ground to pass through an 8-mm mesh, and analyzed for organic C and total N by automated combustion. The MHA and CaHA fractions were extracted based on an established procedure (2).

Stock solutions of humic fractions were made by weighing 10-20 mg of sample in a 2-mL micro tube and then adding appropriate volumes of 0.05 M NaOH to make a final concentration of 10 mg dry matter mL^{-1}. For UV irradiation treatments, 1.0 mL of a stock solution was placed in a closed quartz cuvette. A Spectroline 11SC-1 short wave UV pencil lamp (254 nm, 4.5 mW cm^{-2}, Spectronics Corporation, Westbury, NY) was placed near the cuvette (1 cm away) and the sample was irradiated for 4 h at 22^oC (3). The untreated and irradiated samples were then subjected to CE and EEM analysis.

The CE instrument was an Agilent capillary electrophoresis system (Agilent Technologies, Wilmington, DE). The running buffer was 3 mM KH_2PO_4-6 mM $Na_2B_4O_7$, pH 8.9 (refered to as borate buffer, hereinafter). The capillary column (fused silica, 56 cm length x 75 microm internal diameter) was flushed for 3 min with the borate buffer prior to sample loading (1). The samples for CE analysis were further diluted with borate buffer so that it would have an ultraviolet (UV) light absorbance at 240 nm of about 0.1. Fluorescence measurements were obtained using a Hitachi F-4500 spectrofluorometer. The excitation-emission matrix (EEM) fluorescence landscape was obtained by setting the EX range from 240 to 400 nm and EM range from 300 to 500 nm in 3 nm increments. The PARAFAC modeling was conducted with MATLAB version 7.0.4 , Release 14 (Mathworks, Natick, MA) using PLS_Toolbox version 4.0 (Eigenvector Research, Manson, WA) (1).

3. RESULTS AND DISCUSSION

The CE profiles of MHA and CaHA of the control soil were different (Fig. 1), but they were typical for soil humic and fulvic fractions (1, 4). The CE profiles of the two soil humic fractions with either synthetic fertilizer or cattle manure application were more similar to those of fulvic acid (data not shown). The common feature of the humic fractions of the three soils was the absence of a “neutral peak”, which was due to non-ionic components in humic fractions. UV irradiation remarkably changed the CE profiles of these soil humic fractions (Fig. 1). UV irradiation made the CE profiles of the MHA and CaHA fractions more similar. As show in Fig 1b, extra peaks appeared in both CE profiles, and these peaks were much stronger than those of the un-irradiated samples. The CE profiles of these soil humic fractions after UV irradiation seemed to contain more features of peat humic fractions than the corresponding CE profiles of un-irradiated fractions.

PARAFAC modeling analysis of EEM fluorescence spectroscopic data indicated that four fluorophore components were present in each sample. The characteristics of the four components were similar but not exactly the same as those reported previously (1). UV irradiation changed the distribution of the four components in these humic fractions (Fig.

2). Component 1 was most sensitive to UV irradiation. For all three MHA fractions, Component 1 decreased while component 4 increased after UV irradiation. Conversely, UV irradiation increased the content of Component 1. This increase was mainly due to the decrease in Component 2 and a minor contribution from Component 3.

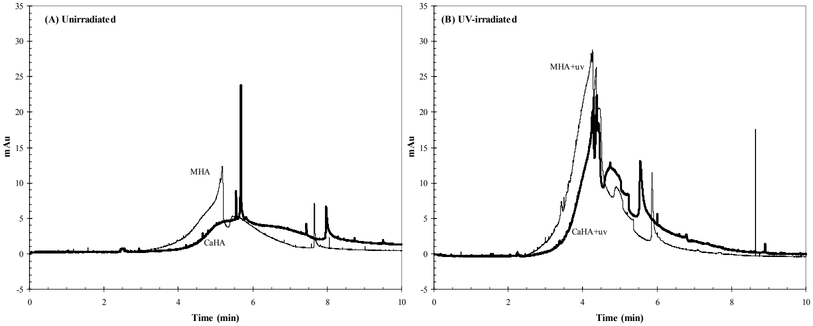

Figure 1. Electropherograms of MHA and CaHA of control soil. Humic fractions were unirradiated (A) or irradiated (B) by UV light for 4 h.

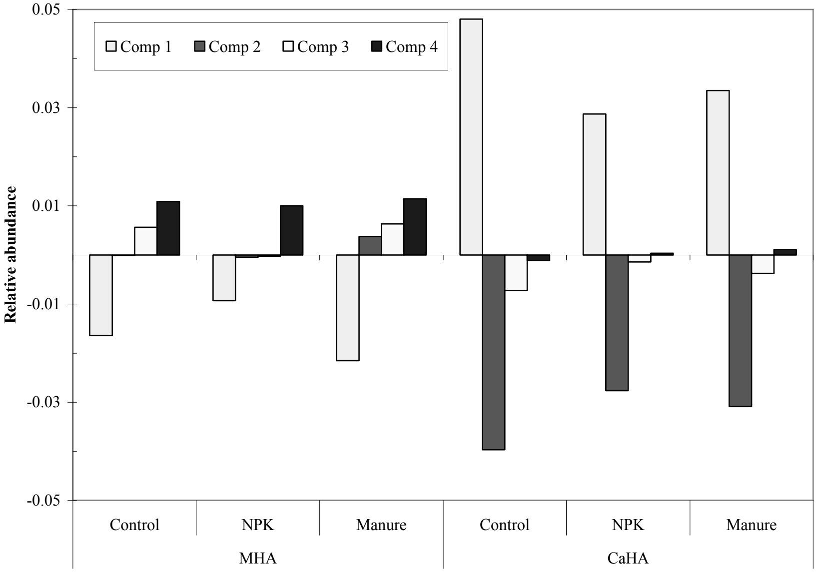

Figure 2. Change of the four EEM components of MHA and CaHA of soils due to UV irradiation. Soils were control, synthetic fertilizer applied (NPK), and cattle feedlot manure (Manure) applied.

4. CONCLUSIONS

The mobile and recalcitrant calcium humic fractions extracted from soils possessed characteristic capillary electrophoretic and EEM fluorescence profiles. UV irradiation caused these profiles to change,. However, no remarkable difference in profiles among soils with differentnutrient managements suggested that crop management practices under the experimental period (6 years) did not significantly alter the chemical nature of humic fractions in these soils.

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Characterization of Synthetic (Core) Humic Substances Made from Dihydroxylated Phenylpropanoids

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Keywords: synthetic humic substances, dihydroxylated phenylpropanoids, HPSEC, TMAH thermochemolysis, FT-ICR mass spectrometry

1. INTRODUCTION

Humic substances (HS) are known to influence various chemical and biochemical processes in the environment: they protect organisms from dangerous UV radiation; interfere with virus-cell interactions, stimulate or inhibit enzymatic reactions, toxify or detoxify heavy metals and catalyze the degradation of undesirable xenobiotics (1). Despite substantial progress in identifying low-molecular components of natural HS, the knowledge on the basic (core) structure of HS does still not meet the requirements for quantitative structure-activity relationships. Therefore, we have followed another path as we made substances of the humic acid (HA) type by oxidation of well-defined diphenolic compounds and analyzed both the chemical structure and the biological activity of the synthesized HS (2-4). To gain more insight into the structure of synthetic HS and to provide a basis for future studies in structure-activity relationships we synthesized a panel of simple (core) HA by oxidation of *only one* diphenolic compound at a time.

In this study we address the molecular structure of three HA-like polymers synthesized from closely related dihydroxylated phenylpropanoids (Figure 1). High performance size exclusion chromatography (HPSEC), pyrolysis/thermochemolysis-

GC/MS and ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) were used for the analytical investigations.

MATERIALS AND METHODS

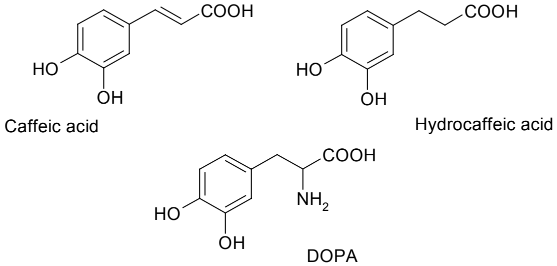

Figure 1: Diphenolic starting compounds.

HA-like polymers were synthesized by periodate (p) (5), air (a) (6) or tyrosinase (t) catalyzed oxidation (modified according to 7) of the following o-diphenolic starting compounds: hydrocaffeic acid = 3-(3,4-dihydroxyphenyl)propionic acid (→ HYKOP 441 p); caffeic acid = (E)-3-(3,4-dihydroxyphenyl)-2-propenoic acid (→ KOP 451 e, KOP 466 p); L-DOPA = 3,4-dihydroxy-L-phenylalanine = *(S)*-2-Amino-3-(3,4-dihydroxyphenyl) propanoic acid (→DOPA-OP a).

HPSEC: The molecular mass distribution of HS was investigated by using a high-performance liquid chromatograph from Hewlett Packard (HP Series 1050) equipped with the Diode Array Detector Agilent 1100 (Series G1315B) and fitted with a PSS Hema Bio column 8 x 300 mm (Polymer Standard Service Mainz, Germany). The stationary phase consisted of a hydrophilic copolymer network of ethylene glycol dimethacrylate and hydroxymethacrylate. As mobile phase 20% v/v acetonitrile and 80% v/v of an aqueous solution of NaCl (0.34% w/v) and K_2HPO_4 (0.2% w/v) adjusted with 0.2 mol/l NaOH at pH 10 was used. The column was calibrated using polystyrene sodium sulfonate standards (0.24 – 145 kDa).

Tetramethylammonium hydroxide (TMAH) thermochemolysis-GC/MS: The reaction was carried out in a 0.53-mm-i.d. stainless steel capillary tubing (Silcosteel) using a sample mass of 50 µg HS. Thermochemolysis was conducted by capacitive discharge at 500°C according to (8). The TMAH solution (2 µl 20% v/v in methanol) was directly injected into the pyrolysis capillary. Separation and identification of the analytes were done by GC-MS analysis in a temperature programmed working procedure as described in (2).

FT-ICR Mass Spectrometry: FT-ICR mass spectra using electrospray ionization (ESI) were acquired on a home-built 9.4 T FT-ICR mass spectrometer at the National High

Magnetic Field Laboratory in Tallahassee, FL. The use of this instrument to acquire ultrahigh resolution mass spectra of humic materials has been described previously (9). Molecular compound information for each synthetic HA sample was summarized in Kendrick and van Krevelen plots for comparative purposes.

RESULTS AND DISCUSSION

The molecular mass distribution of the original, non-hydrolyzed polymers HYKOP 441 and KOP 466 seem to be relatively homogeneous, as they have only a single peak at 1.950 and 2.636, respectively. DOPA-OP, by contrast, shows in addition to two low-molecular peaks at 0.597 and 1.074 kDa an equal third one at 33.17 kDa. This may be due to the presence of the NH_2 group in the side chain enabling further processing via dopaquinone to eumelanin which is stabile even under the strong alkaline HPSEC conditions.

Another essential question of this study was the kind and uniformity of the TMAH thermochemolysis products of the synthesized HS. Figure 2 shows, as an example, the TIC of HYKOP 441. In total, 22 of 24 low molecular products of KOP and 26 of 26 low molecular products of HYKOP were identified. Thereby the dimethoxymethyl esters of the starting compounds caffeic acid (about 50%) and hydrocaffeic acid (up to 95%) were found to represent the main part of KOP and HYKOP, respectively. In addition, the TMAH thermochemolysis revealed cyclization reactions (→ cyclopentenones) as well as ring openings of aromatic structures (→ aliphatic dicarbonic acids) taking place during diphenol oxidation.

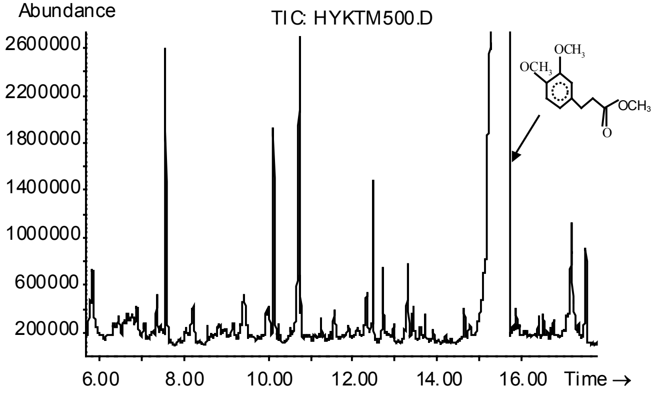

Figure 2: Total ion chromatogram (TIC) of TMAH thermochemolysis of HYKOP.

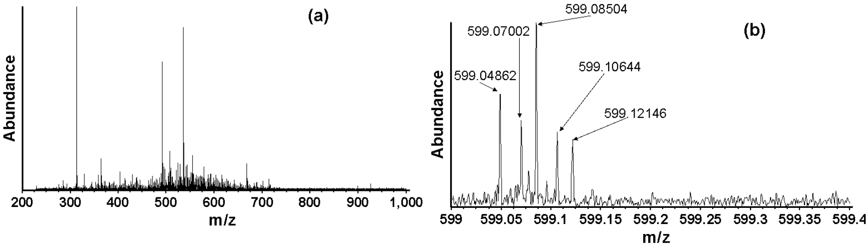

Figure 3. FT-ICR mass spectrum of TMAH thermochemolysis of KOP466: (a) mass range 200-1,000 Da, (b) isolated mass range 599.0-599.4.

FT-ICR mass spectra of the synthesized HS were used to corroborate the molecular mass data. Mass distributions from MS data were lower than those observed by HP-SEC (Figure 3a), in agreement with previous studies. We also observed unique mass spacings in addition to the 0.0364 Da difference characteristic of substitution of CH_4 for O that is common in natural humic and fulvic acids. The exact mechanism(s) responsible for these variations is not yet known.

CONCLUSIONS

The synthesis of simple diphenolic-based (core) HS offers a new opportunity to study the structure as well as possible structure-activity relationships among substances of the HA type. In particular, the results of TMAH thermochemolysis and FT-ICR mass spectrometry analyses provide information about the preservation of starting compounds in the synthetic (core) HS and on reactions potentially taking place during humification.

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High Resolution Ultrasonic Spectroscopy as Aid in Study of Complexation of Humic Acids

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Keywords: humic, modelling, copper, ultrasound spectroscopy

1. INTRODUCTION

The high affinity of humic acids (HA) to transition metals is well known. There is a large number of various coordination sites that are able to bind transition metals by various strengths. In previous works, quantum chemical calculations of interaction enthalpies (1) and also measurement of complexation kinetic (2, 3) were carried out. On the basis of obtained results the following models have been chosen for this study: citric acid, hydroquinone, pyrocatechol, salicylic acid and EDTA. The formation of complex of model with cupric ions has been observed only for hydroquinone and pyrocatechol by UV/VIS spectrometry (new band in spectra was detected). Others were chosen mainly for their high affinity to metal ions, e.g. salicylic acid is frequently considered as the most suitable coordination site in structure of humic acids, which has been confirmed also by quantum chemical calculations in our works (1, 3).

2. MATERIALS AND METHODS

Ultrasonic spectrometer with high resolution HR-US 102 (Ultrasonic Scientific, Ireland), was utilized for measurement of basic ultrasonic parameters. The device consists of two independent cells tempered at 25°C. Both cells were filled by the same model compound (e. g. hydroquinone) and then 1,25 M $CuCl_2$ (20 μl) was added into one cell. Velocity (U) and attenuation (N) in both cells was measured, the resulting differences ΔU and ΔN we can see at graphs.

UV/VIS spectra were measured by means of U-3300 Hitachi spectrophotometer.

3. RESULTS AND DISCUSSION

The examples of data measured for pyrocatechol are shown at Figs. 2 and 3. It was found that model compounds with new band observed in UV/VIS spectra (hydroquinone, pyrocatechol) need longer time for achievement of equilibrium than others. Changes in measured attenuation are also higher for these two models. On the other hand, significant increase of ΔN values was observed only immediately after addition of $CuCl_2$ solution for all used model.

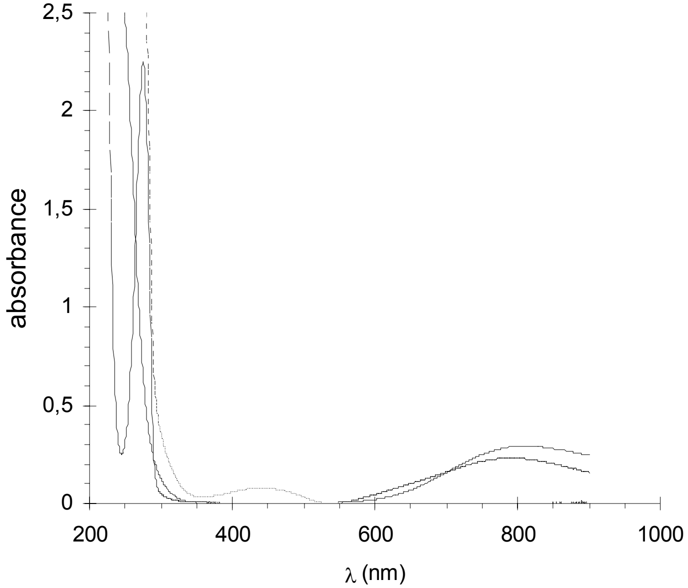

Figure 1. UV/VIS spectra of $CuCl_2$ solution (full line), pyrocatechol (dashed line) and formed complex (dotted line).

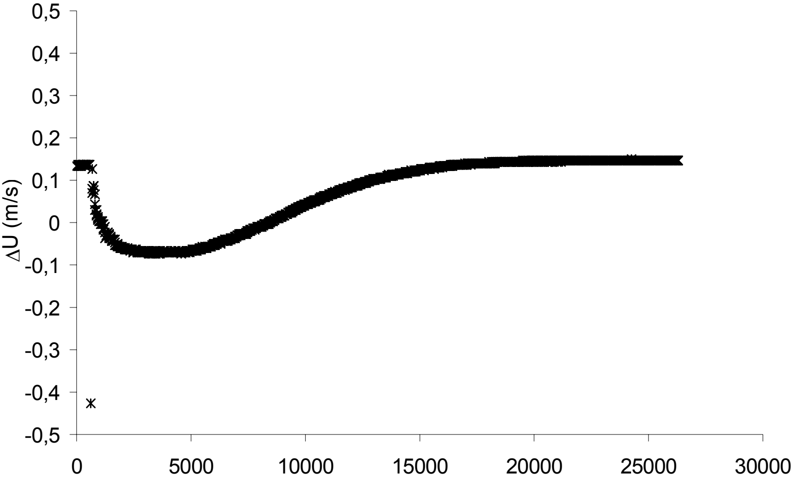

Figure 2. The time dependence of ΔU for hydroquinone after addition of $CuCl_2$.

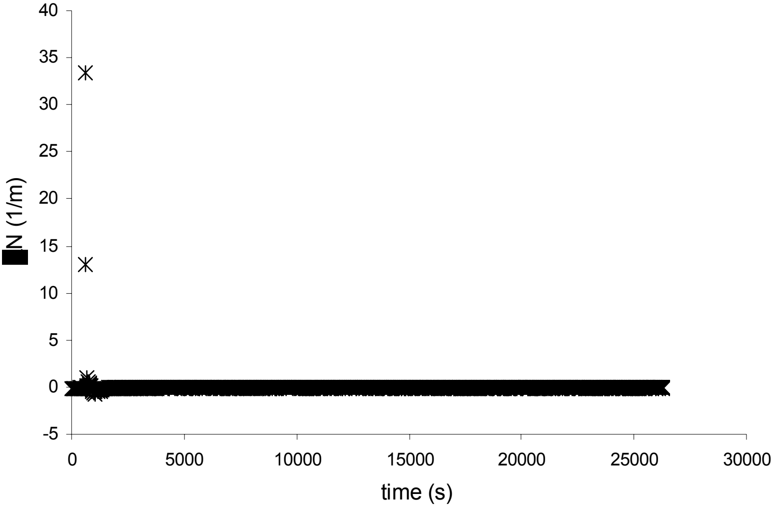

Figure 3. The time dependence of ΔN for hydroquinone after addition of $CuCl_2$.

High velocity and short time measured (Fig. 4) for salicylic acid confirmed high ability of this structure to bind cupric ions in stable complexes. Quantum chemical calculations of interaction enthalpies (1) resulted in model of salicylic acid as a most attractive for complexation of metal ions. High affinity of cupric ions to this structure probably causes that their interactions are faster than others.

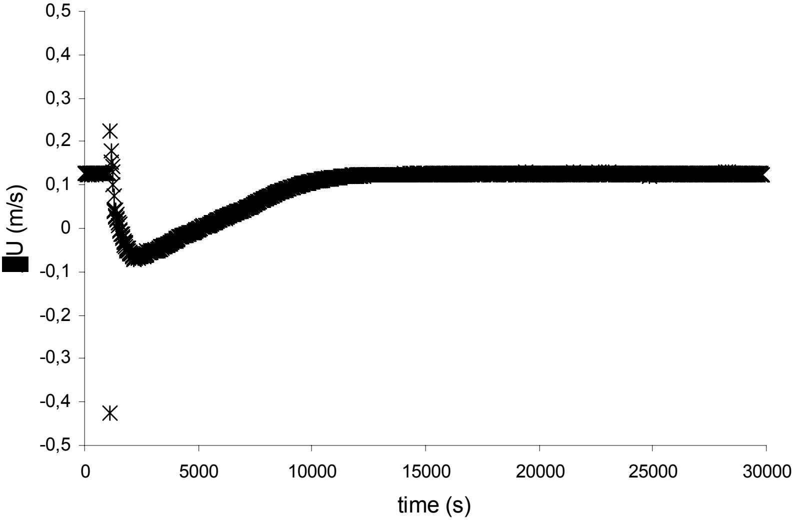

Figure 4. The time dependence of ΔN for salicylic acid after addition of $CuCl_2$.

4. CONCLUSIONS

Preliminary results of high resolution ultrasound spectroscopy are presented. Obtained data correspond with conclusions in our previous works (1-3). Salicylic acid seems to be attractive coordination site for complexation of cupric ions, but the most interesting results were obtained for hydroquinone and pyrocatechol. Even though, their computed interaction enthalpies (1) are much lower than value determined for salicylic acid, these structures contribute also to complexation capacity of humic acids. It corresponds with experimentally measured interaction enthalpy oh humic acids (3), which is approximately three times lower that that of salycilic acid, and EPR and FT-IR spectra of formed complexes discussed in our work (4).

ACKNOWLEDGEMENTS

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Characterization of Mumijo (Shilajit) from Different Regions by FTICR Mass-Spectrometry

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Keywords: mumijo, mumie, shilajit, FTICR mass-spectrometry

1. INTRODUCTION

Native Mumijo is a blackish-brown exudation, of variable consistencies, obtained from steep rocks of different formations found in the Himalayas at altitudes between 1000-5000 m, from Arunachal Pradesh in the East, to Kashmir in the West. Mumijo also is found in other mountain ranges of the world, e.g. Afganisthan (Hindukush, Badakh-Shan), Australia (Northern Pollock Ranges), and in the former USSR (Tien-Shan, Pamir, Caucasus, Ural) (1).

2. MATERIALS AND METHODS

Kyrgyz, Altaic, Kazakh and Indian mumijo from drugstore were used for analysis without further purification. All experiments were performed on a commercial mass-spectrometer 7 Tesla Finnigan LTQ FT (Thermo Electron, Bremen, Germany) equipped with electrospray ion source (Finnigan Ion Max Source). Mumijo samples were dissolved in 1:4 water-acetonitrile solution and analyzed using electrospray ionization both in positive and negative modes. For accurate molecular mass measurements FTICR mass spectra were acquired using selected ion monitoring (SIM) scanning with 100 Da mass range.

For interpretation of FTICR data and comparison of different samples Kendrick and van Krevelen diagrams were used (2). All FTICR mass spectra were also processed using FIRAN software for determination of stoichometric formulas (3).

3. RESULTS AND DISCUSSION

It was found that all mumijo samples could be efficiently ionized by ESI both in positive and negative modes. Data from different mumijo samples are occupying the same regions on van Krevelen diagram. Differences between Indian and kirghisian samples were found in broader variety of observable ions in Indian sample in region higher then 800 Da.

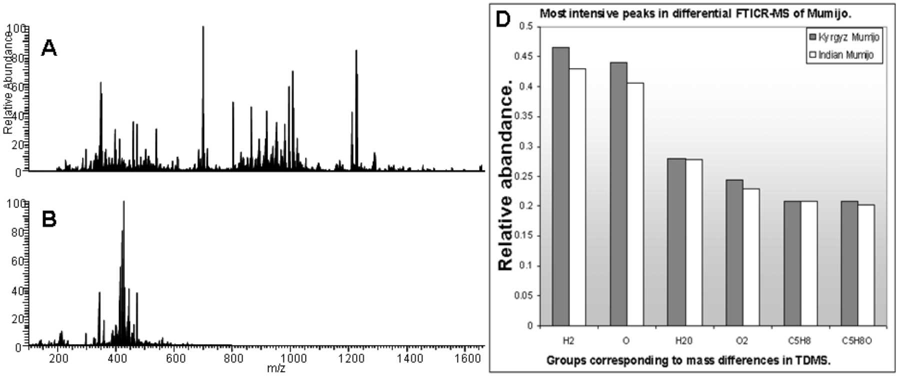

Figure 1. FTICR Mass-spectra of Indian (A), and Kyrgyz mumijo (B), (negative mode, equal concentrations of sample). Most intensive peaks in differential FTICR-MS of mumijo samples (D).

Stoichiometric formulas for ions in this region shows them to be highly saturated with low-oxygen contains (fatty acids esters etc.). Total mass difference statistics (TDMS) for different mumijo samples gives the same set of characteristic functional groups with very similar abundances (Figure 1).

4. CONCLUSIONS

Mumijo from different regions differ significantly in mass spectra but obey similar general stoichiometric characteristics. Mass spectrometry gives advance in understanding of its composition variety.

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Extraction and Characterisation of Humin Fraction from Amazonian Anthropogenic Dark Earths Soils (“Terra Preta de Índios”)

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Keywords: “Terra Preta de Índios”, humin, NMR, pyrogenic carbon, black carbon

1. INTRODUCTION

Humin, the humic fraction that is insoluble in water at all pH values, typically comprises more than 50% of soil organic matter (1). Because of the difficulties in isolation, humin compositions and properties have been less extensively studied than those of humic and fulvic acids (2). On the basis of the accepted definition, humin can include any humic-type substances that are not dissolved during exhaustive extractions with aqueous base.

A solvent system composed of a mixture of aqueous base/urea (0.1 M NaOH + 6 M urea, base/urea) has been reported to isolate material that would be classified as humin in the classical definition (3, 4, 5). Because urea is a powerful hydrogen bond breaker, it is considered that soil organic components were released from associations with humin materials either by the breaking of hydrogen bonds, or by the disruption of steric constraints by materials that had trapped the components released.

Dimethylsulfoxide (DMSO), a dipolar aprotic solvent, is an excellent solvent for cations but a poor solvent for anions (6). It is a good hydrogen bond breaker and the non-polar backbone (as distinct from the S=O face) of DMSO can be considered to have affinities for less polar humin components. A solvent mixture of DMSO with HCl (6%, v/v) was reported to have isolated less than 22% of humin material from soils (7, 8, 9). However H_2SO_4 is a very polar liquid with a high dielectric constant (~100), and due to the process of autoprotolysis (10), protons are highly mobile in H_2SO_4. This enables it to protonate the conjugate bases (carboxylates and phenolates), thereby increasing the solubility of the organic material (4, 5), since anions are weakly solvated in DMSO.

2. MATERIALS AND METHODS

The humin from three (samples 1, 2 and 3) Amazonian Dark Earths ("*Terra Preta de Índios*"), soils rich in pyrogenic carbon, was sequentially extracted using: 0.1 M NaOH (Humic Acids - HA); the soil residue was extracted with 0.1 M NaOH after the soil was dispersed by ultrasonication (HAD); then with base/urea (Humin - HuU); with a mixture of DMSO and H_2SO_4 (94:6, v/v) (HuDMSO); and finally the residue was treated with 10% HF (Hu). The coarse char fraction was carefully recovered from the soils by sieving (63 µm sieve) and sedimentation. Characterisations of the organic components of the humin fractions were carried out by Variable Amplitude Cross-Polarisation with Magic Angle Spinning (VACP/MAS) ^{13}C Nuclear Magnetic Resonances spectroscopy, and additional experiments were carried out using the Chemical Shift Anisotropy (CSA) (spectra of C-sp^3 only) and the Dipolar Dephasing (DD) (spectra of non-protonated and mobile C only) filters. The spectral data were analysed by Principal Component Analysis.

3. RESULTS AND DISCUSSION

Spectra are shown only for the HA and HAD samples (Figure 1). The HAD presents more aliphatic compounds, mainly crystalline poly-methylene structures (33 ppm) and with lesser of the amorphous structures (30 ppm). It is also richer in carbohydrates (102 and 73 ppm, as evidenced in the CSA spectra) and lignin residues (~150 ppm and 56 ppm, as evidenced in the DD spectra). These features indicate that the aggregate rupture releases relatively unaltered organic matter (cellulose and lignin derived materials) and also a

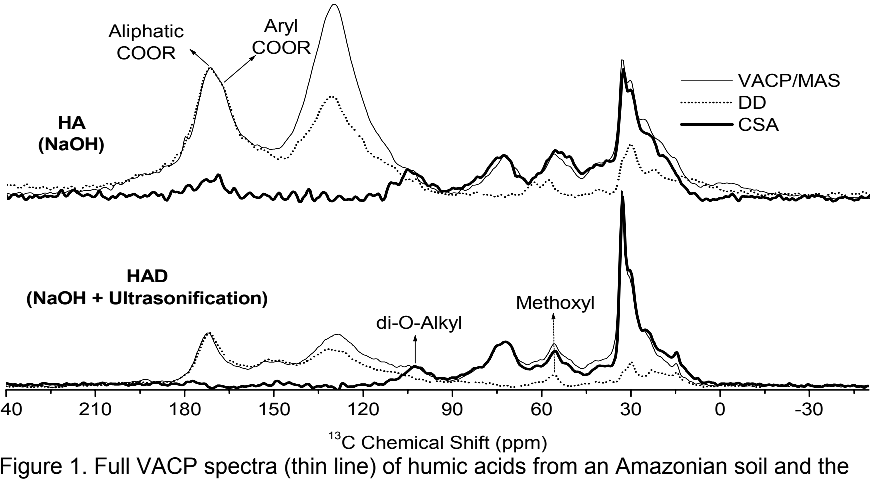

Figure 1. Full VACP spectra (thin line) of humic acids from an Amazonian soil and the corresponding CSA (thick line) and DD spectra (short dot line).

possible aggregating compounds, such as long-chain fatty acids (the 33 ppm signal associated with the aliphatic carboxyl groups at 172 ppm).

The first Principal Component (PC1) accounted for 78% of the total variance and is characterised (Figure 2, right) by positive loadings for crystalline poly-methylene structures (33 ppm) and smaller positive loadings for terminal methyl (15 ppm) and aliphatic carboxyl (175 ppm). On the other hand this PC presents negative loadings for pyrogenic carbon (aryl – 129 ppm and aromatic carboxyl-168 ppm) and ligno-cellulosic residues (153, 105, 73 and 55 ppm).

The scores for the PC1 (Figure 2, left) were greater for the samples extracted with the H-bond breakers (Urea and DMSO), and also for the samples submitted to ultrasonication. This indicates that the mechanical dispersion aided the solvent systems in cleaving H-bonds, and especially the penetrating solvent (DMSO) which facilitated the extraction of a humic material closely associated with the soil mineral matrix. The small scores for PC1 for the Hu, HA and coarse char samples indicate that the residual humin (insoluble in the solvent systems used) is similar to the coarse char (rich in pyrogenic carbon and ligno-cellulosic material). The second PC (13% of the total variance) only differentiated samples from the different soils (1, 2 or 3).

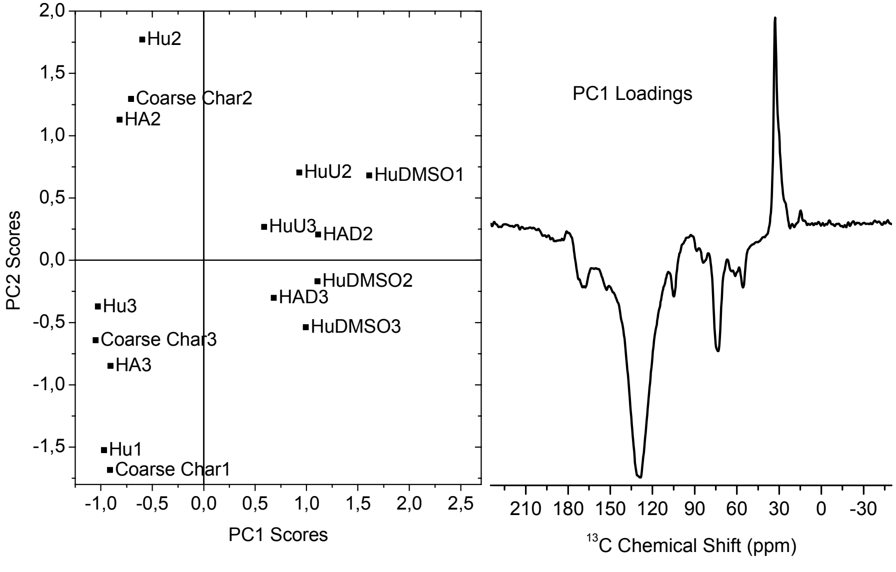

Figure 2. PCA loadings (right) and scores (left) from full VACP spectra.

4. CONCLUSIONS

The use of extraction systems that promote cleavage of H-bonds facilitated the extraction of additional humic substances that are humin in the classical definition. This material is more hydrophobic than the fractions obtained by the classical alkali solution method. The additional fractions contained significant amounts of long-chain fatty acids, mainly with crystalline poly-methylene structures, indicating that these compounds are important for the soil aggregation. The residual insoluble humin is probably colloidal charcoal and derivatives of vegetable debris (lignin and cellulose).

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Measuring Soil Amino Compounds by Anion Chromatography-Pulsed Amperometry

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Keywords: amino acids, amino sugars, anion chromatography, pulsed amperometry

1. INTRODUCTION

Soil amino acids and amino sugars are thought to comprise the vast bulk of soil N, and they are commonly measured by extraction with hot HCl, separation by cation exchange chromatography, derivatization by ninhydrin, and detection by visible light absorption. Yet HCl destroys some amino acids before they can be measured and amino sugars in the HCl hydrolyzate must be measured separately from amino acids. A recent alternative procedure allows simultaneous measurement of amino acids with amino sugars and better preservation of the acid-sensitive amino compounds (1). Soil is extracted by methanesulfonic acid, followed by anion chromatographic separation and detection with pulsed amperometry. This approach enabled slightly more efficient extraction of amino-N from agricultural soils of the U.S. Midwest than has generally been reported in a wide range of studies using the conventional HCl extraction (1, 2). Here, we further evaluate the anion chromatography/pulsed amperometry approach on a separate set of soils.

2. MATERIALS AND METHODS

Nine diverse surface soils were collected from low-input land uses (native vegetation, uncropped borders of crop production fields) in six states of the U.S. (3). For each soil, 250 mg was weighed into glass tubes with screw caps, in four laboratory replicates. To each tube was added 2 mL of a 4 M methanesulfonic solution having 0.2% tryptamine to suppress decomposition of the acid-sensitive amino acids. The soil samples and acid solutions were autoclaved for either 16 h at 121°C and 207 kPa pressure or 90 min at 136°C and 248 kPa pressure, while previous work recommended solely the higher-temperature autoclaving (1, 2). Following autoclaving, tubes were centrifuged and their supernatants were transferred to clean glass tubes. Tubes were washed twice with water and centrifuged, and these supernatants were combined with the original supernatant, then neutralized with 1 M NaOH to pH 5 to 6, and diluted to 10 mL. Of this solution, 1.5 mL was diluted with clean water in a second tube to 10 mL volume, and this solution was injected into a Dionex DX-500 anion chromatograph that was equipped with an AminoPac PA-10 column for separation of the amino compounds. Concentrations of amino compounds were measured with a disposable gold working electrode using triple-pulsed amperometry, i.e. a waveform that uses a stepped sequence of electrical potentials in the detector to cyclically cleanse the electrode surface.

3. RESULTS AND DISCUSSION

Chromatography peaks were distinct and well resolved for many of the amino compounds, including the two amino sugars galactosamine and glucosamine (Fig. 1). With continued use of the chromatography column, though, peaks for serine and proline merged with each other and peaks for ornithine and phenylalanine became indistinguishable from random baseline fluctuations.

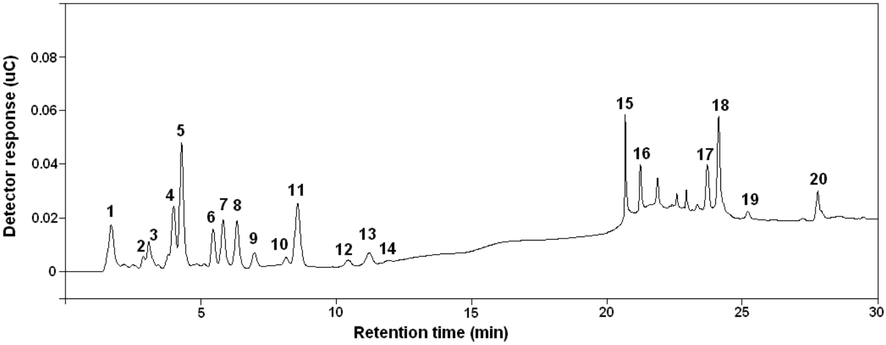

Figure 1. A representative chromatogram of extracted amino acids and amino sugars. Peaks are numbered to represent the following compounds: (1) arginine, (2) ornithine, (3) lysine, (4) galactosamine, (5) glucosamine, (6) alanine, (7) threonine, (8) glycine, (9) valine, (10) hydroxyproline, (11) serine/proline, (12) isoleucine, (13) leucine, (14) methionine, (15) histidine; (16) phenylalanine, (17) glutamate, (18) aspartate; (19) cystine, and (20) tyrosine. The detector response is in units of charge.

On average, 1% more amino-N was extracted by autoclaving for 16 h at 121^{o}C/ 104 kPa pressure than for 90 min at 136^{o}C / 112 kPa pressure (range of -12% to +14%, standard deviation 6%). This similarity in total extractable amino-N masked modest but consistent differences between the two autoclaving methods in the extracted amounts of individual amino compounds (Table 1). Autoclaving for the shorter duration (90 min) at 135°C provided for more efficient extraction of alanine, ornithine, and those amino compounds that are unstable in acidic solutions: serine, threonine, both amino sugars (glucosamine and galactosamine), and the S-bearing amino acids (methionine and the cystine dimer). For all other amino compounds showing a significant effect of autoclaving, the 16-h heating at 121°C provided for more efficient extraction. The interaction of autoclaving with soil type was highly significant (*P*<0.01) for every amino compound. Combining the results from both autoclaving methods for all nine soils, on average 41-43% of total soil N (range 31-63%) was identified as amino-N.

Table 1. Proportion of total extractable amino-N for each amino compound in nine soils and levels of significance (*P*) for two autoclaving methods

Amino compound	P level for autoclaving method	Percent of total amino-N in soil	Amino compound	P level for autoclaving method	Percent of total amino-N in soil
Arginine	<0.0001	25.8	Histidine	0.005	3.5
Glycine	0.211	10.2	Glutamate	<0.0001	3.3
Lysine	0.182	9.6	Leucine	<0.0001	2.9
Glucosamine	<0.0001	9.1	Isoleucine	<0.0001	1.6
Alanine	<0.0001	7.1	Ornithine	<0.0001	1.5
Serine/Proline	<0.0001	5.4	Phenylalanine	0.582	0.9
Aspartate	0.006	4.8	Tyrosine	0.0004	0.8
Threonine	<0.0001	4.1	Methionine	0.012	0.7
Galactosamine	<0.0001	4.0	Hydroxyproline	0.050	0.5
Valine	0.001	3.8	Cystine	<0.0001	0.3

Expressed as proportions of total extractable amino-N, the relative abundance of arginine was greater than has been reported in previous applications of the conventional HCl approach (Table 1). In contrast, the relative abundances of aspartate and glutamate were considerably smaller than previously reported. The most likely explanations are: (i) this approach is less efficient at extracting from soil or measuring aspartate and glutamate than is the conventional HCl approach; and (ii) the low-input history of these soils (no fertilizer or manure, and hence likely small amounts of incorporated plant residues) slowed the formation of new soil amino acids, leading to long-term depletion of these two acidic amino acids, consistent with the results of (4).

Dairy manure slurry was added to these soils at a rate corresponding to 300 kg N ha^{-1} and incubated for 28 days. Analysis for soil amino compounds associated the slurry addition with a general enrichment of most amino acids (3). By the end of the incubation, the N content of three amino compounds that are associated with microbial activity (ornithine, glucosamine, and galactosamine) had increased by 3% for both manured and unamended soils, while the N content of all other amino compounds had decreased by 11% in the manured soils (data not shown), likely due to either mineralization or binding to soil surfaces beyond extraction by methanesulfonic acid.

4. CONCLUSIONS

Meaningful concentrations of soil amino acids and amino sugars were estimated by soil extraction with methanesulfonic acid followed by anion chromatographic separation and pulsed amperometric detection. The results of this method differed somewhat from those generally reported for the conventional HCl analysis. As with the HCl analysis, the main problem remains the modest proportions of total soil N that can be identified as amino N.

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Membrane Processes Applied to the Study of Marine Dissolved Organic Matter

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Keywords: dissolved organic matter, seawater, fluorescence, reverse osmosis, electrodialysis

1. INTRODUCTION

Aquatic dissolved organic matter (DOM) represents one of the major carbon reservoirs on Earth: seawater contains approximately 700 gigatons of dissolved organic carbon, which is comparable to the amount of inorganic C present in the atmosphere (1). Changes in marine DOM production or consumption can have a significant effect on the carbon cycle on a time scale as short as years. Therefore, the study of DOM is important, particularly in coastal waters, where substances from the terrestrial environment are transferred to the marine environment.

The isolation, characterization and study of DOM properties still constitute a real challenge nowadays. In fact, one of the main obstacles in the study of DOM is to isolate sufficient amounts of this chemically complex material, which is present at low concentrations in waters and very often associated to high salinities. Over the last thirty years, several techniques such as extraction on XAD resins or tangential ultrafiltration (UF) have been used to isolate marine DOM. These techniques provide very useful information on this material but only a small fraction of marine DOM (about 10-30% of dissolved organic carbon (2, 3)) is finally recovered. As a result, the composition of a large amount of marine organic matter is still unknown

The aim of this study was to develop a method of concentration of DOM from marine and brackish waters, without modifying or fractionating the organic material and while minimizing the salt content of the samples. Reverse osmosis (RO) is now a well-developed technique for the isolation of DOM from freshwaters (4). But to date only a few attempts of concentration of DOM from salty waters were achieved: RO cannot be used directly to concentrate DOM from seawater, due to the fact that salts are retained exactly like organic matter during RO. Therefore, seawater has to be desalted before the concentration of marine DOM by RO.

Electrodialysis (ED) appears as an interesting alternative to desalt seawater before the concentration step by RO. ED is a gentle electrochemical process allowing the separation of ionic species from aqueous solutions by using an applied direct current potential through anion and cation-exchange membranes. Thus, two solutions are obtained: a demineralised solution containing organic matter (diluate compartment) and a solution containing the extracted salts (concentrate compartment). Over the last ten years, ED has been used in several studies to desalt freshwater (5) or more recently seawater (6) samples before or after their concentration by reverse osmosis. The combination of ED and RO was shown to be a valuable approach to isolate and concentrate DOM from freshwaters, since the losses of DOC during the whole process were relatively low (less than 25%). But it has still to be shown that the quality of DOM is not modified during ED.

During this work, DOM of fresh, brackish and marine water samples collected in coastal environments was concentrated by nanofiltration (NF) and/or RO. At the same time, the development of a desalting step of seawater by ED was achieved. Several aliquots were collected during the RO and ED experiments and they were analyzed by three-dimensional excitation-emission matrix (EEM) spectroscopy. Thanks to this technique, potential modifications or losses of DOM during the concentration and desalting steps can be detected. The DOC content of the water samples was also measured, in order to calculate the amount of organic carbon recovered at the end of the experiments.

2. MATERIALS AND METHODS

The freshwater sample was collected from the upstream part of the Gironde estuary (La Réole; South Western France) in February 2007. The brackish water sample was collected from the maximum turbidity zone (MTZ) of the Gironde estuary in October 2006. Last, the seawater sample was collected from the Bay of Balaguier in Toulon (South Eastern France) in May 2006. Seawater was desalted by electrodialysis, with an ED stack (Eurodia Industrie SA) equipped with AMX and CMX membranes (Tokuyama Corp., Japan) and consisting of 10 cell pairs. The total membrane surface area was 0.2 m^2. As for the reverse osmosis pilot used for DOM concentration, it was constructed by a French company – TIA. Two membranes were used during this work: a NF membrane (Osmonics DL2540) and a RO membrane (SW30-2540; Filmtec) designed for seawater desalination.

The fluorescence spectra were recorded with a Fluorolog SPEX FL3-22 Jobin Yvon Fluorometer. The fluorescence EEM spectroscopy involved scanning and recording of 17 individual emission spectra (260-700 nm) at sequential 10 nm increments of excitation wavelength between 250 and 410 nm. Last, DOC measurements were achieved with a Shimadzu Total Organic Carbon analyzer (TOC-V CSN), in NPOC mode.

3. RESULTS AND DISCUSSION

The results of the concentration of the freshwater sample are summarized in table 1. The average concentration factor of fluorescent DOM was calculated by dividing the fluorescence intensity of the concentrated sample (expressed in cps/s) by the one of the initial sample. This calculation was carried out for the four main fluorophores characteristic of DOM.

Table 1. Results of the concentration of the freshwater sample by RO

		Before concentration	After concentration	Concentration factor
Fresh-water sample	Volume (L)	332 ± 2	3.0 ± 0.03	110
	DOC (mg/L)	1.86 ± 0.07	111.0 ± 0.6	60
	Fluorescence Intensity (cps/s) Ex310/Em400nm / Ex370/Em450nm	1306452/ 939392	85328234 / 60338599	64
	Salinity / Conductivity (mS/cm)	0 / 0.335	4.0 ± 0.1 / 7.3	22

About 55% of DOM was finally recovered at the end of the concentration. The quality of DOM was not significantly modified: the 3D fluorescence spectra of the initial sample and the one of the concentrated sample have a similar shape.

Table 2. Results of the concentration of the brackish water sample by RO

		Before concentration	After concentration	Concentration factor
Brackish water sample	Volume (L)	342.5 ± 2.0	12.5 ± 0.1	27.4
	DOC (mg/L)	2.2 ± 0.1	44.4 ± 0,4	20.2
	Fluorescence Intensity (cps/s) Ex310/Em400nm / Ex370/Em450nm	1799870 / 1398433	42092239 / 32842987	23
	Salinity	2.6 ± 0,1	47.5 ± 0.1	18.3

In the same way, about 340L of the brackish water sample were concentrated by RO (table 2). This technique presents two advantages: the yield in DOC is high (more than 75%) and fluorescent DOM is not altered during the concentration.

RO cannot be used directly to concentrate marine DOM. That is why we combined nanofiltration and RO to concentrate 940L of Mediterranean seawater. The 940L were first reduced to 32L by NF and then to 10.5L by RO. Even though a large amount of organic matter was lost during the NF step, we finally obtained a significant concentrated DOM sample representative of the initial seawater organic matter.

In order to improve concentration yields, ED was considered to desalt seawater before the concentration by RO. Our results show that ED is a fast and efficient technique to desalt seawater: about 6 hours were required to desalt 20 liters of Mediterranean seawater from a salinity of 37 to a salinity of 3. During the ED, the DOC content of the diluate (desalted seawater) stayed approximately constant (figure 1) and modifications of fluorescent DOM were limited.

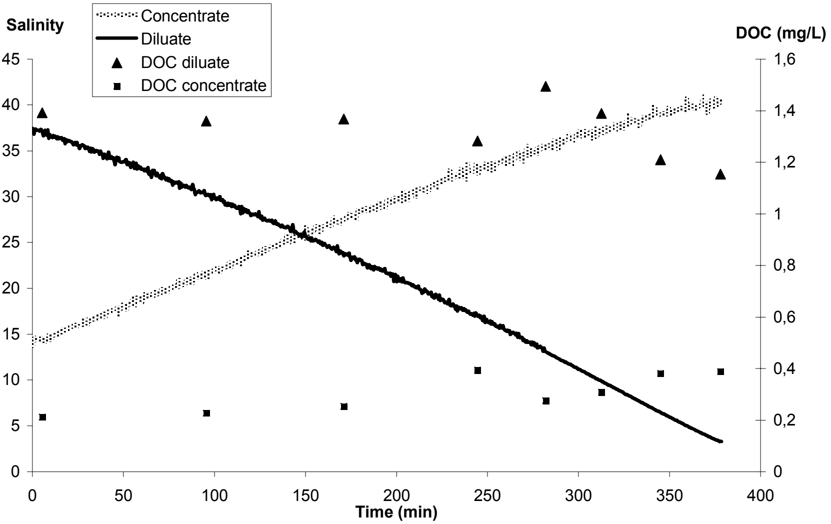

Figure 1. Variations of the salinity and of the DOC content in the diluate and concentrate compartments during the desalting of 20 liters of seawater by ED.

4. CONCLUSIONS

RO is a very efficient method to concentrate DOM from fresh and brackish waters. It could be combined with ED, which allows to greatly reduce the salt content of seawater while minimizing DOM modifications and losses. Thus, sufficient amounts of marine organic matter could be obtained and then analyzed by NMR, IR, or Py-GC/MS.

ACKNOWLEDGEMENTS

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Studies of the Structure of Extracted Humic Acid from Naharhoran Forest Gorgan's Soil

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Keywords: forest soil, extraction of humic acid, IR, potentiometeric titration, CHNS , GPC, XRD, GC/MS, benzene 1,3–dietheyl, N.N dimethyl formamide

1. INTRODUCTION

The humic acid are organic complex organism that is derived from soils, sediments and natural waters. Its composition and structure is still very complex and unknown, so that the suggested models by dragonoy, christamen, gismi, Stevenson and others are not the same (1). They contain functionalized aliphatic-aromatic backbones that may be neutral, acidic or basic and have low or high molecular weight (2).

2. MATERIALS AND METHODS

2-1 Procedure. The samples were derived from soil with high organic and low iron content, taken from Naharkhoran forest(Gorgan-golestan province) in the North of Iran. The separation of organic fractions is after an initial physical (sieve) removal of stones and leaf litter. Then washed with 0.50 mol L^{-1} HCl and several times with DDW. According to the protocol of the IHSS (3) . This acid has been extracted using 0.50M NaOH and 6M HCl and then was purified by 0.3M HF(Merck darmstade Germany) and 0.1M HCl. It should be powdered to 75-150 μm before using.

2-2 Apparatus. Extraction of humic acid was performed agitator (ELM 1400rpm, Germany). IR spectrum of the preparation sample by disk IR-470, elemental analyses system GmbH vario EL, Thermal analysis 1500 STA (England), X-ray D&Advance (Germany).

3. RESULTS AND DISCUSSION

The IR spectrum of this sample perfectly was match with that of Gardea (4) and the presence of benzoic-carboxylic and phenolic functional groups were confirmed in it (Fig. 1). The total cationic exchange capacity and capacities of each functional were determined by potentiometeric titration method in the presence of 0.20 M NaOH in aqueous media (Tab. 1, Fig. 2). By logarithmic interpretation of potentiometeric titration curve the acidic constant (pKa) of benzoic carboxyl and phenol were calculated to be

4.60 ± 0.25 and 8.54 ± 0.35. respectively. Fractions contain larger amounts of the unsaturated groups such as aromatic moieties in the structure (2). Shows obtained percentage of CHNS and C/N ratio (Tab. 2) for humic acid extracted from Gorgan’s (5).

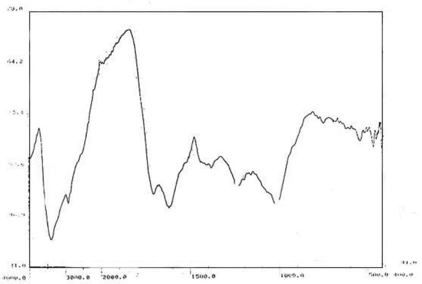

Figure 1. IR spectrum of humic acid.

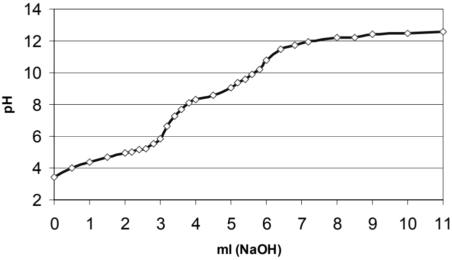

Figure 2. Potentiometeric titration of 0.5 g humic acid By 0.2M NaOH in aqueous media.

Table 1. Cationic exchange capacity for each functional group

Functional groupe	COOH	OH	total
CEC (mmol/g)	3.10 ± 0.30	2.10 ± 0.25	5.20 ± 0.40

Table 2.Elemental composition

Weight of humic acid(mg)	C%	H%	N%	S%	Ash%	O%	C/N
10	49.400	5.100	3.300	0.269	1.500	40.430	15.000

The XRD pattern of this acid has indicated the data so obtained were mainly aromatic nuclei and it has colloidic property of particles (Fig. 3). its scanning electronic microscopy image showed the particle sizes were about 0.2 μm (Fig. 4).

TG thermograms showed in the 50-150°C range a weight loss representative of the dehydration reaction (Fig. 5a). the decomposition process Between 200 and 500°C occurred mainly in two steps. The DSC thermogram of this acid exhibit three peaks (Fig. 5b). The first peak centered at 80°C attributed to the removal of the water, while the second peak centered at 300°C, attributed to the combustion of the carbohydrates. The third peak centered at 400°C may be due to the evolution of CO_2 (7, 6).

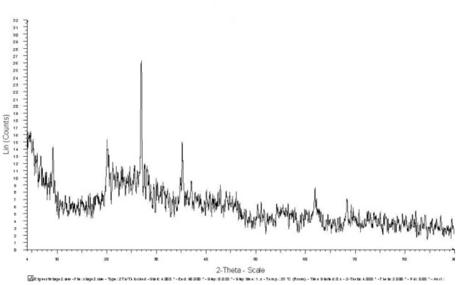

Figure 3. The XRD pattern.

Figure 4. Scanning electronic microscopy image (reference:Al_2O_3, V = 10°C/min).

The molecular weight and viscosity of humic acid in THF solvent of was determined by GPC method (8). The result confirmed the properties of HA typical for high molecular polyelectrolytes (Tab. 3, Fig. 6).

Table 3. Molecular weight and viscosity of humic acid

Factor	Viscosity	Molecular Wt(g/mol)
Data	2327	2153

Chemical composition was determined in DMF solvent by mean GC/MS technique (9) One characteristic fragment molecule was monitored in addition to the molecular. Benzene 1,3-dietheyl, eiccosane 2-methyl, N-tridecane, pentadecane, butanamine, acetaldehy demethoxy ,N.N dimethyl formamide.

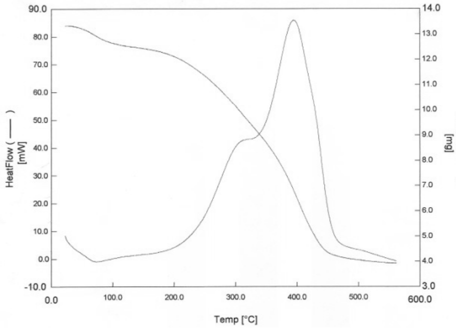

Figure 5. a) Thermogram TG;
b) Thermogram DSC

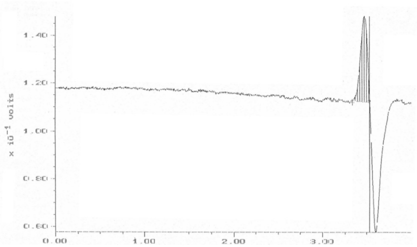

Figure 6. Gel permeation chromatography in THF solvent.

4. CONCLUSIONS

As in soil, the beneficial effects of humic acid in aquatic systems can probably be ascribed largely to its metal-binding and cation-exchange properties may be another useful function of humic acid has proposed that humic acid be employed in the disposal of radioactive wastes. In conclusion, it would seem that the humic acid of soil has great ecological and geochemical significance, but it has not been thoroughly investigated. The results of research indicate that properties of humic acid from northern Iran's forest soil obeys Stevenson model more than other (1).

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Application of a Novel Polarity Method for the Analysis of Natural Organic Matter during Water Treatment

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Keywords: NOM, PRAM, source water, water treatment

1. INTRODUCTION

The accurate characterization of the properties of natural organic matter (NOM) in environmental samples continues to be a challenge. Advanced analytical techniques such as resin fractionation and mass spectrometry are commonly used to characterize NOM (1). However, these methods require extensive preparative steps to obtain solid phase extracts and limit the analysis to a select number of samples. Also, sample pH is modified and this does not accurately represent the environmental conditions of NOM. An additional approach to the characterization of NOM is the development of simple methodologies which could be used to evaluate water treatment performance and reactivity at natural pHs during the treatment process. Recently, an alternative approach to the characterization of the polarity of NOM was presented (2,3). The polarity rapid assessment method (PRAM) permits NOM characterization under ambient conditions in less than one hour, allowing for the timely analysis of multiple samples. This approach enables researchers to evaluate the temporal variability of NOM characteristics and the effect of this variability on the changes of NOM polarity within the water treatment systems.

The objective of this study was to present the applicability of using PRAM to understand polarity changes of chromophoric NOM that occur in surface waters and during water treatment. For this, a pilot system simulating conventional treatment with pre-ozonation and biofiltration was studied. This study emphasizes the characterization of NOM polarity under ambient using this simple methodology.

2. MATERIALS AND METHODS

Three water samples were collected from a dual-train conventional treatment plant (rapid mix/flocculation/sedimentation/filtration) with a flow rate of 17 L/min (4.5 gal/min) per train. The pilot plant included pre-ozonation and biological filtration. The source water for the pilot plant included a variable blend of Colorado River Water (CRW) and State Project Water (SPW) from the San Joaquin Delta in California with a TOC of approximately 3 mgC/L.

PRAM characterization was done following the procedure described previously(2,3). Briefly, solid-phase extraction (SPE) cartridges (Alltech Associates, Deerfield, IL, USA) were used to quantify the amount of NOM adsorbed onto each sorbent. The used sorbents included non-polar (C18 and C2,), polar (CN, Diol and Silica) and anionic exchangers (NH2, SAX). Water samples were run through each cartridge and the UVA at 254 nm was obtained versus time. Flow through each SPE cartridge was maintained at 1.2 mL/min (except for CN, with a flow of 1.67 mL/min) using a syringe pump (KD Scientific Model 100, Holliston, MA, USA).

The UVA breakthrough curves were normalized to the initial conditions. Analysis of these breakthrough curves provided a measure of the total material adsorbed, described as a retention coefficient (RC) and defined as one minus the maximum breakthrough level ($C_{max}/C_{initial}$). The results are presented as RC for each SPE sorbent. These RCs have been indirectly correlated to physicochemical properties of NOM (2).

3. RESULTS AND DISCUSSIONS

Figure 1 presents the PRAM results describing the RCs obtained for the analysis of the raw water during the three sampling events. The observed ranges in RC were 0.50 - 0.70 for the weak anion exchange (NH2), 0.86-0.89 for the strong anion exchangers (SAX), 0.00 - 0.10 for the polar sorbents (CN, Diol and Silica) and 0.05-0.25 for non-polar sorbents (C18 and C2). The high RCs for the anion exchangers were due to the negative charges associated with NOM that are caused by the de-protonation of carboxylic acids and phenolic groups at ambient pH (4).

There was variability between all the samples analyzed. For example, the RCs for C18 were 0.24, 0.17 and 0.14 for the three samples, with a standard error <0.01. This variability could be ascribed to NOM changes caused by fluctuations in the CRW and SPW blends, which varied during testing, as well as to temporal variability of the character of NOM itself. It is believed that part of the variability was caused by other environmental or geochemical effects involved in the creation and modification of the NOM, including differences in the chemistry within the water column.

In addition to characterizing the polarity of the raw waters, PRAM can be used to follow changes in the polarity of NOM during treatment. Figure 2 shows the changes in RC between the ozone contactor influent and effluent for the three sampling events. A negative difference in RC for a sorbent indicates ozonation decreased the NOM polarity fraction that interacts with that sorbent. The opposite is true for a positive change in the RC value.

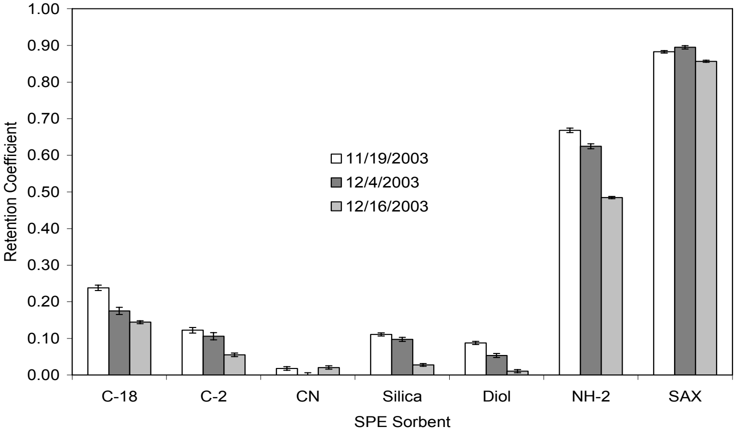

Figure 1. PRAM results for three raw water samples collected from the pilot plant during six weeks of testing.

As Figure 2 shows, the C18 RCs decreased for two of the three samples and practically remain unchanged for the last sample. These results indicate NOM oxidation reduced hydrophobicity, which agrees with other reports that used resin fractionation for NOM analysis through ozone treatment (5). However, a similar trend was not observed for C2 RCs. Since the capacity of C2 is less than that of C18 for the adsorption of hydrophobic compounds, the data suggest hydrophobicity changed within the core of the NOM mixture and beyond the reach of the shorter C2 chain.

The RCs obtained for Diol increased during the last two samples and remain unchanged for the first one. This change corresponds to an increase of the overall proportion of polar functional groups, confirming previous research demonstrating ozonation causes an increase in the overall NOM polarity. This increase is also observed for the weak anion exchanger, NH2 RCs. On the other hand, the stronger anion exchanger, SAX does not follow the increase demonstrated by NH2. For the first sample, SAX shows a decrease in the proportion of negative charges compared to the other two samples in which no change is observed. Additional work in this area will elucidate these differences and their significance.

4. CONCLUSIONS

This paper presents the first time PRAM has been used for the characterization of the polarity of NOM across a treatment system. The application of PRAM offers the advantage of allowing the characterization of numerous samples, resulting in the evaluation of variability of NOM. This is advantageous for drinking water utilities interested in understanding the variations in NOM in their water supplies and how these variations affect treatment systems at ambient pH.

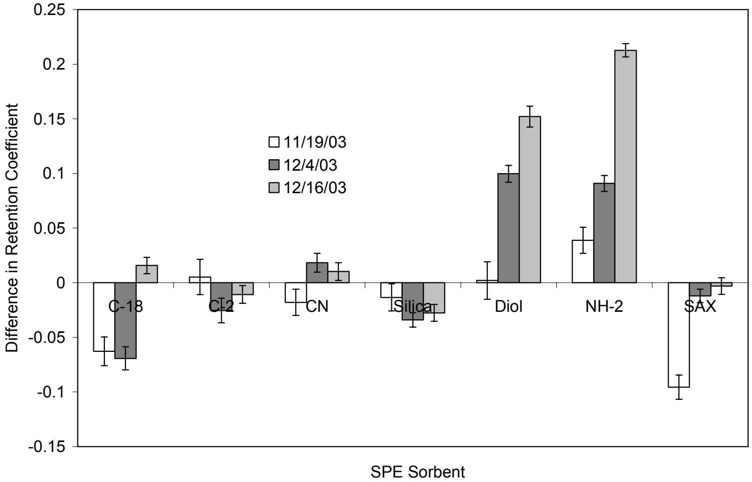

Figure 2. Difference in retention coefficient between raw water and ozone effluent. A positive difference indicates an increase in the specific polarity fraction. A negative difference indicates a decrease in the specific polarity fraction by removal or chemical transformation.

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Separation and Isolation of Soil Humic Acid Fluorescence Species by Tandem Ultrafiltration-PAGE

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Keywords: humic acid, fluorescence, PAG electrophoresis, ultrafiltration

1. INTRODUCTION

Soil humic acids (HAs) are fluorescence organic compounds which emission spectra generally characterized by a broad band showing a maximum that depends on the origin and nature of the HAs. It is well known that soil HA fluorescence is probably the sum of many different fluorophores presented in humic molecules (1, 2). However, the information about soil humic material with different fluorescence properties up to present day is limited. Recently the distribution of soil HAs on the fractions with different fluorescence intensity has been done (3, 4). However, due to the chemical complexity, variable chemical composition and molecular size (MS) polydispersity of soil HA, the separation of soil HA fluorescence on the different species have not been realized still. For solving of this problem the combination of modern fractionation methods with rapid detection of results of fluorescence distribution between fractions seems to be very promising.

In this study ultrafiltration (UF) following by polyacrylamide gel electrophoresis (PAGE) with detection in white and UV-light was used for separation of soil HA fluorescence on the different species.

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 has been done according to IHSS extraction procedure. The HA has been dialyzed through the dialysis membrane 5kDa during seven days against distilled water. Some characteristics of soil and soil HA were previously reported (5).

Ultrafiltration of HA was carried out using Amicon Diaflo membrane YM5 with nominal retention size 5 kDa (pore size was calibrated for proteins by manufacturer) in nitrogen gas atmosphere at a pressure of 5psi (1p.s.i.=6894.76 Pa). The membranes used were 42 mm in diameter. The 50 mg of HA has been dissolved in 50 ml 7M urea and put into the ultrafiltration cell. After finishing of the utrafiltrafiltration procedure 30 ml of ultrafiltrate (marked UF1, MS<5 kDa) and 20 ml of retentate (marked R1, MS>5 kDa) have been obtained. Further retentate R1 was dilute with 7M urea up to 50 ml and again pass through the same 5 kDa UF-membrane. This procedure has been repeated 30 times until the clear ultrafiltrate solution has been obtained. At the end of the experiment we have got 30 ultrafitrates (from UF1 to UF30, the volume of each was 30 ml) and last retentate R30 (volume 20 ml). The ultrafiltrates from UF1 to UF30 have been combined into fraction named UF(1-30). Than retentate R30 and combined ultrafiltrate UF(1-30) were dialyzed through the dialysis membrane 0.5 kDa during seven days against distilled water, lyophilized and assayed by PAGE.

To investigate the effect of urea treatment on HA chemical structural changes, 50 mg of unfractionated HA was dissolved in 7 M urea for two days, then dialyzed seven days against distilled water (cut-off of the dialysis membrane was 5 kDa), lyophilized, and then used for further physical-chemical analyses.

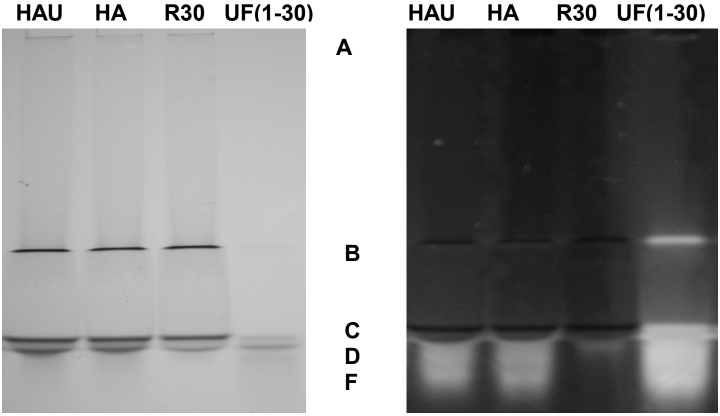
The method of PAGE which used for analysis of HA samples has been reported previously (5). 9.7% acrylamide and 0.3% bisacrylamide were dissolved in 89 mM Tris-borate, pH 8.3, with 1 mM EDTA and 7M urea. Electrode buffer was 89 mM Tris-borate, pH 8.3, 1 mM EDTA. For electrophoresis we have applied on 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, 7 M urea, 1% SDS and 1 mM EDTA.

Immediately after electrophoresis the results of HA fractionation in polyacrylamide gel were reviewed in a dark room using a TFP UV/WL – mixed transilluminator both in white and UV (312 nm) light. The photos have been done using Nikon COOLPIX L3 digital photo camera (5.1 Megapixels, 3x optical zoom).

3. RESULTS AND DISCUSSION

The chernozem soil HA, humic acid after urea treatment (HAU), retentate R30 (MS>5kDa) and combined ultrafiltrate UF(1-30) (MS<5kDa) were tested by PAGE according to Trubetskoj et all (5). The novelty and advantage of our PAGE method for HA fractionation rests primarily on the presence of urea, which added at a level of 7M both in sample buffer and gel, and sodium dodecylsulphate (SDS), which added to a sample buffer at a level 1%. Urea assists in the rapture of intra and/or intermolecular hydrogen bonds in humic matter and prevents interaction between fractionated HA and the PAG. Disaggregating effect of SDS based on displacement of polyvalent cations from HA. The proposed PAGE procedure allows separation of primary disaggregated structural humic components, thereby solving some of the key problems occurring in fractionation of HA.

The result of electrophoresis has been reviewed on transilluminator in both white and UV (312 nm) light and presented on Figure 1. It should be noted that using PAGE with coupling of the transilluminator technique is quite routine methodology for study of proteins and DNA. However, there are no reports in literature about using of this set-up respect to humic substances.



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

Figure 1. Electrophoresis of 0.1 mg HA, HAU, R30 and UF(1-30). A, B, C, D – naturally colored zones, detected in white and UV light. F – fluorescence fraction, detected in UV light.

In white light the original HA sample was separated into four discrete fractions: A - or start zone, that did not move into the gel and three intensively naturally colored zones B, C and D. Zone B differed greatly from zones C and D in EMs. The similar electrophoretic pattern has been obtained for HAU. However, in retentate R30 the fraction D was very pale and for ultrafiltrate UF(1-30) only very weak fraction D and traces of fraction C have been detected. Identically marked fractions have similar EMs. It have been shown previously, that MS of electrophoretic fractions decreased with increasing of their EM (5). Fraction A had the highest MS and fraction D - the smallest MS.

In UV light the original HA sample revealed the new fraction F, which possess the highest EM and shows intense overlapping yellow and blue fluorescence. Fraction D revealed pale red fluorescence. Fractions A, B, and C did not show fluorescence. The similar electrophoretic pattern has been observed for HAU. On the other hand retentate R30 demonstrated only narrow red-fluorescing band associated with the fraction D. Ultrafiltrate UF(1-30) shown the yellow-blue fluorescence associated with fractions C, D, F and small part of blue fluorescence formed narrow zone in the mid part of the gel. We could visualize that humic material actually contain at least a few different fluorescence species (red, yellow and blue). Unfortunately, from the monochromatic colour of the Figure 1, it was not possible to show the natural colour of the fluorescence.

In the work presented here the ultrafiltration of HA was carried out using membrane with nominal retention size 5 kDa in 7M urea solution. It should be suggested that humic material which came through the membrane (nearly 20% of the hole weight of HA) and possess yellow-blue fluorescence had nominal MS less than 5 kDa and connected with HA matrix through intra and/or intermolecular hydrogen bonds. Interestingly that urea treatment of HA with subsequent dialysis through 5kDa membrane was unsuccessful for separation of fluorescence of humic material.

4. CONCLUSIONS

The combination of ultrafiltration in 7M urea following by PAGE with detection in white and UV light is very promising for the rapid and visulizable investigations of the HA fluorescence and separation of fluorescence material from the not fluorescence one.

ACKNOWLEDGEMENTS

The work has been supported by INTAS grant 06-8055 and Russian Foundation for Basic Research (project 06-04-48266-a).

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NMR Approaches for Characterization of Soil HA Fractions Obtained by SEC-PAGE

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Keywords: humic acid, PAGE, SEC, ^{13}C –NMR, ^{1}H- NMR

1. INTRODUCTION

The NMR spectroscopy is one of the technique that has been most successful in structural investigation of soil HAs fractionated by different approaches. The aim of this study was to examine by both solid ^{13}C-NMR and ^{1}H-NMR in D_2O and DMSO-d_6 the chernozem soil HA and its different in molecular size (MS) and electrophoretic mobility (EM) fractions, obtained by combination of size exclusion chromatography and electrophoresis (SEC-PAGE).

2. MATERIALS AND METHODS

The sample was taken from the A horizon of a chernozem soil (Kursk region, central European section of Russia). Methods for humic acid extraction, purification and fractionation by SEC-PAGE was previously reported (1). Three fractions A, B, and C+D with different MS and EM were obtained. To investigate the effect of urea treatment, unfractionated HA was dissolved in 7M urea for two days, then dialyzed against distilled water and lyophilized. ^{1}H-NMR spectra was made on a Varian UNITY plus spectrometer, solid-state ^{13}C-NMR spectra - on a 400 MHz Bruker AVANCE II spectrometer.

3. RESULTS AND DISCUSSION

The PAGE analysis of the whole HA and each individual fraction A, B, and C+D, obtained by tandem SEC-PAGE, is shown in Figure 1. On the basis of fraction elution volumes during chromatography we suggest that the MS of electrophoretic fractions are in decreasing order A > B > C+D.

The ^{1}H-NMR spectra of the chernozem bulk HA in D_2O and DMSO-d_6 are strongly influenced by the solvent used (Figures 2 and 3). While ^{1}H-NMR spectra of bulk HA and HA treated by urea (HAU) in D_2O have no any differences (Figure 2), a major contrast is observed between ^{1}H-NMR spectra of these samples in DMSO-d_6 solvent (Figure 3). The HAU reveals a significantly higher proportion of an overlapping group of peaks between 6.9-7.4 ppm, corresponded to exchangeable groups like phenolic, carboxylic and amidic. We suggest that the increased intensity for these signals is due to the rupture of H-bonds and creation of additional exchangeable sites in HA after urea treatment. Addition of a small amount of D_2O to any investigated samples dissolved in DMSO-d_6 induced complete lose of the signals between 6.9 and 7.4 ppm, indicating that these signals are from the exchangeable hydrogens.

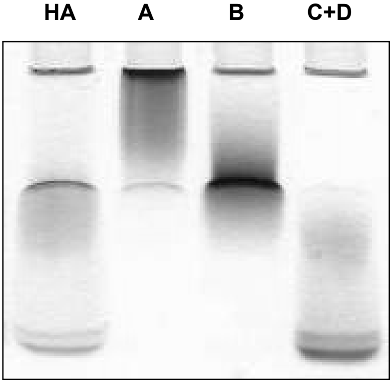

Figure 1. Electrophoresis of the chernozem HA and fractions A, B, C+D.

When comparing the ^{1}H-NMR spectra in D_2O of fractions A, B and C+D, there are some noteworthy differences. There is relatively higher amounts of aromatic ^{1}H's, denoted by the broad band between 6.5 and 9.0 ppm, in fraction C+D. In this fraction C+D there is proportionally less in the resonance range 3.5 to 4.5 ppm (carbohydrates, proteins) than in fractions A and B. Fraction A is more enriched in signals for polymethylenic structures (1.3 ppm) than the other two fractions.

The spectrum of fraction B in DMSO-d_6 shows appearance of sharp signal at 7.4 ppm with shoulders in the region of 7.3-6.9 ppm. The spectrum of fraction C+D in DMSO-d_6 demonstrate high intensity of peaks in region 6.9-7.3, but sharp peak at 7.4 ppm is absent. We could assume that exchangeable groups in fraction B differ greatly from exchangeable groups of fraction C+D. The fraction A was not dissolved in DMSO-d_6 and did not analyze in this solvent.

Solid-state ^{13}C-NMR spectra of the bulk HA and HAU exhibit common features for soil HAs (2), and the ratio between different peaks change dramatically from fraction A to C+D (Figure 4, Table 1). This suggests that there is significant heterogeneity of molecular structure and functionality among the various fractions, which differ in both EM and MS. The spectrum of fraction A is dominated by H,C-alkyl and O,N-alkyl carbon. The percentage of aromatic carbon (108-165 ppm) drastically decreases in fraction A in comparison with the HA sample. In fraction B, the percentage of both H,C-alkyl and O,N-alkyl carbon essentially decrease in comparison with fraction A, but is nevertheless more than in the unfractionated HA. The aromatic character of HA becomes pronounced in fraction C+D, where the O-alkyl carbon peak at 100 ppm disappears and alkyl carbons between at 0 and 50 ppm are considerably less intense than in the unfractionated HA, HAU, and fractions A and B.

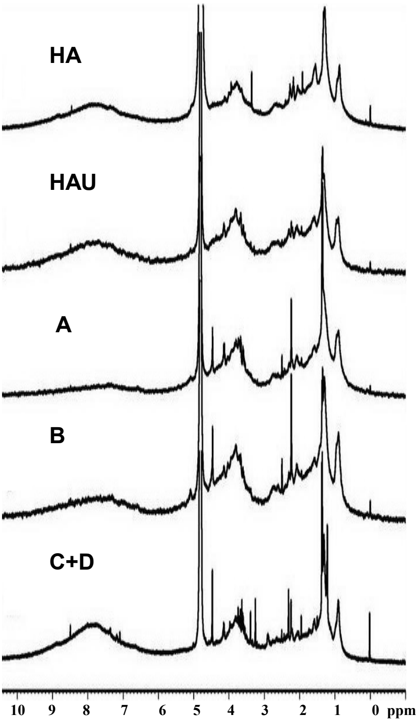

Figure 2. ^{1}H-NMR spectrum in 0.1N NAOH/D_2O of chernozem HA, HAU and electrophoretic fractions A, B and C+D (2.5 mg/ml). The peak at 4.8 ppm corresponds to the traces of water.

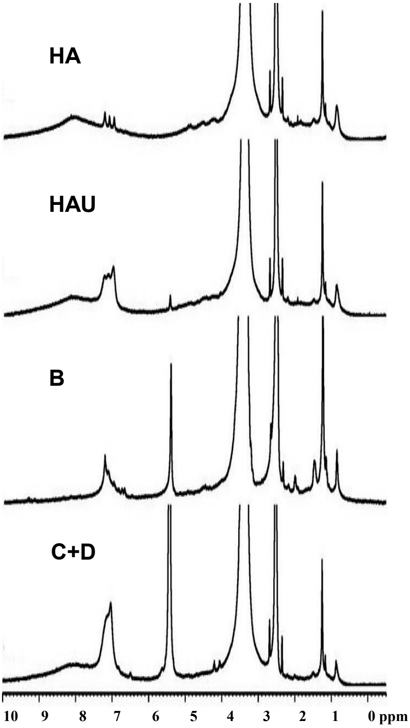

Figure 3. ^{1}H-NMR spectrum in DMSO-d_6 of chernozem HA, HAU and electrophoretic fractions B and C+D (2.5 mg/ml). The peaks at 2.6, 3.4, and 5.5 ppm correspond to DMSO, the traces of water, and urea, respectively.

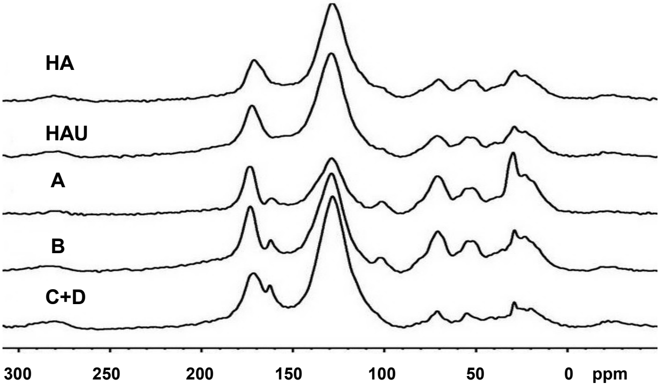

Figure 4. Solid-state ^{13}C-NMR spectrum of chernozem HA, HAU and electrophoretic fractions A, B and C+D (about 80mg of each sample used for analysis). A distinct peak at 161 ppm correspond to urea.

Table 1. Percentage of carbon in the main structural fragments of the chernozem HA and its electrophoretic fractions A, B and C+D

Sample	$C_{C=O}$ 220-187	$C_{COO,CON}$ 187-165	$C_{Ar-O,N}$ 165-145	$C_{Ar-H,C}$ 145-108	$C_{Alk-O,N}$ 108-50	$C_{Alk-H,C}$ 50-0	C_{Alk}/C_{Ar} 0-108/108-165
HA	3.4	10.3	7.5	48.7	13.4	16.7	0.5
HAU	3.8	12.1	8.2	48.0	12.7	15.2	0.5
A	3.6	10.9	5.8	28.3	23.8	27.6	1.5
B	5.6	13.1	8.0	37.6	17.8	17.9	0.8
C+D	3.9	14.2	9.2	53.5	7.6	11.6	0.3

4. CONCLUSIONS

The ^{1}H-NMR spectra in D_2O and DMSO-d_6, and solid ^{13}C-NMR spectra of chernozem HA and its fractions, obtained by combined SEC-PAGE, show significant differences in chemical structure of fractions with different MS and EM. Low MS fraction C+D 5 times considerably more aromatic than high MS fraction A.

ACKNOWLEDGEMENTS

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Comparison of Humic Acid and Metal Salt Interactions in Capillary Electrophoresis

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Keywords: capillary electrophoresis; electrophoretically mediated microanalysis; humic acid

1. INTRODUCTION

The complicated chemical structure of humic acids (HA) depends on their source. Due to the polyfunctionality and the zwitterionic character of humic substances (HS) allows the interaction of anions with positively charged groups of humics and cations with negative charged groups of these substances (1). In addition, they tend to form aggregates. The formation of aggregates is a highly metal dependent process (2).

Recently we have found that electrophoretically mediated microanalysis (EMMA) offers a chance to follow the chemical reactions of Pb^{2+} and fulvic acid ions inside capillary (3). In order to minimize the HS sorption effect on the uncoated capillary, HS diluted solutions were used as background electrolyte (BGE) (3, 4). In the present study, differences in the interaction of metal salts with standard HA (sHA) and natural HA (nHA) from the Baltic Sea sediment were investigated, using those solutions as BGE, in order to find possibilities to characterize the binding sites of HA.

2. MATERIAL AND METHODS

Standard HA from Fluka and analytical grade reagents were used. Natural HA was extracted from the sediment of the Baltic Sea (Haapsalu Bay). Separation procedures are described in a previous paper by Übner et al. (5). The concentration of HA in BGE was 0.1 mg/ml and pH was adjusted to pH = 7 (Na-salt form). Metal salt solutions were prepared in the range 1×10^{-4} M to 4×10^{-4} M. All the CE experiments were performed with an ISCO CV^4 Capillary Electropherograph model 3850, which was thermostated with forced air by a fan. The bare fused-silica capillary 75 µm I.D (Polymicro Technologies, Phoenix, AZ, USA) had a total length of 80 cm (48 cm to the detector). Procedure is described in our previous work (3, 6).

The velocity of electroosmotic flow (EOF) was estimated by the use of nitromethane as a marker compound. The calculated EOF mobility value for sHA was $(1.24 \pm 0.01) \times 10^{-3}$

cm^2/V s and for nHA was $(1.36 \pm 0.05) \times 10^{-3}$ cm^2/V s. All experiments were done in triplicate and perform at room temperature.

3. RESULTS AND DISCUSSIONS

Natural and standard HA solutions with two metal salts solution, $Pb(NO_3)_2$ and $NaNO_3$, form characteristic reproducible electropherograms (Figure 1).

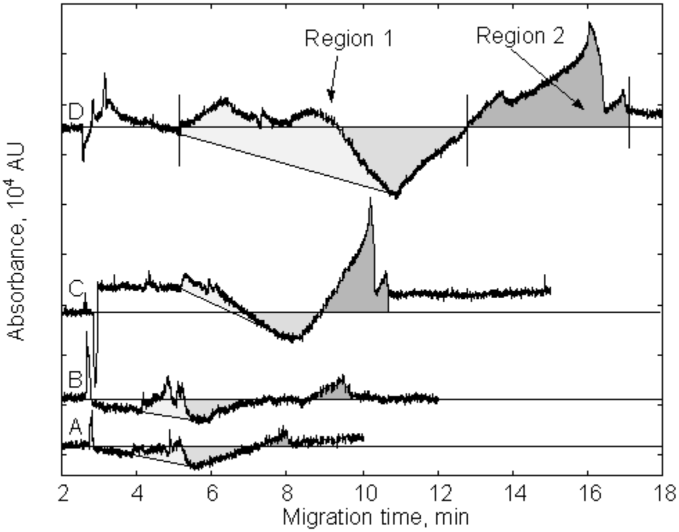

Figure 1. Electropherograms: A – $3 \cdot 10^{-4}$M $NaNO_3$, nHA as BGE; B – $3 \cdot 10^{-4}$M $Pb(NO_3)_2$, nHA as BGE; C – $3 \cdot 10^{-4}$M $NaNO_3$, sHA as BGE; D – $3 \cdot 10^{-4}$M $Pb(NO_3)_2$, sHA as BGE. Separation voltage 20 kV; hydrodynamic injection, 15 s; detection at 226 nm; fused-silica capillary 80 cm × 75 µm.

All electropherograms can be divided into two main regions: 1 – broad negative peak with positive hump on that; 2 – a wide triangular-shape peak. Two humps in region 1 and 2 indicate the formation of at least two different types. Areas of the regions 1 and 2 were calculated.

Area of the whole hump in region 1 (Figure 2) showed good correlation with inserted $Pb(NO_3)_2$ for sHA and for nHA ($R^2 = 0.9807$; $R^2 = 0.9580$, correspondingly). Natural HA do not have linear correlation and the area value have maximum. The slope value is 8.1 times more for sHA. Whole hump of $NaNO_3$ with sHA and nHA showed linear correlation ($R^2 = 0.8246$; $R^2 = 0.9626$, correspondingly) in region 1. The slope values are 1.6 times more for sHA.

The negative area after the hump in region 1 should express the vacancy of charged particles, which is formed by removing HA anions from the reaction zone in both directions – towards the hump in region 1 and in region 2. Area values of that negative area are correlated with inserted metal salt (Figure 3). Natural HA has non-linear correlation with inserted $Pb(NO_3)_2$ and $NaNO_3$ ($R^2 = 0.8078$; $R^2 = 0.8771$, correspondingly). Standard HA has linear correlation with inserted $Pb(NO_3)_2$ and $NaNO_3$ ($R^2 = 0.9895$; $R^2 = 0.9852$, correspondingly).

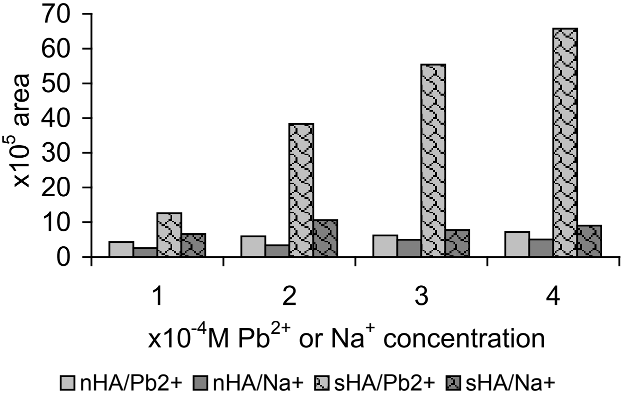

Figure 2. Dependence of the area of whole hump in region 1 on the concentration of inserted $Pb(NO_3)_2$ or $NaNO_3$ in different background electrolytes, nHA or sHA.

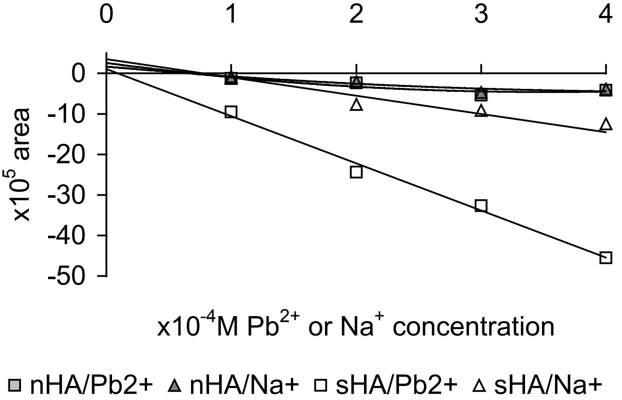

Figure 3. Dependence of the negative area after the hump in region 1 on the concentration of inserted $Pb(NO_3)_2$ or $NaNO_3$ in different background electrolytes, nHA or sHA.

Area of region 2 in both cases showed linear correlation with the injected metal salt. Standard HA has more functional groups that may interact with different ions than natural HA from Baltic Sea. Estimating the slope value for $Pb(NO_3)_2$, it is 14.5 times more for sHA and for $NaNO_3$ it is 1.3 times more for sHA than nHA.

Our previous data showed that nHA had more aliphatic structure than sHA. So, the formation of inner sphere complexes turn outside long carbon chains and make the natural HA molecule with lead more hydrophobic. Standard HA has more carboxyl structures than nHA (Table 1) because the content of oxygen is 1.7 times higher.

Table 1. Elementary composition of standard and natural HA (5, 7)

Humic substances	Ash	C	H	N	O	[H]/[C]	[O]/[C]	[N]/[C]
sHA	20%	35.7%	3.3%	0.6%	39.6%	1,11	0.83	0.01
nHA	33%	33.7%	5.0%	4.8%	23.4%	1.79	0.52	0.12

Slope values in region 1 and 2 in both HA and metal salts and our previous data showed that Pb^{2+} prefer to form fast moving complexes with HA. It is related to the highest content of carboxyl groups in region 1. Region 2 is more complicated and affected with NO_3^-.

4. CONCLUSIONS

On-column complexation of natural and standard HA with two different metal salts characterize the HA fractions. Standard HA contains more carboxylic groups and binds more Pb^{2+} than natural HA. Nitrate anion affects the formation of second type complexes in the region 2. This approach may use in studying the structure of different HS fractions.

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Comparison of Mumijo (Shilajit) and Humic Acids (HA) Chemical Composition Using FTICR Mass-Spectrometry

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Keywords: humic, mumijo, mumie, shilajit, FTICR mass-spectrometry

1. INTRODUCTION

Humic substances (HS) are complex mixtures formed during decay of plant and animal materials. HS are generically classified into fulvic and humic acid fractions according to their solubility properties. The particular features of HS are polydispersity and extreme structural heterogeneity. The biological effects of humic substances can be different, depending on their chemical structure and physicochemical properties. Chemical composition, structure, and functional groups can vary greatly, depending on the origin and age of the humic substance and the conditions of the humification process (humidity, aeration, temperature, mineral microenvironment, etc.) (1). Mumijo has been used in traditional medicine for thousands of years, but there are only a few research devoted to the investigation of humic substances from mumijo.

2. MATERIALS AND METHODS

We used well characterized samples of Suwannee River fulvic and humic acids of the International Humic Substances Society (IHSS). Kyrgyz mumijo from drugstore were used for analysis without further purification. All experiments were performed on a commercial mass-spectrometer 7 Tesla Finnigan LTQ FT (Thermo Electron, Bremen, Germany) equipped with electrospray ion source (Finnigan Ion Max Source). Mumijo samples were dissolved in 1:4 water-acetonitrile solution and analyzed using electrospray ionization both in positive and negative modes. For accurate molecular mass measurements FTICR mass spectra were acquired using selected ion monitoring (SIM) scanning with 100 Da mass range.

For interpretation of FTICR data Kendrick and van Krevelen diagrams were used (2). All FTICR mass spectra were also processed using FIRAN software for determination of stoichometric formulas (2).

3. RESULTS AND DISCUSSION

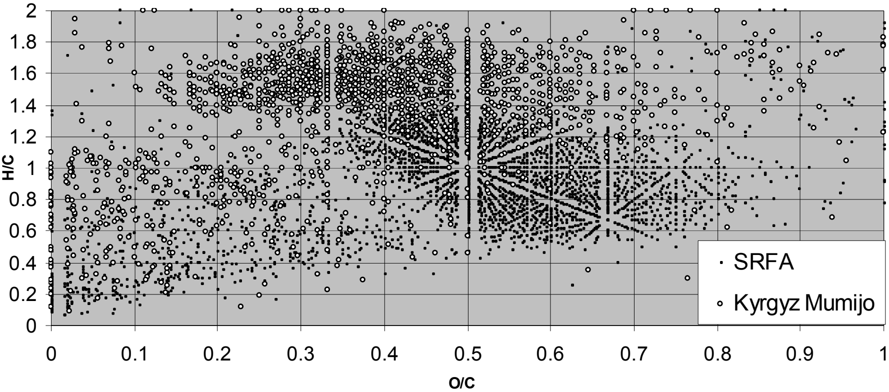

Figure 1 Van Kleveren diagram for kyrgyz mumijo and Suwannee River fulvic acid (for comparison).

Comparison of kyrgyz mumijo with fulvic acid (FA) shown on figure 1. It was found both mumijo and fulvic acid contain a lot of ions tending to form groups in certain region of van Krevelen diagram. This may be interpreted like these substances were formed in stochastic synthesis process and consists of molecules which are close one to each other in their functional composition and differ only by simple structural modifications (e.g., carbon chain length, a number of double bonds, oxidation level). Kyrgyz mumijo seems to differ from humic substances by higher H/C ratio and lower O/C ratio.

4. CONCLUSIONS

Comparison of mumijo composition determined by mass spectrometry with composition of humic substances gives advance in understanding of mumijo origin and its relation with humic substances. Both of these substances seems to be formed from organic remains in slightly different conditions, so, processes which take place during mumijo formation may be very similar to processes of HS formation.

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Solid Phase Micro Extraction (SPME): A Powerful Tool for Complex Natural Organic Matter Investigation

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Keywords: SPME, chemical activity, biological availability

1. INTRODUCTION

Humic acid is a generic term for extremely numerous chemical species of complex organic compounds resulting from natural decomposition processes of organic matter. They are present in soils and natural waters and play an essential role in the matter transfer mechanisms between living organisms and their terrestrial environment. As a consequence of the complexity of the naturally occurring fulvic acid, humic acid and humin mixtures, individual qualitative and quantitative determination of all occurring species generally does not provide any relevant information about the actual properties with regard to their capability of storing or chelating either useful of harmful species. It seems much more important to get a good description of the overall properties of the actual mixture of humic acids as occurring in each case. As a consequence of the wide range of molecular weights, increasing from fulvic acids over humic acids to humins by a factor higher than 10^3, the variations of the proportions of these constituents in each sample will induce very different physico-chemical behaviours with regard to their interaction with living organisms.

Solid Phase Micro Extraction is a technique capable of giving much information about the actual state of the species present in complex natural media. It is based on the preferential adsorption of some species on a well-chosen specific substrate. The substrate is generally a small surface coated fibre. The surface affinity of the fibre for various chemicals depends on the surface coating. Ion exchangers are more selective for mineral species while grafted surface functions render the fibre organic matter specific.

Humic acids can transfer both organic matter and mineral elements to living species. Although chelating mineral elements is an interesting property of humic acids, this contribution will more focus on organic matter behaviour, although this latter will have important consequences on the behaviour of mineral species. The aim was to characterize the physico-chemical interaction of dissolved species in complex aqueous media at a solid interface, in order to enhance the understanding of the way living organisms can extract matter from natural humic substrates. The aim was *not* to characterize the individual products, but to pay attention to their *relative* behaviour, that is to analyse how the presence of some species can modify the behaviour of others. In order to overcome the complexity of natural humic acids, we used as models some well characterised biomarkers such as squalene, selected for their easy identification and their high stability in natural environment.

2. MATERIALS AND METHODS

The study was carried out by means of an SPME extraction and injection device as designed by VARIAN, coupled to capillary gas chromatography fitted with a mass spectrometer. The complete experimental device as modified in order to fit with our experimental requirements has been thoroughly described in (1), with special attention to the thermal desorption process in the injection head of the chromatograph.

The principle of the technique is the following: an adsorption fibre (about 1 cm long and 1 mm in diameter) is exposed to the sample solution for variable periods of time.

The dissolved species present in the analyte will diffuse towards the fibre surface resulting in adsorption processes. The adsorbed amount of each species on the fibre surface at the end of the exposure time depends on three main parameters:

– the diffusion rates of the analytes from the solution towards the fibre surface
– the thermodynamic stability of the adsorbates on the fibre surface
– the exposure time of the fibre surface to the analyte.

These processes are the same as those occurring in natural environment between living organisms and the aqueous environment to which they are exposed in humid soils or in natural waters.

The fibre is then transferred to the chromatograph injection head, where the adsorbed species are thermally desorbed, separated by the column and characterizer by mass spectrometry.

In this work, we just will depict some particular aspects of the chemical physics describing the organic species in natural environment which must be taken into account in order to understand properly why slight changes in the composition of a fertilizer can affect on a huge manner its biological effects.

Separation of the adsorbed species has been carried out by thermal desorption of the analytes, followed by GPC separation and MS characterization. In this work, squalene, a stable naturally occurring biomarker, has been used as a test molecule, and has been quantitatively introduced to water samples after being dissolved in acetone.

3. RESULTS AND DISCUSSION

Our first study concerns squalene, which is a hydrophobic lipidic species. In waters without other organic content, it is extremely strongly adsorbed on the SPME fibre surface. The adsorbed amount depends on the fibre exposure time, on the sample size and on the initial sample concentration. This can be related to the hydrophobic character of the test molecule, strongly rejected by pure waters such as spring waters. Thus, such hydrophobic molecules will undergo exogenous nucleation and droplet growth on interfacial surfaces. At very low concentrations, (typically below one ppm) nucleation cannot occur in homogeneous solutions as a consequence of the kinetic limiting factor. Since in natural environment (especially in mountain lake waters), large volumes of water can be far from any solid surface, some hydrophobic species can be present as metastable supersaturated aqueous solutions (2) due to supersaturation at low concentration levels. They will adsorb strongly but with low kinetic rates on any solid surface such as, for instance, living cell membranes. This phenomenon is clearly shown by the huge adsorption peak observed on figure 1 for water containing 1 ppm squalene.

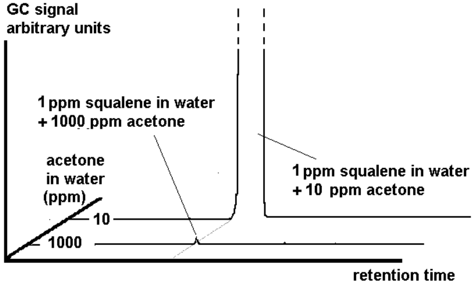

Figure 1. Influence of the presence of acetone in water on the adsorbed amount of squalene.

The same water to which 1000 parts per million (0,1%) by weight acetone have been added results in an extremely weak adsorption amount for squalene on the fibre, showing clearly the lowering of the chemical potential of the squalene in water, due to the presence of a ketone function on an aliphatic propane substrate (amphiphilic character of acetone). Such an interaction is very similar to those resulting from the polar functions of humic acids on hydrocarbon-like molecular skeletons. It clearly shows by which means humic acids can protect living organisms from cell membrane hydrophobic adsorptive coating. Thus, they enhance the cell membrane exchange capability with its aqueous environment. Further, by the variety of chemical sites (hydrophobic, hydrophilic and even polar ionic sites) humic acid molecules can provide, nearly all minor species present in humic aqueous solutions will be fixed on a reversible manner by the humic substrate. Only a small amount of each type of molecule will remain free in the aqueous phase. Due to the thermodynamic equilibrium involving the free species and their humic complex, the former will not predominate so as to completely block the exchange possibilities with cell membranes. Figure 2 shows (for simplicity reasons) just part of the chromatogram of the adsorbed species of a shallow natural lake water containing humic acid, for various exposure times : the two left side lines correspond to kinetic species, with high diffusion rates but weak adsorption equilibrium constant. The three middle lines, continuously increasing with time, correspond to low concentration species, with low diffusion rates but high adsorption constants. The right hand line corresponds to an intermediate species, the surface concentration of which is nearly time independent.

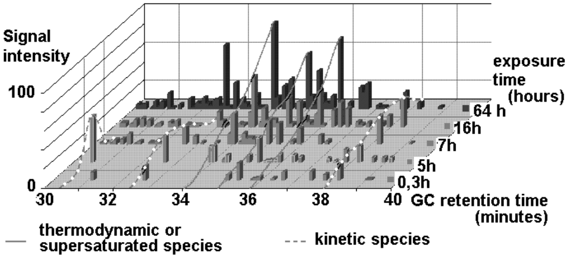

Figure 2. Compared adsorption amounts of organic components as a function of exposure time.

4. CONCLUSIONS

The SPME analysis shows how humic acids can enhance natural equilibria between the numerous species occurring in natural environment, preventing some of them to become predominant during their interaction with living cells. The interactions ranging over an extremely wide range of properties due to the variability of functions that humic acids can provide (aliphatic, aromatic, alcoholic, ketonic, acidic…), most of the existing species can be stored by them under a form exhibiting medium range chemical potentials. In other words, humic acids behave as a storage tank that maintain constant the low concentration level of all species the living organisms need. They thereby prevent the cell surfaces from complete coating by a predominant species.

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Section II

Natural organic matter and humic substances as markers of climate change and anthropogenic repercussions

Evolution of Soil Humic Substances

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Keywords: humic substances, evolution, ^{13}C NMR, anthropogenically disturbed soils

1. INTRODUCTION

Humic substances (HS) is an absolutely special class of natural compounds which, as V. I. Vernadsky has told, are formed both under the influence of abiotic factors and processes, and with direct participation of living organisms.

HS are obligatory participants of not only terrestrial and water ecosystems in small biological circle, but also and in the big geological circle at a level biosphere and geosphere as a single whole. Although quantitatively they compose a little more than half of soil organic matter content in soils and ground waters, their role in functioning of containing them ecosystems immeasurably exceeds their quantitative participation.

HS are the unique natural accumulator of the biochemical energy created by green plants during the uptake the of Sun energy due to photosynthesis. They are not only an universal depot of carbon and energy, but possess also a big biological potential that makes them an analogue of ATP for biosphere. HS cover a contact surface of soil minerals with the environment and thus act as a chemically active film which defines the character of interphase interactions in top the most chemically active horizons of a soil profile. Actually HS are the original biochemical mediators with a help of which a contact of an alive and inert material, bio- and lithosphere is realized in a soil profile. In this connection the problem of estimation of evolutionary trends of HS is important not only for soil science, but also for a lot of related subjects: biology, geology, etc.

HS are presented in biosphere not only in concentrated state (soil humic horizons, peat, etc.) but also in an dissipated state (the bottom soil horizons, surface and ground waters, etc.).

For an adequate estimation of HS evolution an improvement of methodological approaches to their studying is necessary. Unfortunately, many traditional methodical approaches which have been developed in soil science connected with the extraction methods do not quite correspond to a modern level of methodology of the biochemical researches, one of which important bases is using of not destroying methods. They allow to study either completely native forms of substances in points of their localization, or to investigate minimally altered extracted forms.

Many methods of soil chemistry (such as, for example, the analysis of humic fractions) in many respects have depleted their opportunities (2, 3), whether a lot of widely used physical and chemical methods of HS structural investigations, appeared to be either difficult to use, or indirect and difficultly interpreted. Therefore in studying of HS transformation researchers often face the hardly comparable, and often also contradictory data.

Thus, the new level of methodical maintenance of soil HS studying should be connected, firstly, with the use of direct methods of studying of HS molecular structure and functional properties, and secondly, with studying of HS in native state directly in a soil sample at various levels of their structural organization – from soil horizon up to humic acids macromolecules.

2. MATERIALS AND METHODS

We carried out researches of soils HS structurally functional parameters in soils of natural climatic belts from taiga up to a forest-steppe and steppe. Investigations have been compiled both in natural, and antropogenically altered soils - arable and buried. The data about HS molecular structure obtained by 13C-NMR and ESR) and the results of studying the important functional parameters of HS (1, 3), allow to formulate the basic stages of their evolution in soils.

3. RESULTS AND DISCUSSION

The first stage is the formation of an original matrix which consists from rather low-molecular organic ligands, occupying the strongest complexing positions on a surface of a mineral particles. These fractions are present not only in a soil profile, but frequently also in top subsoil layers of parent materials, getting there with soil solutions. Very small on weight from 0,05 up to 0,3% C (from soil weight) these compounds are rather inert. However the following strengthening and completion of high-molecular HS during humus formation take place namely on this matrix.

The second stage is the formation of soil HS system. The basic backbone of their macromolecules is rather closely connected with soil mineral part, both through the matrix mentioned above, and due to new organic-mineral bonds formed during humus formation and distribution of humic film on a surface of mineral particles. The peripheral part of these biochemical active macromolecules possesses a lot of free radicals and high physiological activity.

It is exposed to constant biotical attack and stays in dynamic balance with prehumic compounds of soil solution through the mechanism of constant fragmentary updating. There is a loss of the certain part of the open peripheral chains and heteroatomic fragments of HS, and the tendency to increase in HS molecular weight is observed.

The further development of soil HS system depends in a strong degree on volume and character fresh organic residues obtained. If the hydrothermal conditions of humification and the quantity of these residues is enough for humic-accumulative process, then a gradual escalating of spatial "skeleton" of humic acids macromolecules occur, that leads to increase in thikness of humic coating (“raincoat”) on a surface of mineral particles.

The third stage of HS evolution is connected with processes of the further transformation and humification the frame structure of "raincoat" in a direction of selection of biothermodynamically steady products. These processes are especially accelerated in conditions of sharp deficiency of fresh residues and lead to strong polycondensation of macromolecules, formation of polyaromatic systems, growth of interface to simultaneous increase in quantity free electrons. Formation and relative accumulation of such polycondensed structures of coal type was observed by us at studying spectra ^{13}C-NMR humic acids in arable and buried soils (3). For structures of such type appreciable decrease in biochemical and physiological activity is tipical.

It is possible to draw a conclusion, that at this evolutionary stage properties of HS are determined not so much by functional groups, but the general features of redistribution of electronic density in molecular structure of HS.

4. CONCLUSIONS

On the basis of the analysis of processes of evolution and definition of a level of HS biothermodynamic stability it is possible to create correct forecasts of their behavior during the change of biohydrothermal conditions of humification which can be result of change of land use or global climate change is the latter is especially important, as the contribution of a soil HS mineralization in CO_2 emission in atmosphere is great enough to influence the fate of climate of our planet.

5. ACKNOWLEDGMENTS

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Humus and the Birth of Pedology : Müller, Dokuchaev and Successors

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Keywords: humus history, pedology, Müller, Dokuchaev

AN OVERVIEW

The origin of Pedology is generally linked with Dokuchaev (1) and with his famous work on Tchernozem published in 1883. Nevertheless, the book of the Danish forester Müller, “Natural forms of humus” (2) based on two articles published in 1879 and 1884 is not widely known though translated into German in 1887 and into French in 1889 (3) . This book demonstrates that, as early as 1879, Müller adopted a sincere pedological approach to soil formation and soil-plant relationships. Field observations and Müller’s thinking essentially focused on soils under natural vegetation in Denmark. His book deals with important processes such as brunification, leaching and podzolization, using a dynamic approach (transition from a brown soil to a podzol – Fig. 14 and dating using archaeological remains). All his reasoning was based on the nature of humus, with a special focus on morphology, biological activity (fauna and fungi), and biochemistry. This book displays numerous soil profiles, with much information being synthesized in Table III, which is extraordinarily informative and comprehensive. Although published in the 1880’s, the book can be read today as an almost modern text. We therefore suggest that Müller has been the most important scientist formulating early to modern Pedology. On the other hand the Chernozem’s classification and distribution proposed by Dokuchaev in his famous thesis is based on humus content with the famous isohumus belt. A conflicting debate about the role of climate in comparison with other pedogenesis factors on the accumulation of humus in Russian soils occurred between Dokuchaev and Kostychev early in 1889 (4) and 1890 (5). The theories of Kostychev were reported in French by Feltz in 1887. But, finally, from the time of birth of pedology, humus was in the centre of concepts of pedogenesis and soil classifications. However, the humus quality is rarely taken into consideration in the Dokuchaev’s thesis and generally in the Russian school before the First World War and the Soviet Revolution with the exception of some works between 1893 and 1900 i.e. Kozlovskii, Lesnievskii and Naletov. These three authors enhanced the great differences between Chernoziemic and Podzolic humus and established negative correlations between humus content and humus solubility.

The Dokuchaev pupils, in the early 1900's, such as Sibirtsev (6), and the Russian school of Pedology will be later largely involved in Humus research. One of the earliest and historically important is Tyurin. In its famous 1937’s book (7) Tyurin’s tried to collect all the available present world knowledge on soil organic matter (SOM) with citations of more than 210 papers in Russian and 500 in other languages including Müller.

More recently one have to pay tribute too to Kononova (8) and Orlov (9, 10) for their important works on SOM and SOM history.

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Freshwaters: Do ‘Humics’ Always Represent 50% of NOM?

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Keywords: freshwaters, fulvic, humic, lakes, quantification, rivers

1. INTRODUCTION

Many articles devoted to the study of different aspects of natural organic matter (NOM) begin by matter-of-factly stating that “humic substances are the largest fraction of natural organic matter in water” or that they “represent 50% of natural organic matter”. In practice, however, the refractory fraction of organic matter (ROM), usually referred to as humic and fulvic acids, has rarely been quantified since the necessary analytical methods are lacking. Spectrophotometry and fluorimetry have often been used for this purpose but it should not be forgotten that these methods measure only a fraction of ROM and that they provide results that simultaneously reflect the type of ROM and its concentration. The common practice of normalising the signals by the concentration of dissolved organic carbon (DOC) gives an indication of the type of ROM but does not measure its concentration. A few attempts have been made at quantifying ROM after isolation with the resins traditionally used to define humic and fulvic fractions but the procedure is very long and cumbersome and not free of problems (because the resin method was developed as a preparative procedure and as such does not necessarily ensure a quantitative recovery). Recently, two new methods (1, 2) have been claimed to be faster. However, these can only be applied to waters containing high concentrations of ROM (more than 5 $mg.L^{-1}$ in (1) and 50 $mg.L^{-1}$ in (2)) which, in practice, means they are not applicable to most surface waters. In this paper, the results have been obtained by applying a fast, simple electroanalytical procedure based on cathodic stripping preceded by the adsorptive collection of Mo(VI)-humic or fulvic acids complexes (3) to a myriad of freshwater systems. These show that the method is particularly suited to systems containing low amounts of carbon. Application of this method may prove extremely useful across a broad range of fields, including geochemical modelling, climate change-related studies, etc.

2. MATERIALS AND METHODS

Lake water samples were collected using a membrane pump, with the end of the tube directly attached to a CDD probe. River water samples were collected directly into bottles. Samples for organic carbon (OC) determination were collected in precombusted (3 h at 550°C) glass bottles. Samples for ROM analysis were collected in polyethylene bottles. All polyethylene bottles had been cleaned with nitric acid (10%) and 0.5 mol.L^{-1} sodium hydroxide, and rinsed with Milli-Q water. Immediately after collection, all samples were filtered through precombusted (3 h at 550°C) 1.2 μm glass filters (Whatman GF/C filters) by vacuum filtration and acidified to pH 2 with HCl Suprapur.

The electroanalytical procedure for ROM quantification is based on cathodic stripping preceded by adsorptive collection of Mo(VI)-humic or fulvic acids complexes (3). After the sample was pipetted into the thermostated voltammetric cell, Mo(VI) was added to a final concentration of 10 ppb to ensure formation of the complex. Where necessary, the sample was diluted through the addition of 1x10^{-2} mol.L^{-1} HCl. The solution was then degassed for 10 minutes. The potential was set to –0.2 V while the solution was stirred. After stirring for the deposition time (usually 180 s) it was allowed to rest for 20 s. The scan was then initiated in the negative direction by using DPP or SWV. A standard addition method employing IHSS Suwannee River fulvic acid standard (1S101F) was used for calibration.

DOC was determined using a high temperature combustion method employing a TOC 5000-A Shimadzu analyzer (Shimadzu Co. Ltd.). Milli-Q water was used as blank (0.00 mg.C.L^{-1} with a SD of less than 0.005). Calibration was performed with a 1000 mg.C L^{-1} total DOC standard solution (CertiPUR, Merck). Standard solutions as well as blanks were acidified to pH 2 with HCl Suprapur.

3. RESULTS AND CONCLUSIONS

Table 1 shows ROM concentrations and the corresponding percentage of DOC determined in a range of freshwater bodies of contrasting trophic status at different seasons of the year. The results obtained show that there is a high variability in ROM concentrations in freshwaters, indicating that the widespread assumption that ‘humics’ represent 50% of NOM may prove wrong in most surface waters.

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We would like to thank Joseph Youssef and Dina Andriamahady for field and laboratory assistance.

Table 1. Concentration of ROM (expressed as mg C.L^{-1}) and percentage of DOC (both after filtration at 1.2 μm) for different freshwater systems

System[a]	Depth	Season	Type of system	ROM	% DOC	Ref.
Lake Geneva	surface	early spring	mesotrophic lake	0.26	34	(4)
	epi[b]	spring		0.27	33	
	hypo[b]	spring		0.24	33	
	epi[b]	late spring		0.27	28	
	hypo[b]	late spring		0.25	35	
	surface	summer		0.26	14	
Lake Bret	surface	summer	eutrophic lake	1.12	27	(3)
Lake Gruère	surface	summer	peat lake	12.9[c]	100	(3)
Lake Divonne	surface	spring	eutrophic lake	0.69	17	unp.
Lake Brienz	1 m	June	ultra-oligotrophic lake	0.23	38	(5)
	18 m			0.42	70	
	1 m	September		0.10	29	
	100 m			0.16	27	
	1 m	November		0.15	43	
	100 m			0.13	23	
	1 m	February		0.14	41	
	100 m			0.12	35	
	1 m	March		0.12	18	
	20 m			0.09	11	
	1 m	April		0.18	26	
	20 m			0.14	28	
	1 m	July		0.11	26	
	15 m			0.15	32	
	25 m			0.11	21	
	100 m			0.11	15	
	1 m			0.16	31	
	5 m			0.15	36	
	26 m			0.21	46	
	100 m			0.15	28	
Lake Grimsel	1 m		alpine, ultra-oligotrophic reservoir	0.06	31	unp.
River Allondon (Echenevex)		10 April	karstic spring	0.72	40	unp.
		13 April		0.77	54	
		16 April		0.79	43	
		19 April		0.86	52	
River Allondon (Dardagny)		10 April	weakly anthropized karstic river	0.79	43	unp.
		13 April		0.73	42	
		16 April		1.02	45	
		19 April		0.73	34	
River Allondon (La Plaine		10 April		0.60	31	unp.
		13 April		0.58	30	
		16 April		0.86	45	
		19 April		1.05	50	
Nant d'Asy (Corsier)		spring	urban stream	2.04	35	unp.
Nant d'Avril (Satigny)		spring	semi-urban stream	0.15	18	(3)
hydroelectrical plant outlet (Innertkirchen)		March	water from Lake Grimsel	0.26	55	(6)
		April		0.36	68	
		July		0.05	30	
		October		0.76	85	

System[a]	Depth	Season	Type of system	ROM	% DOC	Ref.
River Aare (Innertkirchen)		March	alpine, modified glacial river	0.58	48	(6)
		April		0.40	46	
		July		0.13	49	
		October		1.01	105	
River Aare (Brienz)		June	alpine, modified glacial river	0.49	52	(3)
		September		0.11	48	
		November		0.10	33	
		February		0.08	25	
		March		0.96	88	
		April		0.64	70	
		July		0.07	32	
		October		0.74	88	
River Lütschine (Gsteig)		June	alpine, unmodified glacial river	0.35	66	(3)
		September		0.11	37	
		November		0.13	20	
		February		0.19	25	
		March		1.43	79	
		April		0.62	62	
		July		0.05	16	
		October		0.44	51	

[a]All systems are located in Switzerland except Lake Divonne and a part of River Allondon, both in France; [b]epi: epilimnion, hypo: hypolimnion; [c]calibration with IHSS peat humic acid (1S103H).

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Atmospheric HULIS: How Humic-Like Are They?

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Keywords: HULIS, atmospheric aerosol, polyacidic, fulvic

1. INTRODUCTION

The last decade has witnessed a large increase in studies reporting the prevalence of macromolecular compounds, termed HUmic-LIke Substances (HULIS) in aerosol, fog and cloud water. Interest is fueled by the realization that HULIS may affect important aerosol properties such as their ability to nucleate cloud droplets or their light absorption. The realization that HULIS compounds are most likely formed in aerosol particles exemplifies the fact that atmospheric reactions can result in formation of new constituents of higher molecular weight, and not just result in breakdown of parent organic compounds.

HULIS have been so termed because of their apparent similarity to the ubiquitous humic substances found in terrestrial and aquatic environments. The purpose of this talk is to introduce HULIS to the humic substances community and to review some of the salient properties of HULIS that make it so interesting to atmospheric scientists, including surface activity and hygroscopic properties. The material presented here is derived mainly from a recent comprehensive review paper; references can be found therein (1).

2. DISCUSSION

UV-VIS spectra of HULIS are featureless, with increasing absorptivity towards shorter wavelengths. In this regard, spectra are similar to typical UV-VIS spectra of humic substances, although commonly, spectra of HULIS display relatively more absorbance in the shorter wavelength regions, and less in the longer wavelength regions as compared with terrestrial and aquatic humic substances. Excitation-emission fluorescence spectra for HULIS have peaks at shorter excitation and emission wavelengths than freshwater or terrestrial fulvic acids, suggesting a lower content of aromatic structures and condensed unsaturated bond systems, a higher aliphatic moiety content, and a lower molecular weight (MW). FTIR spectra of HULIS exhibit a broad band at 3400 cm^{-1}, a shoulder in the region of 3000-2850 cm^{-1}, a strong band near 1720 cm^{-1}, a weak band in the 1600-1660 cm^{-1}

region, absorption at 1384 cm^{-1}, a band in the 1220 cm^{-1} region, and C-O stretching band at 1061 cm^{-1}. ^1H-NMR spectra HULIS display a pronounced aromatic region and relatively low abundance of non-exchangeable organic protons. Based on the ^1H-NMR features, a model structure consisting of an aromatic core bearing substituted aliphatic chains with –COOH, $-CH_2OH$, $-COCH_3$, or $-CH_3$ terminals groups was suggested for HULIS. Such a structure is also consistent with electrospray ionization mass spectra and IR spectroscopy. ^{13}C-NMR spectra for HULIS showed a broad range of un-substituted saturated aliphatic components, aliphatic carbons singly bound to one oxygen or nitrogen atom, aliphatic carbons singly bound to two oxygen atoms, and ester and carboxyl carbons. ^{13}C-NMR spectra for biomass burning HULIS and urban aerosol HULIS were both found to have a substantial aliphatic component, and to be quite different in relative carbon distribution from humic acid. Weight average MW (MW_W) of HULIS was estimated by liquid chromatography and electrospray ionization (ESI) tandem quadrupole mass spectrometry in negative ionization mode to range between about 200 to 300 Da. This can be compared to an estimated average MW_W of 456 Da for Suwannee River fulvic acid via the same technique.

Average elemental composition (m/m%) of HULIS from rural fine aerosol particles was reported to be 52% C, 6.2 % H, 2.5 % N, and 39 % O, giving an average organic matter mass to organic carbon ratio of 1.93, an average O/C molar ratio of 0.58, and an average H/C molar ratio of 1.42. While reported O/C molar ratios are similar to those for fulvic acids, the H/C molar ratios are substantially higher, for example, molar ratios for Suwannee River fulvic acid (O/C 0.60; H/C 0.99) and Nordic Lake fulvic acid (O/C 0.65; H/C 0.91). The elemental analysis of HULIS indicates a predominance of oxygenated functional groups and the presence of saturated systems in excess of that for aquatic fulvic acids. This corresponds to spectral results (UV-VIS, fluorescence, ^{13}C-NMR, ^1H-NMR) that indicate a relatively lower aromatic content for HULIS as compared with fulvic and humic acids. Based on elemental and spectroscopic results, it was concluded that HULIS consists of poly-functional compounds bearing poly-conjugated structural elements, and carrying polar groups such as carboxyl, hydroxyl and carbonyl.

The surface activity of HULIS samples has been found to exceed that of humic acid and fulvic acid. For example, HULIS derived from biomass burning smoke aerosol particles, slightly aged smoke particles, and daytime urban aerosol particles lowered the surface tension of water more than did Suwannee River fulvic acid. Therefore, HULIS could enhance cloud droplet activation by depressing surface tension and lowering the critical supersaturation for activation, or alternatively, delay droplet activation due to hindrance of water vapor diffusion through a surface organic film. It was also found that HULIS extracts from ambient aerosol particles are more hygroscopic than fulvic acid and size-fractionated fulvic acid, and that different HULIS samples exhibit varying hygroscopic properties depending on their origin and residence time in the atmosphere.

Potential origins of HULIS in the atmosphere are diverse, including primary terrestrial and marine sources, biomass burning, and secondary organic aerosol formation (condensation, reaction, oligomerization, etc.). Different suggested mechanisms for HULIS generation during biomass burning include: (i) soil-derived humic matter lofted into the air as a result of combustion; (ii) HULIS generation via chemical transformations during combustion and thermal breakdown of plant lignin and cellulose; and (iii) recombination and condensation reactions between volatile, low molecular weight combustion products. Most studies invoke various secondary formation pathways for HULIS, including evaporation, condensation, and aerosol-phase polymerization of polar, low molecular weight organic constituents. These ideas have been supported by laboratory studies that have focused on elucidating photochemical and oxidative processes that could lead to HULIS formation.

Considering the particularly small molecular size attributed to HULIS as compared with fulvic acids and laboratory-generated macromolecular species, can HULIS truly be considered a macromolecular species? This question may be part of a larger issue currently debated in the humic substances literature involving a new model for humic substances structure, whereby they are considered to consist of supramolecular associations rather than macromolecular polymers. If the suprastructure model for terrestrial humic substances is correct, the high mono-and dicarboxylic organic acid content as well as the inorganic acid content of atmospheric aerosols may actually prevent formation of larger humic suprastructures, explaining the observed low molecular weights for HULIS. There are alternative explanations for the relatively small size of atmospheric HULIS. Bearing in mind the reactivity of humic substances in the face of UV radiation, O_3, and hydroxyl radicals, it can be anticipated that conditions in the atmosphere are such that relatively larger macromolecules, even if formed, cannot be long-lived. HULIS, then, may represent some non-equilibrium phase resulting from a quasi-steady state between formation and destruction of larger species. Another alternative to the small size conundrum is that HULIS consists mainly of a complex, unresolved mixture of relatively small molecules rather than a mixture of complex macromolecular entities. The apparent similarity of HULIS to humic substances would then be a fortuitous outcome of the difficulty in comparing spectra of unresolved mixtures of compounds. The evidence for such a claim may lie in the ultrafiltration behavior of HULIS, which has been found to pass in its entirety through ultrafiltration membranes of 500 and 1000 Da. The very different modes of formation and the duration of formation for HULIS as compared with humic terrestrial/aquatic humic substances may also account for the relatively small size of HULIS, as well as many of the other observed differences. Taking into account that the major portion of HULIS is believed to be of secondary atmospheric origin driven by abiotic oxidizing reactions over a short time scale, it should not perhaps come as a surprise that HULIS is smaller and less aromatic than humic substances.

3. CONCLUSIONS

A class of organic molecules extracted from atmospheric aerosol particles and isolated from fog and cloud water has been termed HUmic-LIke Substances (HULIS) due to a certain resemblance to terrestrial and aquatic humic and fulvic acids. While sharing some important features such as polyacidic nature, there are significant dissimilarities between HULIS and fulvic acids, including greater surface activity, greater water sorption capacity, smaller molecular size, lower aromaticity, higher H/C molar ratios, and weaker acidic nature for HULIS. Recently, ultra high resolution mass spectral studies have identified fulvic acid and sulfated and nitrated fulvic acid analogues in atmospheric aerosol (2). Such sulfated and nitrated compounds have not been identified in soil or aquatic environments, supporting a secondary organic aerosol formation mechanism for HULIS. Thus, accumulating evidence suggests that atmospheric HULIS differ substantially from terrestrial and aquatic humic substances.

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Short Time Humification and Mineralisation of Different Pyrogenic Materials under Controlled Laboratory Conditions

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1. INTRODUCTION

Wildfires yield large amounts of CO_2 and NO_x to the atmosphere whereas considerable amounts of severely or partly charred necromass are incorporated into the soil. Due to the highly refractory nature of such thermally-condensed products, they are assumed to play an important carbon (C) sink in the global C cycle. At first sight, this could be considered as a benefit from the viewpoint of mitigating the effects of greenhouse gases from the atmosphere. But assuming that biomass burning occurred at the same rate than nowadays since the last glacial maximum, black carbon and its transformation products should contribute with 25 to 125% to the humic material (1).

The intention of the present study was to elucidate the impact of microbiology activities on the quality and quantity of pyrogenic substances in soils.

2. MATERIALS AND METHODS

We used rye grass (*Lolium perenne*) and pine wood (*Pinus sylvestris*) to produce the pyrogenic organic materials (PyOM). The both plant materials were charred under oxic conditions on a ceramic tray in a muffle furnace at 350°C. Two oxidation times of one (1M) and four minutes (4M) were applied to obtain materials with a different charring degree. The soil matrix for the incubation experiment was obtained from a fire-unaffected Bw horizon of a Cambisol under spruce near Leuk, Canton Valais, Switzerland. The water content of the soil incubate was adjusted to approximately 60% of the maximum water holding capacity and the samples were incubated for 48 d at 30°C under aerobic conditions in a Respicond-apparatus (Nordgren Innovations, Sweden).

3. RESULTS AND DISCUSSION

The grass-derived chars show the highest C mineralisation. During the 48 days of the incubation 3.2% of the OC of Gr1M was converted into CO_2; most of this (> 1 %) occurred during the first 3 days. As indicated by Gr4M, more severe thermal alteration resulted in a decrease of the total C volatilization of approximately 22% to 2.5% of OC, but to a more efficient mineralisation during the first 1.5 days. Consequently, in spite of the higher aromaticity of Gr4M compared to Gr1M, the more severely charred sample contains a higher contribution of labile constituents that are microbiologically available C in particular the beginning of the incubation.

Comparable to the rye grass chars, the pine chars show degreasing mineralisation with increasing burning degree, although the overall mineralisation is much lower. Only 0.66% and 0.46% of the initial C in the charcoals P1M and P4M were mineralised. As for the rye chars, during the first 9 days, a higher microbiological activity resulting in higher mineralisation rates was evidenced for P4M. This confirms that charring yields in pyrogenic substances that are more or less directly available for microbial degradation and the amount of which increases with thermal intensity.

One explanation for the decline of the total mineralisation in the more severely charred materials may be their higher aryl C content (Table 1). However, other parameters must also be involved since Gr1M and P1M have similar aryl ^{13}C intensities, but the effective char mineralisation differs strongly with 3.2% and 0.7%. Even the relatively high O-alky C content of P1M (38%), which is most tentatively attributed to remaining carbohydrate moieties cannot increase the degradation efficiency. Possibly this material is not available for the MO, due to its physical protection within charred domains and/or a low content of available N forms. Note that we added no mineral nutrient solution in order to simulate natural conditions.

The ^{13}C NMR spectroscopic analysis of Gr1M indicates a modification of the chemical composition of the char during the degradation experiment. At the end of the incubation we found a decrease from 27% to 22% in the alkyl-C region (Table 1). This decline corresponds to 5.6% of the bulk char C (Table 2). Gr4M exhibits a comparable consumption of alkyl C. Here its relative contribution to the total ^{13}C intensity decreases from 20 to 16%, which corresponds to 4.5% of bulk char C.

Note that with the exception of P4M the aryl C content shows no major alteration caused by the degradation, but increasing contents of carboxyl/carbonyl C is evidenced for Gr1M, Gr4M and P4M, supporting that oxidation occurred.

For P4M we find a decrease of the intensity in the chemical shift region between 140 to 90 ppm) from 67 to 60%, but concomitantly the intensity in the O-aryl C region increases from 16 to 19%. An explanation for this behaviour may be a modification of the aryl rings by substitution with hydroxyl or carboxyl groups. Considerating both the O-aryl C and aryl

C regions we detected a total reduction of the aromaticity of 3.5%. Since the total C-loss during the experiment comprised only 0.4%, this indicates that some of the aryl C must have been converted into other C groups. As revealed by the increase of carboxyl/carbonyl C by 5.7%, this conversion must include the opening and partial oxidation of aromatic ring structures.

Table 1. Relative intensity distribution (%) in the solid state ^{13}C NMR spectra of the fresh and incubated PyOM. Values are corrected by subtraction of the contributions of organic C in the used soil material

	245-160	160-140	140-90	90-45	45-0
fresh			ppm		
Gr1M	10	11	39	12	27
Gr4M	11	14	49	6	20
P1M	6	13	34	38	8
P4M	8	16	67	5	5
incubated					
Gr1M	13	13	43	9	22
Gr4M	14	16	50	4	16
P1M	6	14	36	36	9
P4M	14	19	60	2	4

Table 2. Changes of the content (%) of the different C groups of the PyOM during the degradation experiment. They were obtained by subtraction the C contents of the degraded from the fresh char, which were calculated by multiplying the relative intensity of the respective chemical shift region with the C concentration of the sample. Thus, positive values signalize neoformation, negative values correspond to losses

	Carbonyl C	O-aryl C	Aryl C	O-alkyl C	Alkyl C	total loss
			% of bulk C			
Gr1M	2.5	1.5	1.8	-3.5	-5.6	-3.2
Gr4M	3.2	0.9	0.1	-2.2	-4.5	-2.5
P1M	0.1	1.2	1.0	-3.0	0.1	-0.7
P4M	5.7	3.6	-7.1	-2.2	-0.4	-0.4

4. SUMMARY AND CONCLUSIONS

Our study demonstrates that PyOM, as it is produced by grassland fires or accumulated after incomplete combustion of litter during forest fires, can be microbiologically attacked and mineralised at rates that are comparable to humic material. Thus, such pyrogenic sources may not be as recalcitrant as commonly assumed and their role as a highly refractory constituent within the humic substance fraction may need revaluation. Our study confirms further that chars derived from different sources or subjected to different thermal treatments show strong differences in their chemical composition. Whereas char from grass material reflects lower C/N ratios than that from pine wood, it comprises higher contributions of alkyl C. This is most tentatively due to its higher peptide content, yielding in chars containing cyclized peptide remains (Knicker and Müller, 2007). On the other hand, pine wood, exposed to charring of low severity still contains residues of carbohydrates, possibly as sugar anhydrides. Increasing charring intensity, however, increases the aromaticity. As demonstrated in this study, the difference in chemistry has some implication on the degradability of the respective chars which affects not only its potential for C-sequestration but also its role during humic substance formation.

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Sedimentary Organic Matter in the Earth System: Origin and Fate

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Carbon is the 4th most abundant element in the universe. The Earth System contains significant quantity of carbon, in its atmosphere and ocean principally in the form of CO_2 and bicarbonates, in the crust, as carbonate-rich and organic-rich rocks, in the mantle and probably also in the core. Abundance estimates in the shallow reservoirs are relatively well assessed, but our knowledge in the mantle and core is far less secure.

As far as reservoirs of organic matter are concerned, living biomass and its detritus including soil humus and oceanic POC (Particulate Organic Carbon) and DOC (Dissolved Organic Carbon) are minute when compared to the huge reservoir of sedimentary organic matter that they have fed throughout geological times.

The purpose of this paper is to review the transformation processes leading to the different forms of fossil organic materials present in the subsurface.

At the geological time scale, if we except the specific case of peat forming coal, the accumulation of organic matter in soils is a transitional state whose fate is to be mineralized and rapidly returned to the atmosphere as CO_2 or transported by watersheds and incorporated into marine or lacustrine sediments. The “fresh” sedimentary organic matter including more or less altered detritus material derived from marine or lacustrine algae, bacteria and terrestrial higher plants (the latter being associated with autochthonous peat deposits or conveyed into aquatic basins) can then be eventually fossilised and preserved in the rock record for tens/hundreds million years before returning to the Earth surface, been oxidized and reintroduced into the atmosphere as CO_2. For depositional environment and sedimentological reasons the geologically preserved organic matter is associated with fine grain mudstones.

In order to be accumulated in sediments the components of the organic matter should be preserved from degradation by heterotrophic organisms during their transfer from the production site to the ultimate location of sedimentation. Different types of mechanisms are involved, acting differentially on the organic components as a function of their molecular composition. To a large extent the chemical structure of the organic compounds derived from a given biomass will control its preservation fate:

- Certain biomacromolecules are originally more resistant to degradation processes than others. This is for instance the case of cell walls of algae, lignin, spores and pollens, cuticules or cell membranes which can benefit from a “selective preservation” keeping their initial structure relatively unchanged.
- A second type of mechanisms of preservation is the polycondensation of the products derived from the decay and transformation of alterable organic remains, forming a more stable heterogenous mixtures of transformed biomolecules (“humic substances”)
- A specific phenomenon occurs in anoxic sea water, involving the reaction of sulfur with functionalized lipids and sugars (“natural sulfurization”) leading to resistant high molecular weight moities resulting from intramolecular incorporation of sulfur
- A last mechanism of preservation is the protection of organics by the mineral fractions through the formation of “organic-mineral aggregates”

During the first step of burial the organic matter remains subjected to bacterial activity. The latter occurs usually up to few ten’s meters below the seabed, although the recent discovery and study of the “deep biosphere” extend such bacterial activity in sediments several hundred meters below seafloor.

This “**Early Diagenesis**” impacts both:

- the properties of the fossilizing organic matter which becomes progressively non-hydrolysable and non-extractable to alkaline solutions (disappearance of the “humic acids” fraction), becoming the so-called “**Kerogen**” in the terminology of petroleum geologists. This transformation is associated with a loss of oxygen-bearing groups, a change which can be also observed at the molecular level with the alteration of functionalized lipids (i.e. sterols, terpenoids) into saturated hydrocarbons.
- The nature of the products released by the degradation of the organic matter including for instance the formation of “biogenic” methane which will contribute *pro parte* to the stock of **Hydrate-bound Methane** within the gas hydrate stability zone.

At the end of the diagenesis step, the organic moieties whose assemblage is making up the “kerogen”, which can be viewed as a set of molecular networks including mainly , but not only, carbon and hydrogen atoms. The ratio of hydrogen and carbon atoms of the bulk kerogen (a critical property for the quantity and composition of the hydrocarbons to be generated during the subsequent geological history of the sedimentary organic matter) is inherited from the nature of the biological precursors and influenced by the type of electron acceptors occurring in the depositional environment.

Beyond this step, the subsequent burial of organic-rich sediments, which are coined as potential **Source Rocks** (for petroleum and gas) is accompanied by a progressive increase of temperature, which is the main factor responsible for the cracking of a part of the kerogen into smaller chemical moieties: liquid **Petroleum** and **Gas**. The relative importance of generated oil and gas is related to the type of kerogen but evolves as a function the thermal history of the source rock. Oil is the major phase during the beginning of the thermal cracking, but progressively gas contribution increases to a point where it is the only released material, up to a stage where only methane is formed. This thermal evolution can be described by a first order kinetic approach and simulated in numerical basin models. The remaining part of the kerogen which is not transformed into oil or gas tends to evolve towards a more and more carbon-rich material. In this respect the **Humic Coal** series derived from the fossilisation of organic detritus of higher plants, including the peat, lignite, brown coal, bituminous coal, anthracite…,.is a pedagogic example of this evolution pathway.

In the **Petroleum Systems** the hydrocarbons generated in the fine grain source rocks (low porosity and low permeability) have to be expulsed into a carrier system made of coarse grain sedimentary bodies, fractures and faults where they can migrate upward due to their buoyancy. Along the migration pathway the hydrocarbons can be trapped as a result of structural features or change in the permeability of the carrier system, associated with a sealing cap rock. They become accumulated in **Oil / Gas Fields** from which, when identified and economically profitable, can be produced by industrial means.

The hydrocarbons which are not trapped will continue their ascending journey toward the Earth surface where they can spring as **Oil or Gas Seeps** which are ultimately oxidized to be transformed in CO_2 joining the atmosphere. In the subsurface, when oil is reaching shallow depth and temperature adequate to accommodate subterraneous biological processes (~< 85°C), this oxidation, which is mainly mediated by bacterial activity, transforms the conventional oil into viscous to highly viscous oil due to loss of light ends and formation of additional high molecular weigh compounds. The biodegradation of migrated conventional oils is the major phenomenon of the formation of the huge resources of heavy oils, extra heavy oils and bitumen. When these viscous heavy oils are mixed with non-consolidated sands (corresponding to non compacted sands, occurring for instance in the distal part of “recent” foreland basin) they are described as **Oil Sands** or **Tar Sands** (i.e. exemplified by the gigantic Alberta oil sands deposits and Orinoco tar belt).

The gas escaping the petroleum system can end up in the shallow thermodynamic stability zone of the gas hydrate and contribute, with the previously mentioned biogenic gas, to the tremendous reservoir of **Methane Hydrate** (probably twice the resource of the other fossil fuels). These hydrates might be viewed as a potential source of energy, assuming our ability to overcome the very important technological challenges associated with its recovery. Moreover it is considered to have play a substantial role in the atmospheric greenhouse gas mass balance during the geological history as a result of episodes of destabilisation.

A source rock which did not undergo this thermal history will keep its petroleum potential and is liable to be industrially mined and retorted in order to artificially produced oil and gas. Assuming that the total energy required to recover the considered source rock (mainly function of mining depth) and to thermally process it, is lower than the energy associated with the generated hydrocarbons (mainly function of the quality of the source rock), this immature source rock can be qualified as an **Oil Shale**. Although their exploitation is still a matter of technical and environmental issues, the current resource in **Shale Oil** is estimated to be around half of the resource of conventional oil.

The low permeability of the source rocks or the absorption capacity of the organic matter require a sufficient energy for the generated oil or gas to be expelled and to reach the carrier system. If the hydrocarbons remain trapped in the source rock, the continuous burial and temperature increase lead to a further thermal cracking and to the accumulation of gas within the source rock itself. A phenomenon accompanied by a progressive decrease of the already low permeability of the hosting rock due to mineral diagenesis. This is the origin of the **Gas Shales** considered as a new frontier for fossil fuels exploration.

Humic Coal, which is a massive organic matter sediment, is generating oil (during the sub-bituminous and bituminous stages) and a large amount of gas during the whole thermal cracking episode. A part of the gas, **Coal Bed Methane** (equivalent to coal mine firedamp), remains trapped and adsorbed within the organic matrix and can be industrially produced from coal fractures by dewatering and pressure drawdown.

From biological tissues to kerogen the chemical change of the organic matter is largely mediated by biological activity resulting mainly in the loss of nitrogen then oxygen during the early diagenesis. The next step of evolution is controlled by thermal cracking releasing, on one hand, different types of hydrocarbons (bringing hydrogen with them) and transforming, on the other hand, the remaining kerogen in a carbonized residue. All of these products being due to be ultimately brought back to the Earth surface and oxydized as CO_2 and H_2O and involved again in the biological cycle.

Biological and Molecular Structure Analyses of the Controls on Soil Organic Matter Dynamics

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1. INTRODUCTION

The dynamics of soil organic carbon (SOC) are controlled by the interaction of biological, physical, and chemical parameters. These are best measured by a combination of techniques such as long-term field sites with a $C_3 \leftrightarrow C_4$ plant switch. Acid hydrolysis and ^{14}C- dating measure the mean residence time (MRT) of the resistant fraction. Long-term incubation allows the *in situ* biota to identify and decompose the labile SOC components. Statistical analysis (curve fitting) of the CO_2 release curves, determines the pool size and of the two labile fractions (1). The effect of chemical structure is measured with pyrolysis-molecular beam mass spectrometry (py-MBMS). The dynamics of charcoal, clay and silt are measured with both ^{13}C and ^{14}C.

2. MATERIALS AND METHODS

We determined SOC dynamics on continuous maize plots on formerly forested soils, in Ohio and Michigan and a Colorado, grassland loam site. Studies on the Colorado, grassland soils in a wheat-fallow rotation used ^{13}C and ^{14}C and acid hydrolysis to characterize the non-hydrolysable C (NHC) and incubation and ^{13}C to investigate the contribution of the microbial biomass (2, 3). Methodology for the laboratory incubations, fractionation procedures, and tracer analysis are reported by Follett et al. (2), Paul et al. (4), and Collins et al. (5). Methodology for Py-MBMS analyses are reported by Magrini et al. (6).

3. RESULTS AND DISCUSSION

The wetland forest in Ohio had an original SOC content of 75 g C kg^{-1} soil. Drainage and cultivation dropped this to 18 g C kg^{-1} with a MRT of 920 ± 53 yr in the 0-20 cm layer and 6607 ± 79 yr at 50-100 cm. Acid hydrolysis that removed ~ 45% of the SOC resulted in an MRT of 1770 ± 70yr at the surface and 9875 ± 75 yr at lower depths (4)). Continuous maize for 30 yr labeled 21% of the soil ^{13}C. The C_3 (non-maize) SOC had an MRT of 17 yr for the light fraction (LF), 38 yr for the particulate organic matter (POM), 139 yr for the silt, and 261 yr for the clay (Table 1). Incubation for 850 days and measurement of ^{13}C showed the C_4-C (maize) LF to have an MRT of 3.7 yr, while the plant residues and microbial biomass C within the aggregates (POM) dated 7.8 yr. The maize C in the silt had an MRT of 12.8 yr. The SOC associated with clay was oldest at 26.8 yr.

The Michigan site contained 18 g C kg^{-1} soil in the deciduous forest, but 10 g C kg^{-1} in the cultivated. The 0-20 cm depth of the cultivated soil had a ^{14}C MRT of 422 ± 50 yr and 1712 yr at 50-100 cm. The non-hydrolyzable C (NHC) accounting for 45% of the SOC had an MRT of 977 yr at the surface, and 4406 yr at depth. The ^{13}C measurements show that in both soils, the LF and POM fractions contain considerable, non-maize C in spite of an extensive period of maize production at both sites. The clay fraction contained the largest portion of the SOC. Both the silt and the clay had much slower ^{13}C turnover rates in the OH soils than in the MI soil (7). This reflects differences for these soils also measured by ^{14}C dating. The Colorado soil lost 33% of its SOC during an 853 day incubation (2) relative to the ~20% loss from the forest-derived sites (5). It had ^{13}C MRTs similar to the OH site, but its ^{14}C dates were much older. The microbial biomass carbon (MBC) was comprised of a labile and stable fraction. The high, initial CO_2 evolution is reflected in the low MRT of the active fraction (C_a). The NHC comprising 60% of the soil still lost 30% of its C during the 853-day incubation with an increase in MRT from 3175 yr to 4967 yr. There is microbial production and transfers of materials between pools.

The availability of py-MBMS, that rapidly measures volatile pyrolysis products, allows the determination of the molecular structure of the SOC (6). The NHC, although containing a significant proportion of long-chain alkanes and high molecular weight aromatics still contained proteinaceous and carbohydrate materials protected by the soil matrix during hydrolysis. Acid hydrolysis, in removing some of the interfering minerals, results in higher pyrolysis product recovery (8). Interfering materials can also be eliminated by separation of humic acids under an N_2 atmosphere.

Our use of py-MBMS is shown for a cultivated, Colorado Grassland soil before and after an 853 dy incubation (Figure 1). The positive values for the ion intensity represent m/z species present in higher concentrations at day zero than after 853 days. Peaks for amino acids dropped significantly and show the source of some of the 58% of the soil N that was mineralized relative to the 33% of the soil C lost during incubation. The peaks representing carbohydrates and lignin also dropped during the incubation reflecting the drop in both C_3 and C_4 constituents of the LF and POM during incubation (Tables 1 and 2). Our work has shown microbial production and transfers of materials between pools.

Table 1. Distribution of C_4 and C_3 -C and MRT of the LF, POM, silt and clay fractions of two originally forested soils now in continuous maize

	LF		POM		Silt		Clay		Total
Ohio silty clay loam									
	C gkg^{-1}	MRT yr	C gkg^{-1}	MRT yr	C gkg^{-1}	MRT yr	C gkg^{-1}	MRT yr	C gkg^{-1}
C_4- C	0.6	3.7	1.2	7.8	0.9	13	1.1	27	3.8
C_3-C	0.1	17	0.9	38	3.5	138	9.0	261	13.5
Michigan loam									
C_4- C	0.3	3.9	0.84	11	0.2	11	1.1	16	2.3
C_3-C	0.2	20	1.4	33	0.9	47	4.2	40	6.7

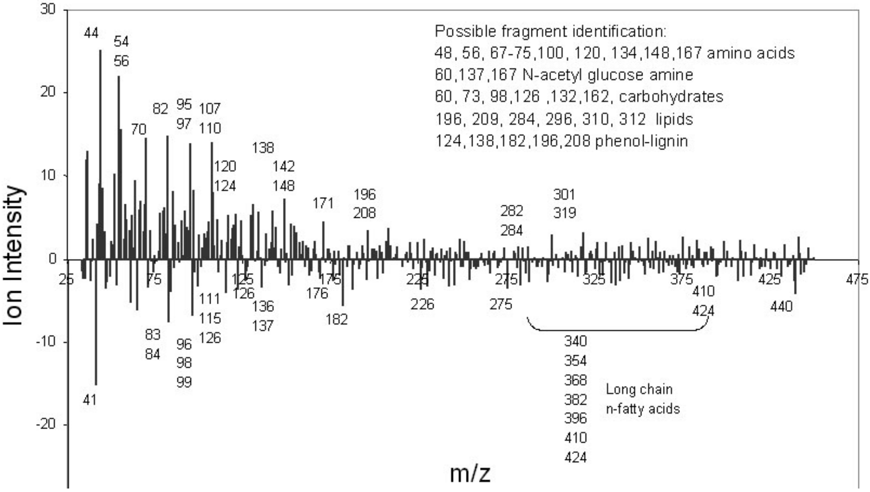

Figure 1. Py-MBMS difference spectrum for a cultivated Colorado grassland (0 day incubation - 853 days). Negative values show species present in higher concentration after 853 days of incubation, positive values show species present in higher amounts at 0 days.

Table 2. The dynamics of analytically-determined fractions of a Colorado silt loam as determined by acid hydrolysis, incubation and ^{14}C and ^{13}C measurements

Fraction	Method of Analysis	C, gkg^{-1}	MRT, yr
MBC labile	Incubation – Fumigation	0.18	0.19
MBC stable	Incubation – Fumigation	0.19	4.6
C_a-SOC	Incubation – CO_2 evolution	0.5	0.67
C_s-SOC	Incubation – CO_2 evolution	3.2	24
C_r-SOC	Acid hydrolysis – ^{14}C dating	5.4	3175
Total SOC	^{14}C dating	9.1	1072
^{13}C from wheat	Incubation – ^{13}C	4.9	7.5
^{13}C from native	Field – ^{13}C	4.2	41
M cr	Incubation-acid hyd- ^{14}C	3.7	4967

MBC = microbial biomass C, Ca = active SOC, Cs = slow C, Cr = resistant C, Mcr = microbial resistant, nonhydrolyzable C

4. CONCLUSIONS

A combination of biological analysis, such as incubation and microbial biomass determination, ^{13}C and ^{14}C tracers, soil fractionation, and matrix analysis (LF, POM, silt, and clay) with molecular structure analysis (py-MBMS) on long-term sites with a $C_3 \leftrightarrow C_4$ crop switch provided the tools for determining the interacting controls in SOC dynamics. Mean residence times vary from a few months to 4967 yr. Long-term incubation, in which the soil microbiota decomposed 33% of the SOC and 58% of its N, produced microbial metabolites and transferred materials between pools.

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Genesis of Cambisol, Luvisol and Rendzic Leptosol of Samara Forest-Steppe Ridges with Special Reference to Characteristic of Humic Acids

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Keywords: soil genesis, humic acids, ^{13}C NMR spectroscopy

1. INTRODUCTION

The Middle Volga transitional landscape region (boreal ecotone) is characterized by very complicated conditions in terms of history of relief, parent material and soil cover. Here three ecosystems (deciduous forests, forest-steppes and steppes) are closely located each other, due to inversions of climatic zonal sequences and very high spatial heterogeneity of geogenic condition. That is why the soils have maximal diversity in higher taxonomic level in Samara ridges in comparison with the same altitudes of Russian plain. For example, there is an unequal (mountain landscape, presened by Luvisols (Dark-gray soils) and Cambisols (Brown soils), which are usually typical for dry continental and wet pacific regions, respectively. In this situation morphological and chemical properties of soil thin fraction are not allowed to conduct a precise and final soil classification in the categories of soil types. Some of these indexes give the evidence of transitions of one soil type to another, i.e. Cambisols can be classified as Gray-metamorphic soils, which have the features of both – Luvisols and Cambisols. Genetic differences between Luvisols and Cambisols can be adequately understood only on the basis of thorough investigation of humic substances (HAs) in nature and structure. Thus, the aim of this investigation is to answer the question if soils of northern slopes of Samara ridges are Cambisols or Cambisols with features of Luvisols (Gray-metamorphic soils). Two catenas on slopes of different aspect of Samara ridges were investigated in terms of soil genesis, morphology and chemical features, i.e. Rendzic leptosols – Cambisols (wet, northern slopes, exposed to Volga thermo regulation influence (1) and Rendzic leptsols – Luvisols (dry eastern slopes with contrasting continental climate). Cambisols and Luvisols are different in morphological features and chemical properties due to different climatic conditions; while Rhendzic leptosols of both slopes seems to be the same on the basis of general morphological and chemical analyses and recognized as intrazonal soils which are known as minimally dependent on climatic conditions. In order to understand the differences in humification process, the soils of two catenas were investigated in terms of characteristics of humic acids (HAs).

2. MATERIALS AND METHODS

HAs powders were prepared by standard IHSS method for soil samples after decalcination. ^{13}C NMR spectra were registered on Brucker Ultra-Shield spectrometer with TMSPNA as a standard. Also the elemental composition of HAs was determined on HP-185B C, H, N-analyzer.

3. RESULTS AND DISCUSSION

The humification process in two catenas with different climatic conditions, i.e. eastern and northern slopes of Samara ridges, is completely different in the formation of HAs (table 1, fig. 1). HAs of Luvisol on eastern slopes are more aromitisized, contains less aliphatic carbon and fewer part of Me-O- connected carbon. In contrast, HAs of Cambisol on northern slopes are essentially higher in the portions of carboxylic carbon and carbonyl species. The same tendencies were revealed for HAs extracted form Rendzic leptosols genetically connected in catenas with Cambisols and Luvisols. These data show that humification process is more intensive and reached to a deeper depth in case of Luvisols which is caused by contrast climatic regime characterized with wide fluctuation of annual and day temperature and moisture. The products of humification in this condition are HAs designaged by the “dark” or “gray” type (2), which is partially dissolved in water and able to move in soil profile, resulting in the formation of deeply developed dark-colored surface horizon of Luvisol. Associated with HAs migration, the formation of cutans is observed in the subsoil of the profile. In contrast, the row, wet and warm stabile climate, which is regulated by Volga river on the northern slopes of Samara ridges leads to the formation of weakly humified HAs. These humic substances are more typical for taiga or humid marine biotopes. HAs here are rather referred to “brown–humus” (3), which is not soluble in water and, therefore accumulates in the upper part of the soil profile. The mobile fraction here is only fulvic acids, which accumulate in subsoil and lead to immobilization of iron, deep weathering, i.e typical cambisol formation. Humus type of soils investigated is also strongly dependent on soil type mainly determined by climatic conditions, i.e. Cambisols and Luvisols are characterized by predominance of fulvic acids group (CHA/CFA=0,4-0,7) and humic acids group (CHA/CFA=0,9-1,2), respectively.

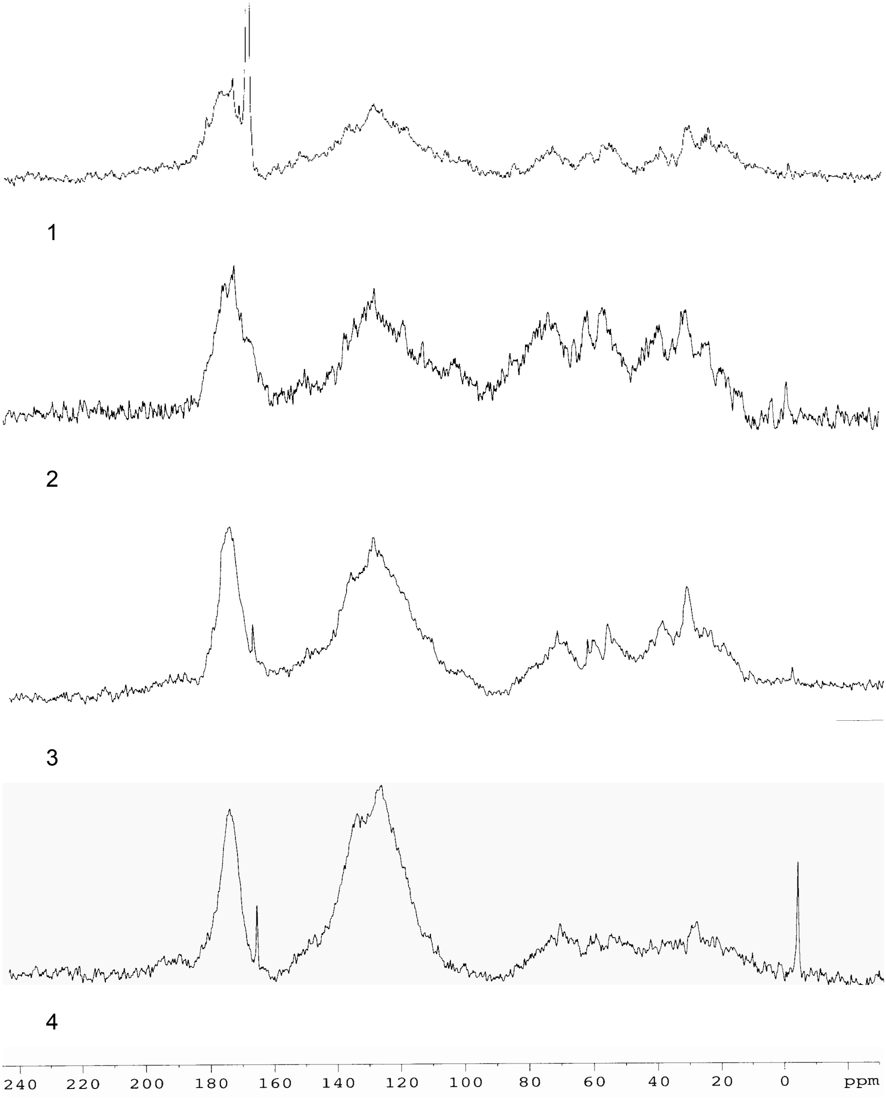

Figure 1. ^{13}C NMR spectras of:

1 – Cambisol, 2 – Luvisol, 3 and 4 – Rendzic leptosols related on the slopes to Cambisol and Luvisol respectively; x – chemical shifts, ppm, y – the signal intensity.

Table 1. The carbon species content in HAs, % to total carbon, on the basis of ^{13}C NMR spectra

C=O	COO-C	phenolic-C	aromatic-C	HCOH, MeO-C	aliphatic-C	Aromaticity
230-190	190-165	165-145	145-110	110-48	48-0	
Cambisol on residual calcaric on slope clayely derivates						
5,8	20,3	6,9	29,4	19,1	18,9	0,6
Rendzic leptosols, upper part of the same slope, eluvium of limestones						
3,5	17,5	5,3	23,4	32,9	18,2	0,6
Luvisol on calcaric on slope clayely derivates						
4,4	16,8	7,7	42,3	16,0	13,3	0,7
Rendzic leptosols, upper part of the same slope, eluvium of limestones						
3,4	18,8	7,7	34,9	17,0	18,4	0,7

4. CONCLUSIONS

Climatic condition in Samara ridges in boreal ecotone causes diversity of humification processes, that is reflected in the characteristics of HAs in structure and its solubility leading the ability to move with soil solutions through a soil profile. This HAs features make difference in soil morphology and horizontal organization not only in full-developed or matured soil profiles, but also in intrazonal soils such as Rednzic leptosols.

The results obtained here suggest that the increase in water storage (moisture condition) due to Volga River locally influences humification processes in the soils in a continental steppe ecosystem and modify morphological and chemical characteristics of soil horizons to form “brown-humus” instead of “dark-humus” which is more common in the soils of continental steppe ecosystem.

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Paramagnetic Activity of Humic Acids in Soil Chronosequence of Former Sandy Quarry, Leningrad Region, Russia

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Keywords: soil genesis, humic acids, 13-C-NMR spectroscopy

1. INTRODUCTION

Rate of primary humus accumulation and chemical mechanisms of humification process are two main questions of modern pedology and humiomic science. These tasks require long term experiments, but in case of chrono sequences it is possible to use the natural time-models of humification process development. Different-age soils, formed in the same bioclimatic conditions on the same spoil heaps parent materials provide exact information about evolution of humic substances. This investigation was aimed to asses a HAs paramagnetic activity changes with time in organogenic horizons of forest floors of young weak-developed and mature Spodosols.

2. MATERIALS AND METHODS

The 10-, 15-, 60- years old and also mature Scots pine forest plots in naturally-revegetated spoil heaps of Maluxa glacio-lacustrine sand quarry in Kirovsky district of Leningrad region (50 km East of St. Petersburg) were studied. The 10 years young soil under the solitary pine trees with spotty grass-mosses cover demonstrate only low humus accumulation and not stabile thin forest floor. Starting from 15-20 years the process of organic accumulation in soil intensifies which results in differentiation of forest floor to subhorizons Oi, Oe and Oh (L, F and H in forest pedology). The HAs, extracted from these three sub horizons are quite different on elemental composition and carbon species based on NMR data. This allows us to suggest that paramagnetic properties of HAs also must show the tendencies to change with the age row and due to forest floor differentiation to tree sub horizons. Previously is was shown that HAs from arable or buried soils characterized by lower portion of free radicals than HAs from natural soils under undisturbed plant cover. Thus, paramagnetic activity is expected to be in inverse proportional to HAs aromaticity and transformation degree.

3. RESULTS AND DISCUSSION

Paramagnetic activity of humic acids, estimated by concentration of free radicals (fig. 1), is quite changeable parameter with the age row. Difference between HAs from 15-, 60-years old and mature Oi horizons are not significant, and, in general the level of HAs paramagnetic activity is stable for fresh organic matter, accumulated of the soil surface with the age row. It is caused by annual renewal of organic matter in forest ecosystem due to input of organic debris. The Oe horizons show the tendencies of strict decreasing of free radicals concentrations. The reason of this trend is intensive transformation of soil organic matter in this horizon of fermentation. This tendency is more expressed in humic acids of forest floor humification subhorizon – Oh.

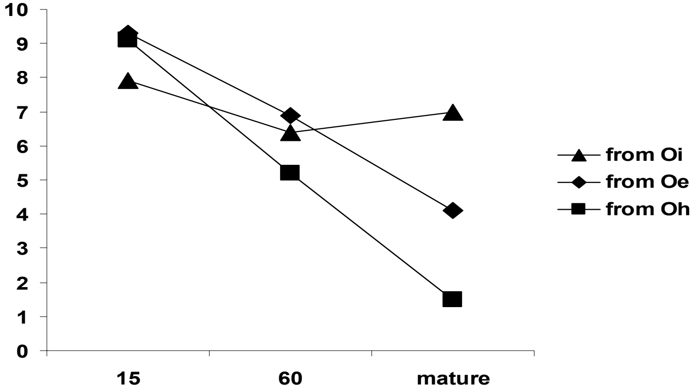

Figure 1 The concentration of free radicals in HAs powder, spin/g.

4. CONCLUSIONS

Data obtained demonstrate that humification process leads to strong and statistically significant decreasing of free radicals concentrations in different-age soil soils of vegetation chronosequence. These data also provide facts the intensity of soil organic matter transformation is in inverse proportion with its paramagnetic activity. On the first stages of ecosystem development the paramagnetic activity of HAs is in maximum. This fact is not only the result of low degree of HAs condensation, but also the index of maximal physiological activity of HAs.

Global Warming and Effects of Sea-Salts on Dissolved Natural Organic Matter (DNOM) Draining From a Sub-Catchment Upstream Lake Terjevann, Southernmost Norway

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Keywords: global warming, sea-salts, DNOM

1. INTRODUCTION

Negative impacts of the observed global warming include increased risk of inland flash floods in most parts of Europe due to storminess (1). During stormy weather sea-water aerosols are transported into the atmosphere and deposited inland. Observed drainage acidification and increased levels of labile aluminium after rainfall events with high sea-salt concentrations have been ascribed to ion-exchange reactions in the humus-rich top soil (2, 3). Effects of sea-salts on DNOM draining from a sub-catchment are reported here.

2. MATERIALS AND METHODS

Lake Terjevann is situated about 2.5 km from the coastline about 8 km west of Kristiansand in the southernmost part of Norway. The catchment consists of four sub-catchments A, B, C+D of 0.50, 0.24 and 0.35 km^2 respectively. The altitude ranges from 22 to 128 m above sea level. The soil cover is thin (20 – 70 cm) and frequently interrupted by outcrops of bedrock, mainly felsic augen-gneiss. The vegetation is dominated by conifers, mainly Scots pine, with elements of deciduous trees, mainly oak and birch. Besides, about 50 % of sub-catchment A was forested with Norway spruce about 50 years ago. A maritime climate prevails in this area with monthly mean temperatures commonly just below 0 ^{o}C in December, January and February and just above 14 ^{o}C in June, July and August. The annual mean deposition is about 1300 mm characterised by maxima during late autumn and winter. Sea-salts as well as long-range transported acidic sulphur and nitrogen compounds in the deposition are common.

Samples of drainage water from sub-catchments A and B were collected almost weekly in clean glass bottles and transported to the laboratory where conductivity, pH, absorbance (200-700 nm) and HPSEC fractions were measured a few hours after sampling. Conductivity and pH were measured with standard Radiometer equipment calibrated against KCl and Radiometer buffers of 4.01 and 7.00 respectively. The UV-VIS spectrums were obtained on filtered (0.45 µm) samples by a Shimadzu Multispec photodiode array spectrophotometer and a 10 mm quartz cuvette. The same instrument with a 10 mm quartz flow-cell was used as a detector (254 nm) in the HPSEC system in combination with a Perkin Elmer Series 200 pump and a Tosoh TSK-G3000SW column (7.5 x 600 mm with a 7.5 x 75 mm guard). A sodium acetate buffer with pH 7.0 was used as mobile phase. DOC was measured on filtered (0.45 µm) and acidified (pH 2) samples by a Shimadzu TOC analyzer.

3. RESULTS AND DISCUSSION

Wind direction and strength control the observed fluctuations in the sea-salt (Cl^-) concentration of the deposition (Figure 1). Strong south-westerly winds in January 2005 combined with relatively large amounts of deposition (Figure 1) loaded the catchment with sea-salts. This was observed as an increase in conductivity of the drainage from about 8 to about 12 mS/m (Figure 1). The minimal decrease in pH (Figure 1) further indicates that the sea-salts resulted in a significant decrease in DOC and besides a significant decrease in the higher molecular weight (HMW) fraction of the DNOM draining from the catchment (Figure 1).

Aggregation of the HMW fraction in the soil/soil-water due to the sea-salts and precipitation could explain the loss of this fraction as laboratory experiments have shown similar effects on adding neutral salts to solutions with humic substances (e.g. 4, 5). This conformational change of the DNOM may have implications for the binding properties and may e.g. explain the observed substantially increased inorganic aluminium concentrations in the stream waters during a similar event in January 1993 (3).

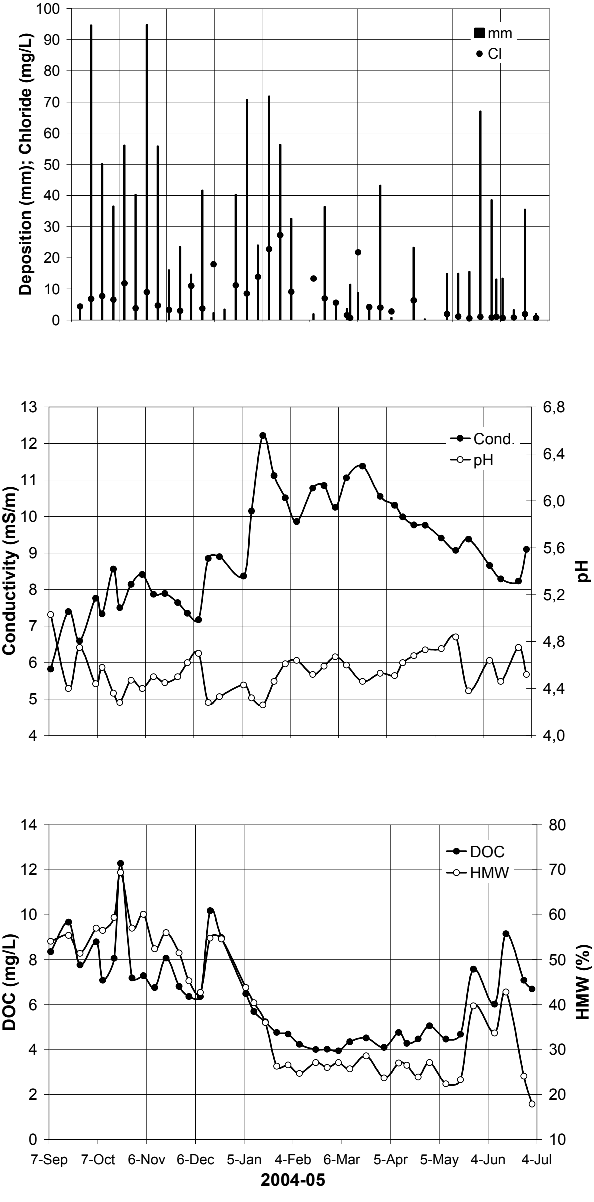

Figure 1. Amount of deposition and chloride concentration in deposition; conductivity, pH, DOC and higher molecular weight (HMW) DNOM in the drainage from the catchment.

4. CONCLUSIONS

Increased frequency of sea-salt events may be expected as more extreme rainfall events is predicted in e.g. the northern part of Europe due to global warming (1, 6). In areas with at least acidic soils probable conformational changes of the DNOM may have implications for its binding properties and the drainage water chemistry.

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Characterization of Soil Organic Matter from Flooded Rice Fields Contaminated by Geogenic Arsenic in Bangladesh

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Keywords: SOM, rice field, fluorescence spectroscopy, UV-vis absorbance, FT-IR, CP-MAS

1. INTRODUCTION

Ground waters containing high concentrations of arsenic have been reported in many parts of the alluvial plain of Ganges-Brahmaputre-Meghna delta. Flooding of rice fields with highly contaminated groundwater may cause an accumulation of As and Fe (fig. 1) in the subsurface which is suspected to increase the severity of the straighthead disease and affect the grain yield of rice plant (*Oryza sativa* L.) (1, 2). The release of As from soil and its transfer to the aquifer is governed by several physico-chemical processes such as adsorption, complexation or oxydoreduction, in which soil organic matter (SOM) is thought to play a central role (3). Hence, this study aims to obtain information about structural and functional properties of SOM in the soil profile of rice fields in order to understand its effect on the fate and mobility of arsenic in flooded soils. Six different sites have been studied, however in this abstract, only the results corresponding to the most contaminated soil ([As]>2000 μg/l) will be presented.

2. MATERIALS AND METHODS

Soil and water samples were collected before rice harvest (April 2007) in a contaminated rice field located in Araihazar Upazila (N23°47'35.7'' E90°37'44.9''), Bangladesh. Soil cores of 14cm in length, divided into seven 2cm thick slices, were collected near the water inlet to the paddy field. Slices were squeezed to extract soil interstitial water containing the dissolved organic matter (DOM). Soil humic substances (HS) were extracted from the solid soil core with NaOH 0.1M (1:2 w/v) during 24h. Humic acids (HA) were separated by acid precipitation (32% HCl, pH = 1) for further analysis. Both DOM and HS were analyzed by measuring UV-Vis absorbance and three-dimensional fluorescence spectroscopy. Furthermore, HA were studied with FT-IR and solid state ^{13}C-CPMAS-NMR using the two pulse phase modulation method. In order to enhance the resolution of the FT-IR absorption bands of the polysaccharide fraction (1300-800cm^{-1}), the 2nd derivative multiplied by a positive factor was subtracted from the raw spectra (4).

3. RESULTS AND DISCUSSION

Total organic carbon (TOC), HS and DOM concentrations within the entire soil profile are presented in figure 1. Contrarily to DOM, which showed a maximum between 6 and 10 cm, HS tended to decrease steadily with increasing depth and exhibited a closely comparable spatial profile to that of total dissolved As and Fe (fig. 1).

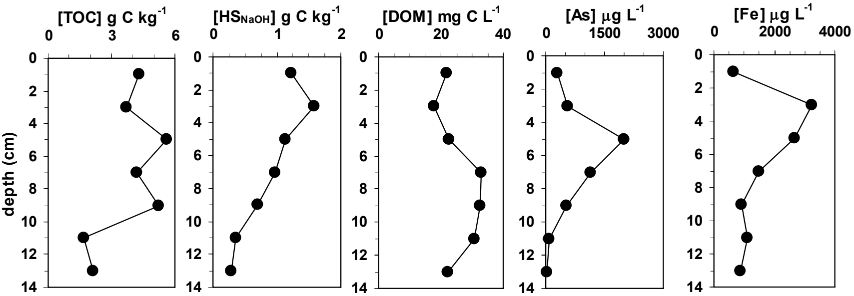

Figure 1. Profile of concentration of TOC, HS, DOM, and dissolved As and Fe.

UV-Vis absorbance spectral analysis of DOM and HS showed an increase of their absorbance ratios A_{250}/A_{365} (E2/E3) and a decrease of their humification index A_{465}/A_{665} (E4/E6) with soil depth, indicating their enrichment in simpler and less humified structures. Typical excitation emission matrix (EEM) fluorescence spectra of DOM (fig. 2.1) revealed the presence of peaks A (λ_{ex}270nm/λ_{em}425nm) and C (λ_{ex}325nm/λ_{em}400nm), respectively related to fulvic acids and protein-degradation derived materials, and to more humified humic acids. At depth ranging from 0 to 4cm, an additional peak, B (λ_{ex}280nm/λ_{em}360nm) most likely related to protein-like moieties (5), was also detectable. Furthermore, traces of a shoulder-like peak, H (λ_{ex}390nm/λ_{em}480nm), corresponding to aromatic compounds derived from lignin degradation was perceptible along the soil profile. On the other hand, the EEM fluorescence of HS (fig. 2.2) were particularly dominated by peaks C and H, and characterized by the complete absence of peak B. In addition, a hardly distinguishable blue-shifted peak A was also noticed.

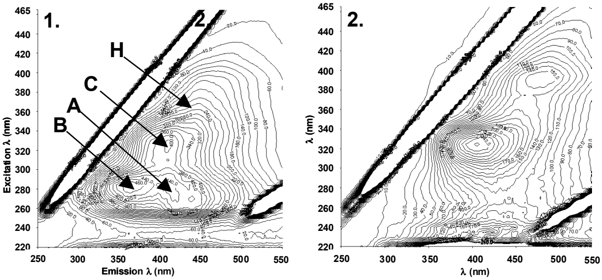

Figure 2. EEM of NOM between 0-2 cm. 1) DOM, 2) HS.

FT-IR spectroscopy was used to characterize the functional groups present in the soil HA fraction. In the FT-IR spectra hydroxyl (3400 cm^{-1}), alkyl (2920 cm^{-1}), carboxyl (1720 cm^{-1}), aromatic (1510 cm^{-1}), alcohol (1150-1050 cm^{-1}) and ester (1250-1220 cm^{-1}) bands were observed. The optical density relative to the aromatic band at 1510 cm^{-1} was determined for each functional group. The relative optical density (ROD) of hydroxyl and carboxyl bands showed significant differences along the soil profile. Indeed, the decrease of ROD of carboxyl and hydroxyl groups reflects aromatization, decarboxylation and dehydroxylation processes due to the natural degradation of HA (4). Furthermore, the absorbance bands at 1330cm^{-1} and 1270cm^{-1}, which are characteristic of the lignin components syringyl and guaiacyl, suggested that HA originate of vegetal biomacromolecules. This finding was further confirmed by the isotopic value of $\delta^{13}C$=-26.7‰ which indicate an essential contribution of C3-plants, such as *Oryza sativa* L, in the formation of these structures in soil.

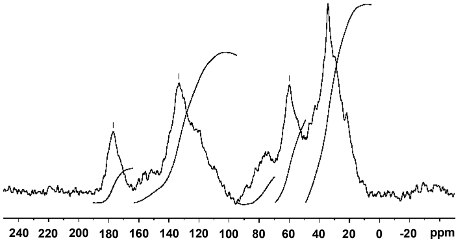

Figure 3. ^{13}C-CPMAS-NMR spectrum of HA fraction of HS.

On the basis of the ^{13}C-CPMAS-NMR, determined for the HA fraction extracted from the entire soil profile, several structural groups were detected and quantified. Indeed, ^{13}C-CPMAS-NMR showed the presence of peaks in the chemical shift regions representative of alkyl (0-50 ppm), O-alkyl (50-70 ppm), carbohydrate (70-95 ppm), aromatic (100-160 ppm), and carbonyl (160-190 ppm) structural groups. The quantification of these groups revealed the dominance of alkyl (40.6%) and aromatic (30.2%) carbon compounds, compared to carbon associated to O-alkyl, carbohydrate and carbonyl groups which represented respectively only 16.9%, 5.3% and 7.0%.

4. CONCLUSIONS

Some striking qualitative and quantitative differences between DOM and HS extracted from the same sampling site have been highlighted by several analytical methods. These differences suggest that these two fractions have different origins and reactivity. However, results show a decrease of the amount of organic carbon, complexity and molecular weight in both fractions with soil depth. This is confirmed by the presence of low ROD values for hydroxyl and carboxyl FT-IR bands and by higher fluorescence intensity of low molecular weight humic-like and fulvic-like compounds in deeper soil layers. Furthermore the chemical characterization of the rice field SOM has confirmed that HS are essentially derived from C3-plants residues.

This research has opened up new prospects in the study of rice field soils. Indeed, the combination of different spectroscopic methods has helped to elucidate in part the nature of SOM in flooded soils. However, in order to advance in our knowledge of the rice field SOM, TOCSY, ^{13}C-CPMAS and other NMR experiments should be used in the future to characterize the complex structure of SOM.

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Soil Organic Matter Chemical and Physical Fractionation: a Comparison on a Developing Soil

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Keywords: humic acid, fulvic acid, free particulate organic matter (POM), occluded POM, MOM

1. INTRODUCTION

Humification processes can be related to biological, microbial, or chemical transformation of plant residues to humic substances which have been defined as the fraction of soil organic matter (OM) most resistant to biodegradation (1). However, if this concept is restricted to the products of OM transformation, it does not explain why it is possible to find in soil old OM rich in easily degradable residues. Different authors proposed that, besides the selective preservation of the products more recalcitrant to degradation, two major mechanisms can contribute to stabilisation of OM (reviewed by 2). The occlusion of OM into aggregates may reduce its accessibility for micro-organisms, while the interaction with minerals and metal cations can limit enzymatic activity.

The chemical separation into humic acids (HA), fulvic acids (FA) and humin (Hu) has been used for long time to evaluate the processes governing humification in soil. Additional information can derive from physical fractionation which takes advantage of a non-destructive technique to evaluate other mechanisms contributing to OM stabilisation in soil. Density fractionation physically separates OM into light fractions representing particulate OM, mainly fresh biomass debris, and heavier fractions comprising OM occluded into aggregates and/or intimately associated to minerals.

This work is aimed to compare the chemical and density fractionation methods and highlight the different information obtained by each approach. For this aim young soils exposed to a natural succession of pure pine for different periods seem a good tool to evaluate the time course of the processes of humification and stabilization of OM.

2. MATERIALS AND METHODS

The studied area is located in Russia, 40 km South Est of St. Petersburg, in a former sand quarry where dumps are under natural revegetation for different years. The chronosequence is composed of a “time zero” plot with no vegetation cover and of four plots covered with *Pinus sylvestris* L. of different ages (10, 20, 40 and 65 years). From each plot soil was sampled from the three organic horizons (Oi, Oe, and Oa) if present, and from the mineral horizons down to the parent material.

In addition to the physical-chemical characterization of the soils, total extractable C (TEC), humic (HA) and fulvic acids (FA) were extracted and purified (1).

On the mineral horizons the density fractionation was carried out with Na polytungstate at a density of 1.6 g cm^{-3} (3) applying a sonication intensity of 175 J cm^{-3}. Three different fractions were obtained: free particulate organic matter (FPOM), occluded particulate organic matter (OPOM), and mineral-associated organic matter (MOM).

Bulk soils and both chemical and physical fractions were analysed for their organic C and total N contents.

3. RESULTS AND DISCUSSION

The parent material was a weakly buffered substrate with a coarse sandy texture, neutral pH, and little OM, probably due to deposition of dust. The Scots pine colonization resulted in the build-up and transformation of surface humus, followed by the incorporation of OM in the mineral horizons, visible already in the 20 year old-site. The result is a continuous increase of the stored C.

From the chemical extraction findings it appeared that the organic material accumulating in soil with time was at a low level of humification without important changes for the first 40 years. Major modifications were observed thereafter in the 40 and 60 year-old plots with increase of the humic fractions and HA/FA ratios. The density fractionation confirmed the presence of a poorly degraded material in the FPOM fraction of the younger plots. With time, following mineral weathering, a larger proportion of OM was associated to the OPOM and MOM fractions, highlighting that besides humification, the formation of organic-mineral association, which became progressively more pronounced, contributed at a large extent to the stabilisation and consequent accumulation of C in soil.

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Study on the Role of Microorganism in the Structure of Soil Humic Acid

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Keywords: microorganism, humic substances, fungi, bacteria, humic acid

1. INTRODUCTION

Microorganisms are the driving force behind the formation, transformation, degradation and mineralization of humic substances (1). Although bacteria dominate the environment and participate in turnover of humic substances, their ability to degrade stable macromolecules such as HA and lignin is limited (2-5, 10, 11). Fungi are the most efficient HS degraders which carried out mainly via secondary or cometabolic processes. Approximately 8,500 described species of basidiomycetes are lignocellulose-degrading saprotrophs, and about half of these occur in soil and on fallen plant litter (7). Until recently, basidiomycetes were considered less common in agricultural soils. A recent study (6) linked to global warming revealed that over a 6-y period under elevated CO_2, soil carbon was reduced by half, with this decline driven by the activity of the soil microbial community. Soils exposed to elevated CO_2 had higher relative abundances of fungi and higher activities of soil carbon degrading enzymes, emphasizing the role of fungi in humic substance turnover. Fungi active in the decomposition process include mainly ascomycetes and basidiomycetes which are common in the upper layer of forest and grassland soils. However, their relative abundance and role during turnover of humic substances are still unclear (8, 9), there is still a gap in our knowledge of their actual diversity and function in OM decomposition. This work was therefore undertaken with the aim of investigating the role of soil microbial communities in changing the structure of soil humic acid and to highlight problems, unsolved questions and hypotheses.

2. MATERIALS AND METHODS

Materials: Sampling site locates in Liujia village, Nong An county, Jilin province, China (N: 44°11’39.7”, E: 125°07’58.8”). Samples for this study were collected from 0-40 cm depth, uncultivated soil.

Microbial species for test were indigenous fungi (*Aspergillus sp.*, *Penicillium sp., Fusarium sp.*, *Phoma sp., Trichoderma sp.*) and indigenous bacteria (*Arthrobacter sp.*, *Agrobacterium sp., Pseudomonas sp.*, *Bacillus* sp., *Cellulomonas sp.*, *Streptomyces sp.*) isolated from the soil samples.

Methods: Soil samples were fully mixed together, and then put it (50 g) into each glass bottle with cotton plug, respectively. Sterilized at 121°C, 2 hours, then inoculated fungi or bacteria pure cultures to each glass bottle, respectively. Water content: 30%, culture temperature: 25°C, 360 days, 3 times.

Humic acids were isolated from the soil samples, mild purification by successive HCl-solution (pH 7.0) and centrifuge (8000 rpm., 20 min.) steps, and final freeze-drying.

FT-IR spectra of HAs were recorded using a Nicolet 5 PC FT-IR spectrometer on KBr pellets obtained by pressing under vacuum uniformly prepared mixtures of 1 mg sample and 400 mg KBr, spectrometry grade, with precaution taken to avoid moisture uptake.

3. RESULTS AND DISCUSSIONS

The FT-IR spectra of soil HAs are substantially similar to each other, whereas the FT-IR spectra of the Fungi-HA (Fig. 1) differs markedly from the bacteria spectra in the relative intensity of several absorption peaks. The main features of these spectra are: (a) a band between 3400 and 3300 cm^{-1}, generally attributed to O-H and, secondarily, N-H stretching of various functional groups, which appears broader but relatively less intense for the Fungi-HAs than Bacteria-HA; (b) two peaks at about 2920 and 2850 cm^{-1} and a peak at about 1450 cm^{-1}, ascribed to aliphatic C-H groups, which are relatively more intense in fungi-HA than bacteria-HA; (c) a peak at 1720-1715 cm^{-1} and a broad absorption at about 1220 cm^{-1}, generally ascribed, the former to C=O stretching of COOH and other carbonyl groups, (d) a peak at about 1618.16 cm^{-1}, ascribed to aromaticity C=C stretching with bacteria-HA (Fig. 2). The results of FT-IR analysis confirm the existence of important structural and functional differences between fungi-HA and bacteria-HA, which mainly consist of a relatively greater aliphatic character, lower aromaticity, higher content of polysaccharide groups with fungi-HA. On the other hand, we can drown the same conclusion from the 2920/1720 rates of Relative intensity of main absorb peak of IR spectrum (Table 1).

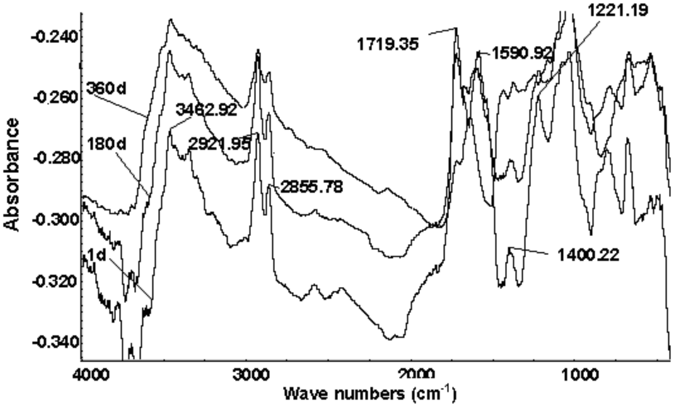

Figure 1. IR spectrum of HA at different time of fungi.

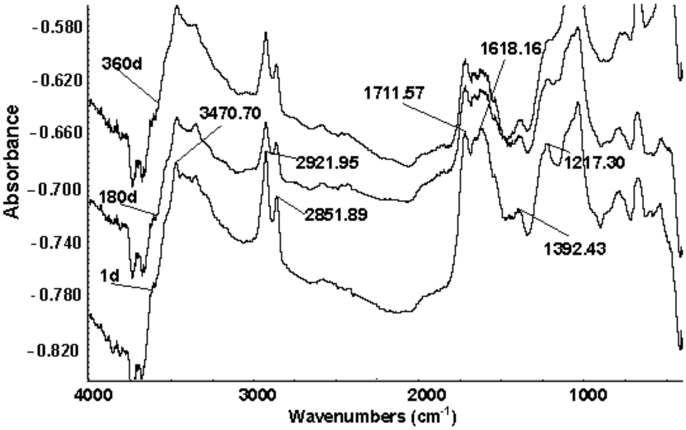

Figure 2. IR spectrum of HA at different time of bacteria.

Table 1. Relative intensity of main absorb peak of IR spectrum in HA.

Microbe	Wave numbers, cm^{-1}				
	2920	2850	1720	1620	2920/1720
Bacteria	41.651	17.908	22.534	17.907	2.643
	32.394	13.249	32.148	22.209	1.420
	33.625	11.410	34.652	20.313	1.300
Fungi	28.393	11.145	38.807	21.655	1.019
	35.951	11.708	34.986	17.356	1.362
	21.879	6.331	5.042	66.748	5.595

4. CONCLUSIONS

The results of FT-IR analysis confirm the existence of important structural and functional differences between fungi-HA and bacteria-HA. fungi-HA mainly consist of a relatively greater aliphatic character, bacteria-HA consist of a relatively higher aromaticity groups .

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Impact on SOM of an Oxisol after Application of Sewer Effluent Treated by Brazilian Septic Cesspool Biodigester

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Keywords: sewer effluent, soil organic matter, humic acids

1. INTRODUCTION

The generation of effluent from the sewage treatment is increasing nowadays and so, its final disposition represents a mandatory question to be answered. An alternative for this residue is its agricultural application as a substitute for mineral fertilizer. This practice, beyond inherent benefits due to nutrient recycling, promotes agricultural water reuse, which is responsible for around 70% of the total water consumption in Brazil.

Embrapa Agricultural Instrumentation was developed a simple and inexpensive continuous system, based on anaerobic process, called “Septic Cesspool Biodigester”, to treat the “black water” sewer (faeces and urine, only) generated by a residence with up to 5 persons (1). Fresh bovine manure is used as an inoculant to optimize the system efficiency. This system is intended to meet demands in rural areas which, in general, do not have sanitation installations.

The effluent generated by Septic Cesspool Biodigester has alkaline character and significant salt concentrations with a high quantity of $N\text{-}NH^{4+}$ and low content of $N\text{-}NO^{3-}$, characteristic of anaerobic systems (2). The effluent has a substantial quantity of plant macro and micro nutrients.

The use of sewer effluent in agriculture is not recent, however, the impact of the use of this residue is not well understood regarding to clay stability (mainly due to sodium ion presence which can decreases the aggregate stability of the colloids) and on soil organic matter (SOM) content. The correct use of the effluent improves soil quality due to the incorporation of nutrients and fresh organic matter. However, excess of sewer effluent can change the SOM characteristics, due to the high availability of nutrients (mainly nitrogen and phosphorus) to the soil microorganisms, favoring the increase in population of degraders using the natural organic matter present in the soil as carbon source. The result could be a decrease of SOM content, leaving only the most recalcitrant carbon.

The objective of this work is to present some results about the impact of the use of treated sewer generated by Septic Cesspool Biodigesters on SOM and humic acids (HA), in a Brazilian tropical Oxisol, using chemical and spectroscopical methods.

2. MATERIALS AND METHODS

The soil, classified as Yellow-red Latosol, sandy phase, was submitted to the effluent application during three years and was used for a guava orchard. The effect of effluent application was analyzed in comparison to NPK mineral fertilization as well as to soil under a native forest nearby the orchard. The three treatments were: soil with addition of the treated effluent (about 50 L/tree each 3 month, during three consecutive years) (Sample "E"); addition of NPK mineral fertilizer as per agronomic recommendation during the same period (Sample "NPK"); and soil in native forest close to the experimental field (Sample "NF").

Soil samples were analyzed for pH, conductivity, macro and micro nutrient content, total organic carbon and humification by Laser Induced Fluorescence (LIF) (4). Soil HA were extracted according to IHSS method (5) and characterized by ^{13}C NMR spectroscopy (VA-CPMAS) (6).

3. RESULTS AND DISCUSSION

After three years of effluent application, the quantity of SOM increases slightly in comparison to the NPK fertilized soil (Figure 1A). This result shows that part of organic matter present in the effluent has becomes part of the SOM. Other soil parameters like electrical conductivity and pH were changed by effluent application. Them most significant change was in increase in pH. (data not shown). The nutrients status of the effluent treated soil was also greater than in the NPK fertilized soil (data not shown).

Laser Induced Fluorescence (LIF) can be used to determine the humification degree of the whole SOM, with no extraction necessary (4). The results in Figure 1B show that the SOM in NPK treated soil has higher values of humification degree (HFIL), in comparison to E and NF samples. HFIL increases with increase the depth for all samples and the NF-SOM had lower values at all depths. E-SOM has intermediate HFIL values between NPK and NF samples, due to incorporation of fresh carbon in the soil, from either the organic matter present in the effluent or the forests litter.

Figure 2 shows relative percentage of aromatic and aliphatic groups present in the HA from different samples, by NMR spectroscopy. In soil E, there was a decrease of aliphatic content in the HA with depth, similar to the observed to the NF soil. The effluent releases organic matter to the soil that is high in simple structures like protein and polysaccharide derivatives from the biodigestion process, which is incorporated to the soil. The E soil had HA with distribution of different chemical groups more similar to the NF soil than the NPK soil, showing an improvement of the organic matter quality due to the effluent. HA from NPK soil presented higher aromatic content (mainly $C_{aromatic}$, $C\text{-}O_{aromatic}$ and $C_{carboxilic}$) when compared to the E and NF humic acids. This result is expected since the use of mineral fertilizers can promotes the degradation of the most labile natural organic matter present in the soil, favoring the aromatic moieties in the remaining SOM.

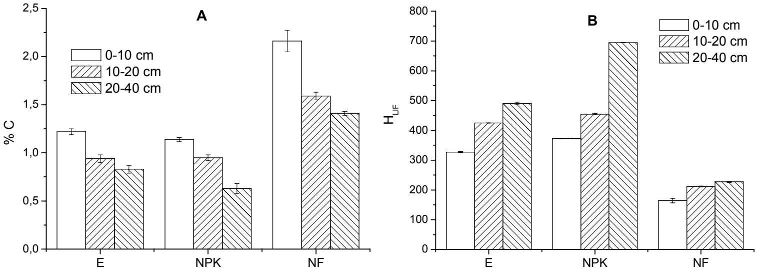

Figure 1. Percentage of total carbon in the soil samples (A) and humification degree of SOM obtained by Laser Induced Fluorescence (LIF) (B). E: Soil fertilized with effluent; NPK: soil fertilized with mineral NPK and NF: soil from native forest.

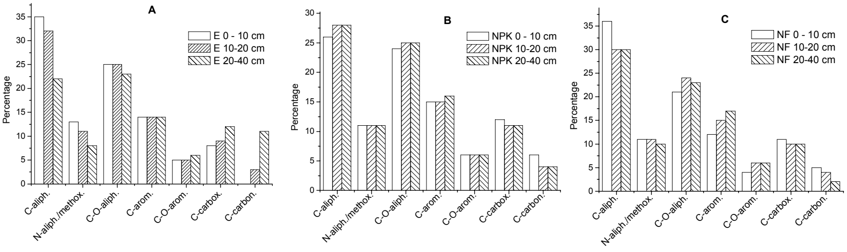

Figure 2. Relative areas from VA-CPMAS ^{13}C NMR spectra of HA extracted from soil fertilized with effluent (A); soil fertilized with NPK (B) and soil obtained from a native forest close to the experimental area (C).

4. CONCLUSIONS

The results showed a tendency for increase of carbon content in the soil after successive application of effluent. Data from FIL showed incorporation of fresh carbon in the soil from effluent or the forest decreased the index of humification, compared to NPK treatment. The use of ^{13}C NMR spectroscopy provides observations on the relative proportion of several functional groups present in the HA from different treatments. The HA in the E soil is more similar to the NF than the NPK treatment.

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Organic Matter in Oxisol Profiles under Pasture Submitted to Periodic Burning in the Past

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Keywords: subtropical highlands, organo-mineral interaction, black carbon

1. INTRODUCTION

The northeastern part of Rio Grande do Sul State (800 to 1300 m above sea level) is one of the coldest regions of Brazil, and the temperature in winter usually drops to below zero. The landscape is covered by native pasture and native forest of *Araucaria angustifoli*, and the fields have traditionally been used for cattle raising, which is the main economic activity of the region. The burning of the vegetation at the end of the winter season is a common and ancient practice, which aims to promote the pasture regrowth in a shorter time than compared with an unburned field. Beside the impact on the biodiversity and the contribution to the greenhouse effect, this agronomic practice may affect the chemical composition of the soil organic matter (SOM). Therefore, the present study aimed to evaluate the effect of periodic burnings on the composition of the SOM at different depths in a high altitude Oxisol (810 m, 28^0 38’ S / 51^0 34’ W) under pasture. The SOM of the same soil under native forest was also analyzed for comparison purposes.

2. MATERIALS AND METHODS

Soil samples from three representative locations in the sampling site were collected in four layers (0-5 cm, 0-20 cm, 20-40 cm, 40-60 cm) of a Red Oxisol (heavy clayey soil) from the following treatments: native pasture under biennial burning for more than 100 years (BP), native pasture without burning in the last 41 years (NP) and native Araucaria forest (NF). The soil samples were treated with 10% (v/v) HF solution to remove the inorganic fraction(1). Previously to the HF treatment the samples were treated with 0,1 mol L^{-1} HCl and the C content in the supernatant was determined (Shimadzu TOC 500). The whole soil and the HF treated samples were analyzed (two replicates) by elemental analyses (Perkin Elmer 2400) and by Fourier Transformed Infrared spectroscopy (FTIR) (Shimadzu 8300). Based on the FTIR spectra an aromaticity index was calculated by dividing the intensity at around 1630 cm^{-1} by the intensity at 2920 cm^{-1}(**$I_{C=C}/I_{C-H}$**). From the elemental composition the following indexes were calculated: C/N ratio before and after HF treatment, R factor ($CN_{soil}/C/N_{HF}$), recovery of mass (M_R), of carbon (C_R) and of nitrogen (N_R) after HF treatment(1).

The electron spin resonance spectroscopy (ESR) measurements of the HF treated samples were performed in a Bruker ESP 300E spectrometer (9.5 GHz at 300 K and 77 K, quartz tubes with 3 mm I.D) (2). The relative concentration for semiquinone free radical (SFR), corrected for the sample carbon content (spin $g^{-1}C$), was calculated using a Bruker strong pitch reference of known spin concentration. The solid state ^{13}C nuclear magnetic resonance spectroscopy (NMR CPMAS) spectra were recorded in a Bruker DSX 200 (50.3 MHz) using zirconium rotors of 7 mm OD with Kel-F caps that were spun at 6.8 kHz. The experiments were performed with a contact time of 1 ms, a 90^o ^1H-pulse width of 6.6 μs and a pulse delay of 300 ms. Between 4500 and 13000 scans were accumulated and a line broadening between 50 and 100 Hz was used. The ^{13}C chemical shifts are reported relative to tetramethylsilane scale (=0 ppm) and their assignments were as follows(3): 0–45 ppm, alkyl C; 45–110 ppm, methoxyl and N-alkyl C + O-alkyl C; 110–160 ppm, aromatic C + phenol C; 160–220 ppm, carbonyl C. The total signal intensity and the relative intensity (proportion) of each functional group were obtained by integrating the spectral regions with the integration routine of the spectrometer.

3. RESULTS AND DISCUSSION

In respect to the SOM content, the treatments differed mainly in the superficial layer (0-5 cm), where C and N contents in BP were lower than in NP (Table 1). The greater values in NF are probably related to higher residue input generally observed in forests. The C_R varied from 33 to 82% and decreased with depth in all three soil uses (Table 1). The HCl pre-treatment removed at most 3.5% of the total C, indicating that great part of the SOM, lost during the HF treatment, was associated to iron oxides through organo-mineral complexes. By the dissolution of these minerals, hydrophilic and low molecular weight compounds dissolved in the acid solution and were removed by sample washing out. In the samples under pasture, the lowest values for C_R were observed for BP, suggesting that in this site the SOM contained higher proportions of mineral associated organic compounds. The line width for Fe^{+3} in the room temperature ESR spectra (around $g \approx 2$) varied between 730 and 1400 G and decreased from NF to BP to NP, showing that the degree of oxidation decreases in the same order (data not shown). Within each treatment, the same behavior was observed along the profile. The values of the line width (G) for the SFR were lowest in the 0-5 cm and highest in the 40-60 cm layer. This behavior was similar in the three soil uses and was attributed to an increase of the interaction between SFR and Fe^{+3} with depth. Concerning the ESR parameters for the organic free radicals, the three samples presented similar values both for the g factor and the spin concentration. The spin concentration varied between 7.5×10^{16} and 1.2×10^{17} spins g C^{-1} and is comparable to those reported in the literature for SOM from other Brazilian Oxisols (1). The values of spin concentration tend to decrease with depth, with a parallel increase of the aromaticity degree, inferred from the FTIR index, and of the content of aromatic C determined by ^{13}C NMR (Table 2). In all sites the content of carboxylic C (NMR) also increased with depth. The ^{13}C NMR spectra of the SOM from the burned and from the not-burned pasture were relatively similar and no major differences in the chemical composition were identified. In all samples the C O-alkyl group was the most abundant and no significant black carbon contribution was detected.

4. CONCLUSIONS

The biennial burning of the native pasture in a subtropical Red Oxisol reduces the content of SOM, mainly in the superficial layer. This effect may be related to the lower residue input in the burned pasture as compared to the not burned one. Major differences in the chemical composition of the SOM due to the burning or due to the soil use were not detected in the present study. Possibly, the fire at the end of winter, when the environment is relatively humid and cold, does not affect significantly the SOM quality. Nevertheless, the burning seems to favor the formation of smaller and more oxidized organic structures, which are more affected by the stabilizing effect of the organo-mineral complexes with iron oxides. The SOM from the native forest showed a higher content of both aryl and alkyl C in comparison to the pasture sites. This higher SOM hydrophobicity in the samples under Araucaria forest is possibly a consequence of the vegetation type.

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Table 1. Sample depth, C and N values before and after HF treatment, FTIR aromaticity index and g factor for the Fe^{+3} of the ESR spectra

Sample	Depth (cm)	C (%)	N (%)	C/N	C/N_{HF}	M_R (%)	C_R (%)	$I_{C=C}$ / I_{C-H}	C_{HCl}/C_{soil} (%)	g factor for Fe^{+3}	
										300K	77K
NF	0 – 5	6.3	0.54	12	14	13.0	70.6	1.3	2.4	2.171	2.308
	0 – 20	4.2	0.34	13	14	9.7	64.0	1.5	2.6	2.244	2.581
	20 – 40	2.5	0.17	15	17	6.8	45.4	2.7	2.5	2.115	2.356
	40 – 60	1.9	0.12	16	17	4.8	36.3	2.5	3.5	2.044	2.057
NP	0 – 5	4.4	0.35	13	13	9.0	63.6	1.5	2.5	2.031	2.057
	0 – 20	3.5	0.28	13	15	7.9	81.7	1.3	2.8	2.035	2.053
	20 – 40	2.6	0.18	14	15	5.7	57.6	1.9	2.3	2.013	2.024
	40 – 60	1.9	0.12	16	15	3.9	49.8	2.9	2.2	2.016	2.024
BP	0 – 5	4.1	0.29	14	16	7.7	47.3	1.2	2.2	2.063	2.066
	0 – 20	3.2	0.22	15	15	7.3	54.5	1.4	2.9	2.066	2.060
	20 – 40	2.5	0.16	16	14	4.8	47.2	1.8	2.9	2.047	2.050
	40 – 60	2.0	0.14	15	14	3.0	32.8	2.3	2.9	2.040	2.024

Table 2. Proportions of C-functional groups determined by ^{13}C NMR

Sample	Proportion of C groups (%) / chemical shift (ppm)				
Depth (cm)	Alkyl C	O-alkyl C	Aromatic C	Carbonyl C	O-Alkyl/Alkyl
NF 0-5	30.0	38.7	19	12.3	1
0-20	25.6	35.9	22.8	15.8	1.1
20-40	21.1	33.7	28.8	16.5	1.2
40-60	19.6	32.6	29.2	18.6	1.3
NP 0-5	22.8	47.7	16.4	13.2	1.7
0-20	23.1	47.8	16.8	12.3	1.6
20-40	19.4	41.9	22.6	16.2	1.7
40-60	20.1	38.6	24.6	16.7	1.5
BP 0-5	21	47.2	17.8	14	1.8
0-20	21.5	46.8	17.7	13.8	1.8
20-40	16.8	42.5	23	17.7	2.1
40-60	20.2	37.9	22	20	1.4

Chemical and Spectroscopic Characterization of Humic Acids Isolated from Urban Soils

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Keywords: forest litter, humic acids, fluorescence spectroscopy

1. INTRODUCTION

Urban soils can be defined as all soils influenced extensively by human activities in the urban landscape (1). Thus, soils in urban areas may contain a mixture of natural humic substances and anthropogenic organic particles, such as carbonaceous material like debris, ash, waste of various origin, as well as airborne particles. Compared to their natural counterparts, such soils may show substantially different behaviour towards environmentally hazardous compounds, and altered microbial activity (2). It is well known that organic matter (OM) plays an essential and dominant role in preserving the soil from pollution of agricultural and anthropic origin, therefore in an urban environment OM is of considerable ecological significance.

The aim of this work was to study the chemical and spectroscopic properties of humic acids (HAs) isolated from urban soils in order to identify their role as potential markers for the environmental risk management and prevention of organic chemical pollution in selected areas.

2. MATERIALS AND METHODS

Soil top surface layers (0-5 cm) were sampled from four sites of the city of Ancona (center Italy) with the following use: a) Palombella (P, an area in the outskirts, disused after the landslide of 1982 and close to a main road); b) Duomo (D, a flower bed on the hilltop overbearing the city, very ventilated area); c) Porta Pia (PP, a flower bed in the urban core area, with intense vehicular traffic); and d) Porto Dogana (PD, a flower bed on the harbour ingress, with very intense vehicular traffic).

HAs were isolated from soil samples according to the procedure suggested by IHSS, and characterized by elemental analysis, ratio of absorbances at 465 and 665 nm (E_4/E_6), functional group content, Fourier transform infrared (FT IR) and total luminescence spectroscopies.

3. RESULTS AND DISCUSSION

Chemical and spectroscopic data shown on Table 1 evidence important differences in the elemental composition of HAs from flower bed with respect to HA from disused soil, which shows the highest C, H and S contents and C/N ratio, and the lowest O content, and O/C and E_4/E_6 ratios. These results suggest a larger amount of C-containing structures with a character prevalently aromatic of the P-HA sample with respect to the other HAs. The highest and the lowest, respectively, COOH and N contents measured in the D-HA sample can be likely correlated to a greater oxidation. This is supported also by the high O content.

Table 1. Elemental composition (mg kg^{-1}) moisture and ash free, atomic ratios, carboxylic group contents (meq_{COOH} $g^{-1}{}_{HA}$) and E_4/E_6 ratio of the HAs examined

HA	C	H	N	S	O	C/N	C/H	O/C	COOH	E_4/E_6
P	635.8	68.4	52.6	16.8	226.4	14.1	0.8	03	3.24	5.2
D	544.6	56.4	51.1	5.8	342.1	12.4	0.8	0.5	3.88	7.4
PP	579.8	64.5	62.2	7.4	286.1	10.9	0.7	0.4	3.29	7.3
PD	571.8	60.6	59.4	8.4	299.9	11.2	0.8	0.4	3.32	5.7

FT IR spectra of the four HAs (Fig. 1) feature some common IR absorptions having similar or different relative intensities, and some relevant differences in the "fingerprint" region. In particular, P-HA and D-HA samples are characterized, with respect to the other two HAs, by slightly more intense absorption bands of aromatic structural moieties (3070 and 1654 cm^{-1}), and COOH groups (1715 and 1227 cm^{-1}) respectively. No distinctive feature are evident in the FT IR spectra of PP- and PD HA samples.

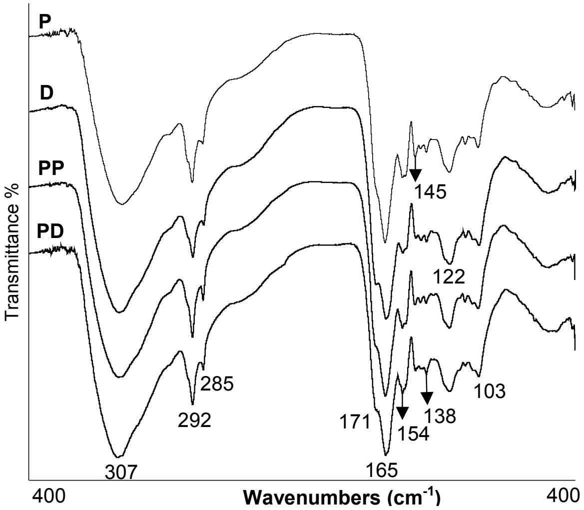

Figure. 1. FT IR spectra of the four HAs examined.

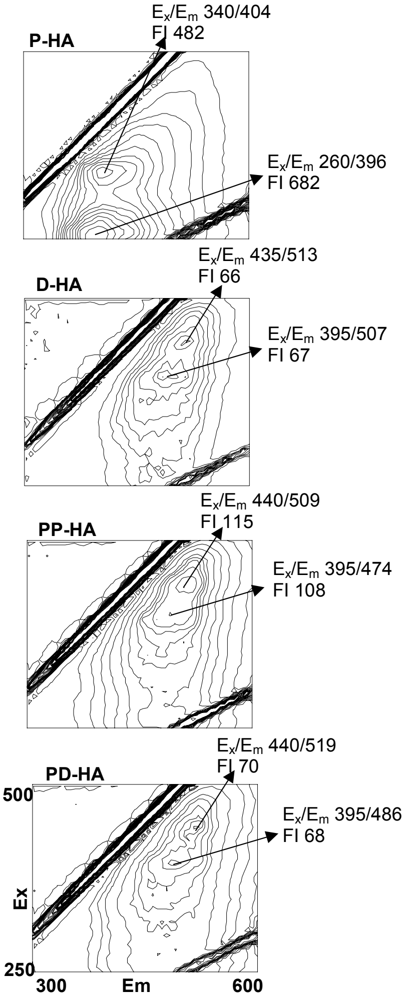

Figure 2. Total luminescence spectra of the four Has examined.

Total luminescence spectra (TLS) of HAs in the form of excitation-emission matrices (EEMs, contour maps) are shown in Figure 2. The maps reported in Fig. 2 indicate the occurrence presence of two major fluorophores in the HA macromolecules, each of them characterized by an own excitation/emission wavelength pair (EEWP). As it is well known, a number of different structures can act as potential contributors to the fluorescence behaviour of HAs, and their identification is intrinsically difficult. In general, the TLS of HAs from the flower beds are characterized by the occurrence of two common fluorophores, localized by EEWPs at about 435-440/509-519 nm and 395-405/486-507 nm, likely attributed to highly conjugated phenols and quinoids (3). Different fluorophores are evident in TL spectrum of P-HA sample, which is characterized by the presence of two main fluorophores in the medium and short wavelenght regions. In particular, the two peak are indentified by the EEWPs at 340/404 nm and 260/396 nm, attributable, respectively, to structural units like to hydroxyquinolines and/or coumarins, and to very simple structures, like components of proteinaceous origin such as tryptophan and tyrosine (4). The fluorescence intensity (FI) values of both peaks result strongly higher in the P-HA as compared to those of the HAs from flower beds, between which the greater values are shown by PP-HA sample, and similar values are measured for D- and PD-HAs.

As reported on literature (5), fluorescence pattern and intensity are strictly depending on the structural and molecular properties of the humic substances, that is on their origin. The shorter EEWPs and higher FI values found in the P-HA sample indicate the occurrence of simple structural components and a low degree of aromatic polycondensation. In addition, the FI values could be ascribed also to the occurrence of electron-donating substituents, such as hydroxyl, methoxyl, and amino groups on low molecular weight molecules. On the contrary, the longer EEWPs and lower FI values measured in the HAs from flower beds suggest the presence of large molecular weight components possessing linearly-condensed aromatic ring systems bearing electron-withdrawing substituents, such as carbonyl and carboxyl groups, and/or to other unsaturated bond systems capable of a great degree of conjugation.

4. CONCLUSIONS

The chemical and spectroscopic data shows important differences between the HA samples examined, and the results obtained are in good agreement with the related soil use. In particular, the HA from the Palombella site is poorly condensed and extremely rich in S content, probably because of the landslide which completely covered all the area. On the other hand, the HAs from the flower beds show similar features, with some differences, especially in the D-HA, which exhibits a greater oxidation compared to PP- and PD-HAs, likely due to less intense disturbance and maintenance.

ACKNOWLEDGEMENTS

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Effect of Different Fruit Tree Cultivation on Humic Substances and Soil Properties

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Keywords: soil humic substances, herbicide fallow, orchard, tree species

1. INTRODUCTION

Long-term herbicide fallow management of orchard leads to soil enrichment with organic matter originated only from cultivated plant, what may influence biological activity (3) and decide on the direction of humification processes. Although input of plant remnants play a crucial role in humification processes, there are very few papers dealing with influence of different plant species on soil humus (2). The objective of the study was to determine the effect of different fruit trees monocultures treated with herbicide fallow on the physicochemical properties of the soil and the composition of humus compounds.

2. MATERIALS AND METHODS

The study was conducted in the Lower Silesia region (Poland), at the Orchard Experimental Station in Samotwor, belonging to the Wroclaw University of Environmental and Life Sciences. Five orchard quarters were established on Haplic Luvisol developed from silt loam (USDA), as follows: cherry tree (*Prunus cerasus L.*) - object 1, natural crossing cherry x sweet cherry tree (*Prunus cerasus L. x Prunus avium* L.) - object 2, peach tree (*Prunus persica* L.) - object 3, sweet cherry tree (*Prunus avium* L.) - object 4, and apple tree (*Malus sp.*) - object 5. The number of trees per hectare were 833 (for objects 1 , 4 and 5), 626 (for object 3), and 333 (for object 2). Trees were maintained in rows on 1.4 m wide strips of herbicide fallow.

Soil samples were taken 15 years after the orchard had been established, from the depth of 5-15 cm. Samples were taken between trees in 5 replications, and the following soil properties were determined: organic carbon (C_{org}), pH_{KCl}, hydrolytic acidity (H_h^+), exchangeable cations (K^+, Na^+, Ca^{2+}, Mg^{2+}) extracted with 1 M NH_4Cl. Cation exchange capacity (CEC), the sum of base cations (BC), and base saturation (BS) were calculated on the basis (H_h^+) and exchangeable cations. Results were statistically verified at the confidence level of $P = 0.05$.

Humic and fulvic acids were separated from the following fractions (except F1), extracted according to a modified method of Tiurin:

(F1): *low molecular fraction*, extracted with 0.05 M H_2SO_4 (so called decalcification),

(F2): *free,* as well as *Ca- and non-silicate* R_2O_3 *bound humic substances*, extracted with 0.1 M NaOH (extracted from residuum after F1),

(F3): *silicate* R_2O_3 *bound humic substances*, extracted by alternately treatment with 0.1 M H_2SO_4 and 0.1 M NaOH (extraction from residuum after F2),

(F4): *free,* as well as *non-silicate* R_2O_3 *bound humic substances*, extracted with 0.1 M NaOH (extraction from non-treated sample - no F1 extraction),

Ca- bound humic acids (CHA_{ca}) were calculated from difference between humic acids in F2 and F4.

3. RESULTS AND DISCUSSION

The results of the study indicate that the 15-year cultivation of fruit trees affected the properties of the soil with different intensity. Soil under natural crossing cherry tree x sweet cherry tree (object 2) and peach tree (object 3) indicated significant increase of hydrolytic acidity, and decrease of base saturation (Table 1). Acidification process observed in these objects (2 and 3) did not affect the sum of base cations (BC), however significantly higher amounts of Ca^{+2} was found in soil under apple trees (object 5).

Table 1. Some physical-chemical properties of soils

Object	pH_{KCl}	H_h^+	Exchangeable cations Ca^{2+}	Mg^{2+}	K^+	Na^+	BC	CEC	BS, %
		cmol (+) · kg^{-1}							
1	5.8-6.4	1.54	4.47	1.48	0.54	0.21	6.70	8.24	81
2	5.1-6.2	2.26	3.64	1.51	0.55	0.20	5.85	8.11	72
3	4.3-5.9	2.53	3.97	1.34	0.57	0.15	6.03	8.56	70
4	5.9-6.3	1.51	4.79	1.75	0.52	0.18	7.24	8.75	83
5	5.9-6.3	1.53	5.16	1.39	0.48	0.16	7.19	8.72	82
$LSD_{0.05}$		0.58	0.99	0.41	0.26	0.08	1.40	1.30	7

The planted tree species affect the level of organic matter influx to the soil, as well as conditions of organic matter mineralization and humification. In result, the content of C_{org} in soil under cherry tree (object 1) and natural crossing cherry tree x sweet cherry tree (object 2) was significantly higher than in other objects (Table 2). Humic substances in these objects (1 and 2) indicated significantly bigger amounts of non-extracted carbon, corresponding to humin fraction. The lowest amounts of this fraction, representing the most stable humus complexes, was found in object 5 (apple trees). On the contrary, the content of so called "fulvic fraction", representing low molecular fraction (F1), did not differ in particular objects, with an exception of object 5 (apple tree), where it reached 7.0 % of $C_{org.}$ Furthermore, the biggest share of fulvic acids found in object 5, particularly in F3, pointed out that organic matter in soils under apple trees is clearly slower transformed into high molecular humics.

In all orchard quarters, as usually in other soils, dominated F2 fraction extracted from the soil after decalcification. Contents of free, as well as Ca- and non-silicate R_2O_3 bound humic substances (F2) were significantly lower in objects 1 and 2 (cherry tree and natural crossing cherry x sweet cherry tree) while objects 5 and 3 (apple and peach trees, respectively) differ markedly form the other by bigger values. Amounts of humic and fulvic acids (CHA_2 and CFA_2) varied proportionally to the content of F2 fraction, and the CHA_2/CFA_2 ratio ranged around 1.5 in all objects.

Table 2. Fractional composition of humic substances

Object	C_{org} g·kg^{-1}	C extracted (% of total C)								
		F1	F2		F3		C non- extr.	F4		F2 - F4
			CHA_2	CFA_2	CHA_3	CFA_3		CHA_4	CFA_4	$CHA_{\mathbf{Ca}}$
1	10.73	4.1	21.4	13.0	6.1	1.9	53.5	10.9	10.6	10.5
2	10.90	3.7	19.3	13.5	4.8	2.5	56.2	11.1	11.7	8.2
3	9.04	4.2	27.7	16.6	6.9	1.3	43.3	19.9	14.3	7.8
4	8.95	4.4	25.8	17.9	8.4	2.1	41.4	12.3	13.1	13.5
5	8.20	7.0	28.2	18.1	6.6	3.5	36.6	11.6	15.2	16.6
LSD	1.05	1.7	4.5	3.3	1.4	0.8	6.5	3.6	2.5	6.3

Accumulation of specific organic substances in soils under particular orchard quarters affects the content of humus compounds bound with mineral components of the soil. In objects 4 and 5 (sweet cherry and apple trees) the content of F3 fraction was significantly higher than in other objects. Notably, humic acids extracted in F3 fraction (CHA_3) clearly predominated over fulvic acids (CFA_3). Particularly high values of the CHA_3/CFA_3 ratio were found in objects 3 and 4 (peach and sweet cherry trees).

As it was mentioned earlier, soils of objects 3 and 2 (peach and natural crossing cherry x sweet cherry trees) indicated significantly lower base saturation (BS), while soil of object 5 (apple tree) indicated significantly higher share of exchangeable Ca^{+2} and BS. These soil properties turned up in humics composition. The share of Ca- bound humic acids (CHA_{Ca}) of object 2 and 3 were significantly lower then those of object 5.

An important role in determining soil properties, apart from those extracted in F2, plays free humus complexes and those bonded with non-silicate forms of R_2O_3 (F4). However, the content of fraction F4 depends on soil physicochemical properties, as well. Those relationships are supported by statistic data, indicating significant correlation coefficients between F4 and values of pH ($r = -0.513$), as well as Hh ($r = 0.518$). These relations were especially marked for Ca- bound humic acids (CHA_{ca}), indicating a significant correlation with pH ($r = 0.478$), Hh ($r = 0.500$), the content of exchangeable Ca^{2+} ($r = 0.44$), and BS ($r = 0.60$).

Presented data confirm that monoculture orchard cultivation with herbicide fallow may result in differentiation of soil physicochemical properties (1), and consequently humus composition. Differences in soil organic matter inputs and turnover, as well as variation in plant residues accumulated under particular monoculture plantations, lead to their different susceptibility to transformation processes. Apart of that, orchard floor management practice affect soil microbial community abundance, activity, and composition (3), that play crucial role in humification processes. The specific chemical composition of organic residue in cherry tree and the natural crossing cherry trees x sweet cherry trees probably slows down their transformations, which may result in a higher content of organic carbon in soil and a lower degree of organic matter humification.

4. CONCLUSIONS

1. Effect of monoculture orchard plantation on soil properties and humic substances composition may be noticed as early as after 15 years, depending on plant species.

2. Plantations of cherry tree and the natural crossing cherry tree x sweet cherry tree contribute to significant increase of soil organic matter, which is dominated by the most stable humic compounds.

3. Character of organic remnants under apple and peach trees stimulate humification processes, which result in bigger amounts of humic acids.

4. Peach and natural crossing cherry x sweet cherry monocultures caused significant decrease of base saturation, and consequently lower amount of Ca bound humic acids.

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Long-Term Effects of Wildfires in the Characteristics of Soil Organic Matter: a Study in Mediterranean Shrublands

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Keywords: wildfires, C availability, labile and recalcitrant C, thermal properties

1. INTRODUCTION

Fire is a major ecological factor in Mediterranean terrestrial ecosystems, and it has been repeatedly demonstrated to have an immediate effect on the chemical characteristics of soil organic matter (1, 2). Nevertheless, Mediterranean ecosystems (and in particular shrublands), which are often affected by recurrent fires, are usually very resilient to fire. If the inputs of fresh organic matter to the soil (or, at least, part of these inputs) are quickly restored, the short-term effects of fire on the soil may be difficult to detect when the study is carried out beyond the first years after the perturbation.

The aim of this communication is to gain insight on this matter. Here we present data about SOM characteristics in a set of shrubland plots characterized by different land-use and fire histories.

We focused on the detection of possible increases in the recalcitrance of SOM, and/or decreases in C availability for the soil microflora due to recurrent fires.

2. MATERIALS AND METHODS

The study was carried out in La Vall de Gallinera, in Alacant province (eastern Spain). The mean temperature is 17.4ºC and the mean annual precipitation is 825 mm. Soils are mostly Leptosols over limestone, and calcaric Cambisols over marls. Most agricultural fields were cultivated until the 1960s. After abandonment, the vegetation reverted to secondary shrubland communities of the *Rosmarino-Ericion* alliance Br.-Bl. 1931. The region has suffered frequent fires since the 1970s.

In the experimental, we selected a set of plots according to two factors:

(a) Land use: never cropped (henceforth, NC), early abandoned (EA: set-aside before 1978), recently abandoned (RA: set-aside after 1978).

(b) Number of wildfires since 1976: either 0, 1 or 2.

The number of plots found for each combination was not the same, and for one of the combinations (RA, 2 fires) no plots were found. On each plot, 4 randomly chosen points (first 5 cm of mineral soil) were sampled. Litter was not included in the sampling. Soil samples were air-dried, sieved to 2 mm, and a subsample was ground in an agatha mortar for chemical analysis. Total organic carbon was analyzed by dichromate oxidation in an aluminium heating block at 155ºC.

Extraction with hot water has been used to obtain the most available fraction of SOM. Hot-water extract was obtained from ungrounded samples, in sealed Pyrex tubes, in a heating block at 105ºC for 1 h, and was recovered by centrifugation and filtration through a Whatman GF/A glass fiber filter. The extract was analyzed for organic carbon by dichromate oxidation (hot-water OC: HWOC), for aromaticity by absorption at 280 nm of a solution previously adjusted to 10 mg l^{-1} of OC, by carbohydrates by the phenol-sulphuric method, and polyphenolics by the Folin-Denis method.

The thermal properties of SOM were obtained in samples previously demineralized by a repeated treatment with HF 10%. Thermal properties are taken as indicators of recalcitrance; recalcitrant forms of C (polyphenolics, lignin, the resistant core of humic substances, and in particular charcoal and other black carbon forms) are highly thermoresistant. Differential thermogravimetry curves (DTG) were obtained in a Mettler-Toledo TGA-851e thermobalance, and differential scanning calorimetry curves (DSC) in a Mettler-Toledo DSC-822e calorimeter. In both cases, the temperature was raised at a rate of 3ºC min^{-1}, up to 600ºC.

3. RESULTS AND DISCUSSION

Data about total C and N accumulation in the studied plots have been published previously (3). Here we give a summarized advance of some of our results concerning SOM quality. As shown in Fig. 1, the changes in organic matter availability (HWOC) due to repeated wildfires seem to depend on previous land use: no detectable changes in never cropped plots, a trend to decrease in early abandoned plots, and an increase in recently abandoned plots (Fig. 1, A). The carbon in the extract becomes more aromatic as fire recurrence increases, even though the increase in aromaticity reaches the significance only in early abandoned plots (Fig. 1, B). As for other indicators of the quality of the HWOC, no significant changes were detected in the proportion of carbohydrate C relative to total HWOC (Fig. 1, C), whereas the abundance of polyphenolics in it seems to increase (Fig. 1, D), in agreement with the results obtained for aromaticity.

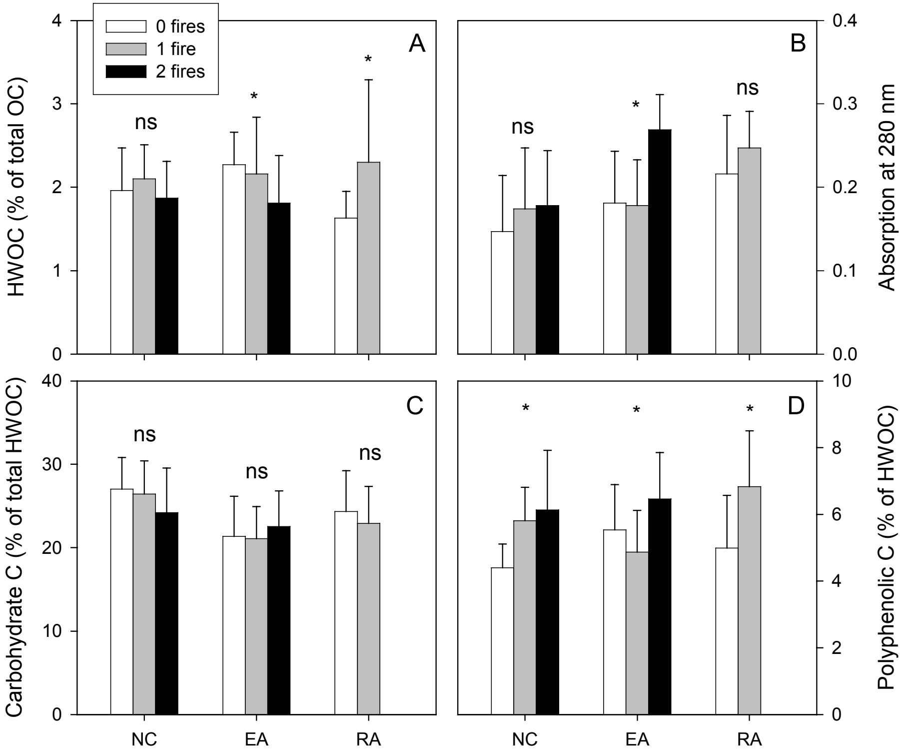

Figure. 1. Hot-water extract. A: relative amount of HWOC. B: aromaticity of HWOC, as measured by absorption at 280 nm. C: carbohydrate C. D: Polyphenolic C. (*) differences are significant at P = 0.05. ns: non-significant.

Table 1 summarizes the results of DTG analysis. T50 is the temperature at which 50% of SOM is lost by volatilization. It represents an indicator of the overall recalcitrance of SOM4. Neither the effect of the number of fires (P = 0.870) nor that of previous land use (P = 0.144), nor the interaction of both factors (P = 0.827) significantly affect T50 values. If the total C is introduced as a covariate in the model, the land use factor becomes highly significant (P = 0.004***), but the number of fires does not (P = 0.257).

The energetic value of SOM (Table 2) is highly affected by the previous land use (P = 0.010**), but not by the number of fires (P = 0.866), nor by the interaction of both factors (P = 0.973). For all land uses the energetic value of SOM increases with the number of fires, even though not enough to reach significance.

Table 1. T50 for DTG. Data are means ± standard deviations. N.D.: no data.

	0 fires	1 fire	2 fires	All
NC	346.6 ± 11.4	348.7 ± 9.3	349.1 ± 12.6	348.1 ± 10.7
EA	355.0 ± 8.2	350.7 ± 7.4	354.7 ± 10.6	353.7 ± 8.7
RA	352.4 ± 10.2	353.2 ± 4.0	N.D.	352.7 ± 7.9
All	351.3 ± 10.2	350.7 ± 7.2	351.9 ± 11.6	351.3 ± 9.5

Table 2. Energetic value of SOM (Joules per mg OM). Data are means ± standard deviations. N.D.: no data.

	0 fires	1 fire	2 fires	All
NC	10.85 ± 1.04	11.43 ± 1.02	11.93 ± 3.36	11.38 ± 2.02
EA	10.55 ± 1.17	10.67 ± 1.24	11.16 ± 2.48	10.79 ± 1.67
RA	8.97 ± 2.14	9.19 ± 3.62	N.D.	9.06 ± 2.74
All	10.12 ± 1.69	10.48 ± 2.31	11.54 ± 2.86	10.59 ± 2.26

4. CONCLUSIONS

Our results suggest that the changes in SOM quality are difficult to detect several years after the fire event. Some indicators of recalcitrance show a slight increase with recurrent fires, but the overall abundance of refractory SOM structures (as measured by their thermal properties) does not suffer clear changes. Our dataset suggests that if the inputs of fresh OM recover quickly enough, the overall SOM quality will behave in the same way, even though the presence of signals of past wildfires can be expected.

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Organic Carbon Stocks in Bulgarian Soils

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Keywords: soil organic carbon, carbon pool, humus substances

1. INTRODUCTION

Soils play an important role in the earth's carbon cycle because they contain most of the earth's carbon pool. One of the important reasons for the assessing of the Soil Organic Carbon Stocks (SOCS) is its role as headline indicator of soil quality and health. Soil organic matter improves the soil physical properties, increases the cation exchange capacity (CEC) and water-holding capacity. Humus substances hold a great proportion of nutrients, cations, and trace elements that are of importance to plant growth. Soil organic matter prevents nutrient leaching; it also buffers soil and increases the ability of the soil to bind chemicals. Globally the upper meter of mineral soils contains 1300-1600 Gt carbon (3, 13). The organic carbon reserve has been estimated in Bulgaria (1, 2, 6, 8, 11). The aim of this paper is to present the organic carbon stocks of Bulgarian soils and GIS maps for organic carbon reserves in Bulgarian soils for the layers 0-25 cm and 0-100 cm.

2. MATERIALS AND METHODS

The presented results are based mainly on the available data of organic carbon and bulk density measurements along the profile depths of different soils grouped according the revised legend to the soil map of the world (4, 5), extrapolation, and interpolation of average data on humus content from the large-scale soil survey and available data on bulk density. Soil organic carbon was determined by modified Turin's method (7; 12) and soil bulk density was determined at field capacity (14). Relevant information was organized in a database. Development of GIS maps of distribution of organic carbon reserves of Bulgarian soils was based on: proper localization of 274 profiles on soil map at a scale M 1:400 000; data interpolation from profiles to soil polygons; data extrapolation for soil contours (polygons) using respective data on organic carbon reserves for other soil contours (3 000 soil polygons); using average data on humus content from the large-scale soil survey and available data on bulk density and land use; removing areas without soil cover.

3. RESULTS AND DISCUSSION

The average carbon density and total organic carbon was calculated for each of the group. These data demonstrate that the greatest carbon density occurs in Gleysols &

Histosols for both layers 0-25 and 0-100 cm. Average organic carbon density ranges from 3.1 kg m^{-2}(Litosols) to 18.6 kg m^{-2} (Gleysols & Histosols) for the surface soil and from 6.7 kg m^{-2} (Litosols) to 33.0 kg m^{-2} (Gleysols & Histosols) for the entire soil.

The total organic carbon stock of the soils in Bulgaria is estimated at 1.3 Gt, which is about 1/1000 of the world reserve estimate of 1576 Gt (3). Percentage distribution of organic carbon stocks among soil groups for 0-25 cm is presented in Figure 1. Cambisols, which occupy about 18.4% of the territory, contribute about 27.6% of the total national carbon stocks (Figure 2). Chernozems, Phaeozems, Luvisols and Vertisols, which cover respectively 7.6, 13.0, 20.2 and 5.8 % of the territory, contribute about 9.4, 14.2, 18.4 and 7.2% of the national carbon stocks. Gleysols &Histosols occupy about 0.6% of the total area but contribute 1,6 % of the national carbon stock (Figure 2). GIS maps of distribution of organic carbon reserves for the layers 0-25 and 0-100 cm are presented in Figures 3 and 4.

4. CONCLUSIONS

The total carbon stocks of Bulgarian soils are calculated to 1.3 Gt. Three groups of soils can be recognized:

(i) First group includes Chernozems, Phaeozems and Kastanozems. These soils have comparatively high SOCS, to a certain extent inherited from the past. The main reasons to SOC decrease: wind and water erosion, tillage, removals of the organic matter by harvest.

(ii) The second group includes Fluvisols, Planosols and Vertisols. In general, these soils are least damaged from erosion. However, the rest SLM options for preservation of SOC are recommended. For Planosols a special attention has the balance of nitrogen amendments.

(iii) Third group unify Luvisols, Cambisols, Regosols and Leptosols. The SOCS in these soils are not high. Cambisols, Regosols and Leptosols are comparatively young soils. All of them damaged from erosion. The balance of SOC removals and nitrogen amendments are especially important.

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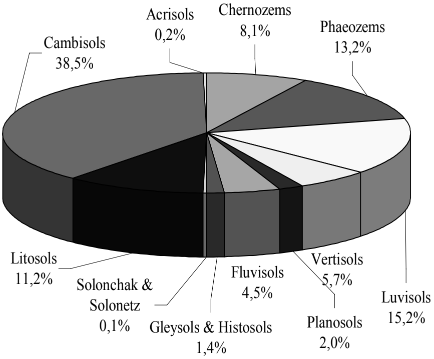

Figure 1. Percentage distribution of organic carbon stocks among soil groups, 0-25 cm.

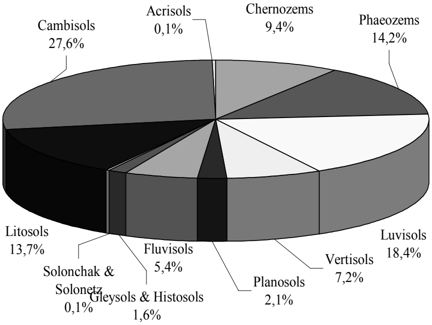

Figure 2. Percentage distribution of organic carbon stocks among soil groups, 0-100 cm.

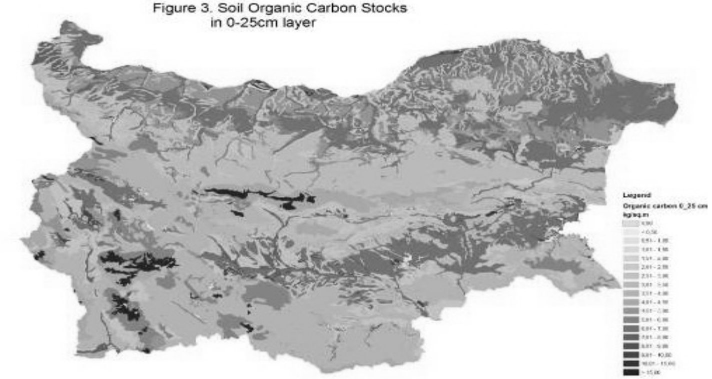

Figure 3. Soil Organic Carbon Stocks in 0-25 cm layer.

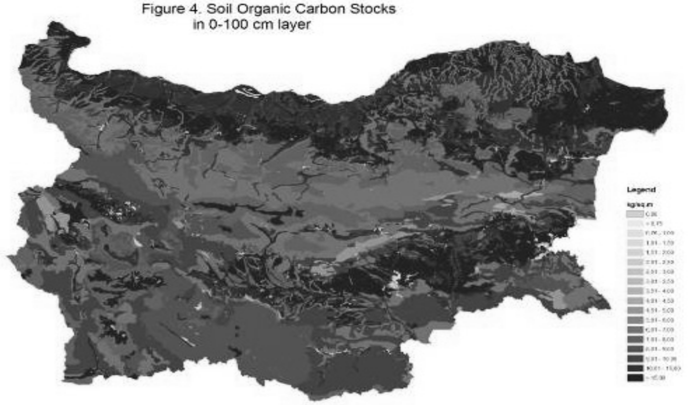

Figure 4. Soil Organic Carbon Stocks in 0-100 cm layer.

Use of Lipid Biomarkers in Sediment and Aquatic Systems

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Keywords: lipid, biomarkers

1. INTRODUCTION

Measuring the diversity of a living community is not an easy task, due to the multitude of organisms and processes involved. In that game, the organic matter present in sediments provides a complex picture of the organisms living in the surrounding ecosystem. Over the centuries, the sediments act as an archive of past events affecting the composition of living communities as well as of biological (reworking of the primary inputs) and abiotic degradation processes. Detailed analyses of the organic matter composition of lacustrine sediments are therefore a powerful tool to assess environmental variations. Within the complex mixture of organic compounds contained in the sediments, lipids are widely distributed and commonly used as 'biomarkers'. Our research project consisted of the study of lipids in aquatic systems in order to evaluate their diversity in terms of space and the impact of environmental change on biodiversity. The method employed to analyse the lipid composition of sediments will be mainly explained in this paper.

2. MATERIALS AND METHODS

2.1. Extraction

In a typical procedure, 10 g of dry sediment were extracted with Chloroform / Methanol (2:1, v/v, 100 ml, 45 min. under reflux, 3 times). After filtration, combined extracts were dispatched into 3 equal parts to be treated by one of the following procedures, one part being stored at 4°C as standard.

2.2. Chemical treatments of extracts

2.2.1. Procedure 1

Crude extracts (100 mg) were methylated with an excess of fresh distilled diazomethane during 3 hours at room temperature. The mixture was evaporated to dryness and consecutively acetylated with a mixture of acetic acid and pyridine (1:1, v/v, 2 ml) at room temperature for 16 hours. This procedure allowed the analysis of n-alkanes as well as lipids bearing an acetylated alcohol or a methylated carboxylic acid group.

2.2.2. Procedure 2

Crude extracts (100 mg) was treated at room temperature for 1 hour with a solution of sodium periodate ($NaIO_4$, 500 mg) in THF / water (9:1, v/v; 10 ml) to yield aldehydes from 1,2-diols. Water (10 ml) was added to the medium and the mixture was extracted with petroleum ether (40 ml, 3 times). The residue was reduced at room temperature during 1 hour with an excess of sodium borohydride ($NaBH_4$, 500 mg) in ethanol (10 ml) to yield primary alcohols. After addition of aqueous potassium hydrogenophosphate (KH_2PO_4, 500 mM, 3 ml), the mixture was extracted with petroleum ether (40 ml, 3 times). Dried organic phase was evaporated to dryness and consecutively acetylated as in procedure 1. This procedure allowed the analysis of n-alkanes as well as lipids bearing alcohol or 1,2-diol groups yielding simple alcohols after the periodate / borohydride degradation procedure (complex hopanoids for example).

2.3. Instrumentation

Gas liquid chromatography was performed on a Fisons Gas Chromatograph 8000 fitted with a DB-5 (30m × 0.32 mm id) fused silica capillary column, an on-column injector and an FID detector. Hydrogen was used as carrier gas. The temperature of the oven was programmed from 50°C to 310°C at 6°C / min. In a typical analysis, 1 µl of a chloroformic solution of the analysed mixture was injected.

2.4. Identification and quantification of biomarkers

After adequate treatment of total sediment extracts, two representative samples for each series and for the procedures detailed above were analysed by GC / MS for identification purposes, the other samples being quantified by GC. Compounds were identified by mass spectrometry and by comparison (mass spectra, retention index of compounds in GC) with authentic samples. A kit of n-alkanes injected twice in each series of chromatograms furnished retention times allowing us, thanked to an automatisized informatic programme developed by our means, to attribute indexes, analogous to Kowats indexes, to all compounds contained in analysed mixtures.

For quantitative analyses, n-tetracontane ($n\text{-}C_{40}H_{82}$) was used as internal standard. Accuracy of quantitative analyses is of 20% in the studied concentration range (from 10 ppb to 10 ppm). The minimal amount of lipid recognizable in a given sample is around 0.2 µg, what means a detection limit of 10 ng lipid / g dry sediment (10 ppb) for 20 g samples.

3. RESULTS AND DISCUSSION

Lipid extracts from sediments were complex mixtures of various classes of lipids. For sediment core interpretation we chose lipids having biomarker value, meaning that:

- they were fairly abundant in the organic matter of the sediments (around 1 ppm)
- they were specific of the organisms synthesizing them or they can lead, through calculations, to conclusions about the composition of living communities
- they were fairly stable so that biotic or abiotic degradation could be neglected
- they could be successfully resolved and identified by GC/MS analysis

In order to achieve a relevant fingerprint of the lipid composition of each studied sediment sample, we analysed more than 100 lipids belonging to 8 structural groups

- An: long chain n-alkanes (from C27 to C36)
- Ao: long chain n-alcohols (from C23 to C34)
- Ai: long chain fatty acids (from C24 to C36)
- S: C27 steroids (like cholesterol I)
- MS: C28 steroids bearing an additional methyl or methylene group at C-24
- ES: C29 steroids, with an additional ethyl or ethylene group at C-24
- H: Hopanoids
- O: Other pentacyclic triterpenoids (amyrines, gammacerane, tetrahymanol (THM)…)

3.1. Surface layers of all aquatic systems

A wide range of lipid concentrations between the top sediment surface samples studied from near to 2 mg/g of dry sediment down to less than 200 µg/g of dry sediment. Whatever, an overall classification in “lipid rich” (more than 1 mg analysed lipids / g sediment), “intermediated lipid richness” (between 400 µg and 1mg/g) and “lipid poor” (less than 400 µg/g) sediments could be done and suggested to use such lipid compositions as fingerprints to characterise the sediments.

Considering the sediment samples and the distribution of the analysed lipids over the 8 defined structural groups, 3 major groups could be distinguished:

- Sediments where linear compounds dominates: long chain fatty acids (>30% of lipids)
- Sediments with high sterol contents (>30% of the analysed lipids)
- Sediments with high hopanoid content (>25% of the analysed lipids)

It was remarkable to observe that sediments belonging to the first group were remarkably stable downcore over the studied period, meaning that no major shifts in the lipid composition occured. On the other hand, sediments where the dominants lipids were sterols or hopanoids showed drastic changes in their composition downcore.

3.2. Establishing specific indicator calculated from the lipid concentrations

The lipid composition and mainly its evolution through time, which could be monitored by analysing the sediments downcore, could be used, by calculating several ratios and bioindicators, to describe the evolution of the compositions of living communities and the various processes happening during the early diagenesis. Following indicators were developed and used to study the sediment downcore:

Contribution of living organisms		Origin of organic matter	
Bacteria	BA = H/F	Odd/Event Index	OEI = 3/2((C_{29}+C_{31})/(C_{28}+C_{30}+C_{32}))
Protozoa	PR = THM/F		
Higher Plants	PL = (ES + O)/F	Eutrophication	EUT = OEI / Saa
Algae	AL = (MS + (S + ES)/3)/F		
Zooplankton	ZO = S/F		
“F = (4(S+ES)/3 + MS + H +O + THM)”			*“Saa = (An+Ao+Ai+ES+O)(S+MS+H)”*

The lipid concentrations and compositions of the sediments were analysed and all the indictors were calculated as detailed above. The results showed clearly the complexity of the aquatic systems biogeochemistry in relation to the environmental factors. Nevertheless some highly interesting pattern could be recognised and the value of lipid biomarkers as “summary of all events, including biodiversity of organisms and diagenetic processes was obvious. Furthermore, the eutrophication indicator was a promising tool for approaching aquatic systems productivity not only in function of total phosphorous but also depending on organic matter inputs.

4. CONCLUSION

As conclusions, clear links of lipid compositions of the sediments to environmental events were obvious even if the statistical tools used so far are unable to demonstrate it clearly. Similarities between aquatic systems, which appeared at all analysed levels, reflected a similar response of the biological communities to a common physico-chemical environment. Undoubtedly, lipid biomarkers were the right tool to quantify such similarities.

Thermal Properties in Soil Particle-Size Fractions of *Andosols*, with Different Land-Use, in the Trans-Mexican Volcanic Range

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Keywords: physical fractionation, thermal analysis, forest degradation

1. INTRODUCTION

In the Cuitzeo Basin (Michoacán, Mexico), land degradation has reduced soil fertility and productivity. The necessity of rural communities of searching resources for improving the local economy has lead to an increase in timber extraction activities during the last years. This practice has been associated to a decrease in the vegetation cover and an increase in the soil surfaces affected by degradation processes. As a consequence, the SOM stored in the soils of this area is in high risk of being lost, especially in the upper soil horizons. Thermal stability of soil organic matter (SOM) refers to its resistance to thermal oxidation during heating treatments and relationships between thermal properties and biological stability have been found (1). Thus, thermal techniques allowing to detect gross changes in SOM as a consequence of land-use changes has also been referred.

The objective of this work was to determine how land use and forest degradation affected the SOM of volcanic soils located at the Mexican Volcanic Belt, using as a tool the thermal responses of the C associated to the silt- and clay- sized fractions.

2. MATERIALS AND METHODS

The study site is located in the Atécuaro catchment, within the Cuitzeo basin (Mexico), 12 km away from the city of Morelia (State of Michoacán, 19º 34' N; 101º 10' W). The dominant soils in the catchment are *Andosols* in the top and slopes and *Acrisols* in the piedmont and valley. The forest succession is pine-oak at the summit, with increasing degradation on the hillside and, finally, maize cultivations on foothills. Six representative plots were selected in the area: a grasslands (P1) previously used as agriculture land and abandoned about 30 years ago; 4 pine-oak forest plots with increasing degradation down slope (P2, P3, P4, and P6); additionally a fuel-wood area (P4) that suffered a forest fire in 2000; and a recently abandoned agricultural land (P5) that followed a fallow management.

Soil samples (0-10 cm) were taken in each site and soil organic C (SOC) was determined. A particle-sized fractionation using ultrasonic energy allowed for the separation of the silt- and clay-sized fractions. Differential thermal (DTA) and thermic-gravimetry (TGA) analysis were performed on the clay- and silt-sized fractions of soil samples. Total weight loss by thermal combustion of samples between 200 and 600ºC was determined; in addition, relationships between the areas of the two main exothermic peaks (EP2/EP1) based on the first derivative of the TGA were calculated according the methodology proposed by López et al. (1).

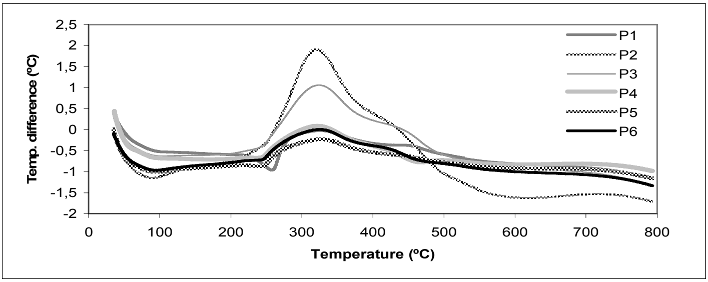
3. RESULTS AND DISCUSSION

- a) Clay-sized fraction: The first exothermic peak (EP1) detected for the clay-sized fraction was very similar for all sites (about 333 ºC; Fig.1); the second exothermic peak (EP2) only appears in P1 (455 ºC); samples of the other sites showed a shoulder more than a real peak. In general, the EP1 accounted for a higher area (i. e., weight loss) than the EP2, excepting the site P4. The best preserved forest area (P2) presented the highest EP1, followed by samples from the P3 and the other forest sites (P4 and P6); these last sites did not show significant differences between them. On the other hand, the weight loss associated to the EP2 was similar for all forest sites. Then, land-use affected the quality of organic C associated to the clay-sized fraction. The soil sample of the P1 (grassland) had a higher EP1, but the EP2 of the P5 (agricultural-land) was higher.

-b) Silt-sized fraction: Samples of the silt fraction showed two well-defined peaks. The EP1 was close to 345 ºC, and the EP2 ranged from 425 to 453 ºC. In this case, the P1 and the P5 presented significantly higher temperatures (>440 ºC) than the forest sites. The EP2 was, in general, wider, indicating that the silt-sized fraction contained more thermal-stable organic C. The height of the EP1 decreased in the order: P2 > P3 > P4 = P6; and that of the EP2: P2 > P3 = P4 > P6. Then, land-use affected more intensely the quality of the C included in the silt-fraction.

EP2/EP1 ratios (relation between the areas of the two exothermic peaks) are exposed on Table 1 and used as a measure of the aromaticity of the organic C (humification index; 2). C linked to the clay-sized fraction seems less aromatic and C associated to the silt-sized fraction was, in general, more aromatic (ratio >1.0). Then, the organic C associated to the silt fraction forms more stable and humified organic-mineral complexes. EP2/EP1 ratios of both particle-size fractions increased moving down slope, which means that the humification intensity of the SOM increases with the forest degradation. That means that labile C is lost when the human activities increase and only the more recalcitrant SOC remains.

a) Clay-sized fraction



b) Silt-sized fraction

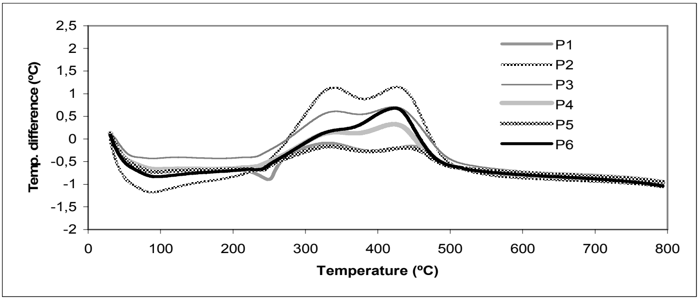


Figure 1. DTA lines of the clay-sized fraction (a) and of the silt-sized fraction (b).

Table 1. Ratios between peaks for the clay- and silt-sized fractions

Sites	Ratios	
Fractions	Clay-size fraction	Silt-size fraction
P1	0.39	0.61
P2	0.36	1.12
P3	0.52	1.36
P4	1.09	1.99
P5	0.70	1.43
P6	0.83	1.48

4. CONCLUSIONS

C associated to the silt fraction is more aromatic (more humified) than the C associated to the clay fraction. Thermal analyses showed that the labile C associated to both fractions is significantly affected by forest degradation. As a consequence of this, only the more stable SOC is stored in the more degraded forest soils. Land-use changes from forest to pasture affected both the amount and quality of C associated to the two considered fractions, but the agricultural use produced even a higher loss of the more thermally stable C associated to the silt fraction. As a consequence of this, a faster decrease of C is promoted by agriculture, remaining only the more recalcitrant SOC.

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Soil Organic Carbon Stocks and Humic Substances in Extremely Acid Soils, Sierra Norte de Oaxaca, México

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Keywords: mountain cloud forest, carbon sequestration

1. INTRODUCTION

The accumulation of organic matter (OM) in the decomposition is a characteristic feature of terrestrial ecosystems, and studies of OM will enable us to develop further management systems that sustain soil productivity. One of the more complex regions of Oaxaca is the Sierra Norte region that is situated at the northeast of the state, with an extension of 9,400 km^2. This study was conducted in the surroundings of Santa Cruz Tepetotutla, located in the catchment of Perfume river in the northern range of Oaxaca state (17°38’ - 17°40’ N, and 96°32’ - 96°33’ W). The lithology of the study area is represented mainly by mica-chlorite schists. This zone is characterized by abrupt topography with slopes ranging between 10 and 50º. The climate has been classified as hyper-humid, transitional temperate one, mean annual precipitation is 5800 mm and mean annual temperature (MAT) is 16.5°C. Vegetation along the gradient consists of a mosaic of undisturbed forest communities displaying an overall elevational arrangement, generally arranged by altitude, of premontane and montane forests. In this paper we present the characterization of the pools of organic carbon reserves, forest floor properties and the humic substances of the soils situated in an altitude gradient.

2. MATERIALS AND METHODS

Two stands at the elevations of 1520 and 2550 m asl. were studied, on each stand five plots were stablished of 25 × 4 m (500 m^2), measuring diameter breast high (DBH), basal area of the trees and stocks of biomass and edaphic carbon. One profile was collected on each stand. In the O layers from 5 plots, pH (H_2O and KCl solutions ratio 1:5) was measured, ash and loss-on-ignition (LOI); Na^+ and K^+ were determined in solution with H_2O and HCl. Previously soils were classified according to WRB1998 (1) as Histic Stagnic Cambisols (stand at 2550 m) and Histic Hyperdystric Cambisols (stand of 1520 m). In each soil sample pH (H_2O and 1M KCl with a soil:solution ratio 1:2.5), organic C (TOC) were determined by wet combustion. TOC reserves were calculated for every soil horizon by multiplying horizon depth to bulk density then the results were recalculated to $kg.ha^{-1}$, and summed for total soil and separately for mineral and organic horizons. Samples for fractionation of SOM according to the Ponomareva & Plotnikova method were selected from some soil horizons.

3. RESULTS AND DISCUSSION

The first site situated at the altitude of 2550 m asl. with a MAT of 11.5ºC, ranging from –2.0 to + 34.4ºC and tree vegetation represented by 22 species; the most important ones were Quercus ocoteifolia Liebm., Clethra galeottiana Briq., Persea chanissonis Vel. Alt. In this stand, 3 soil profiles were studied. All soils are extremely acid both in organic and mineral horizons. TOC content is high in organic layers of the forest floor, and relatively low in the mineral horizons. C:N ratio is > 10 in O horizon, and < 10 in mineral horizons. The content of exchangeable bases in all the horizons is surprisingly low, except in the forest litter. The site situated at the altitude of 1520 m, has a MAT of 15.4ºC, ranging from 4.1 to 34.4ºC. The stand had 52 tree species, among them the most important were Cyrilla racemiflora L., Ticodendron incognitum Gómez-Laurito & L.D. Gómez, Pinus chiapensis (Martínez) Andresen, with a high TOC content.

Conditions of the canopy of each studied stand (Table 1), indicate in general that stands differ in number and species distribution. At the stand 1 (2250 m) basal area without >60 cm BHD is 8.08 $m^2.ha^{-1}$ and 30.65 $m^2.ha^{-1}$ for stand 2.

Soil C stocks (Table 2) are distributed as ± 50% in the first 15 cm deep for the 2550 m stand. At 1520 m, 60% of the C stocks are at 30 cm deep. It emphasizes that DOC could be important on C distribution along the profile and also may be related with the increase in clay and silt contents with deep in this site.

Table 1. Canopy composition and density of the stands

DBH, cm	Stand 1		Stand 2	
	trees No.	%	trees No.	%
0 – 10	40	43.0	103	70.5
11 – 20	22	23.7	24	16.4
21 – 40	20	21.5	16	11.0
41 – 60	6	6.5	2	1.4
> 60	5	5.4	1	0.7
Total	93		146	
$Trees.ha^{-1}$	1860		2920	

Table 2. Stocks of Carbon in the study sites on the montane cloud forest

Forest stand	Tree biomass^{4,5}	Shrubs & herbs^{5}	Litter^{5}	Necromass^{5}	soil	Total
			Mg C. Ha^{-1}			
2550 masl	187.97	1.74	9.81	11.12	143.62	354.27
	113.31	1.41	6.23	9.97	36.71	
	60%	81%	63%	89.70%	26%	
1520 masl	109.67	0.55	14.17	54.48	227.09	405.96
	49.79	0.20	7.25	46.77	38.59	
	45%	37%	51%	85.9%	17%	

(1) mean, (2) standard deviation, (3) coefficient of variation, (4) <60 cm DBH, (5) Coversion factor of C=45

Table 3. Properties of the layers L (Oa) and F(Oe) of the organic horizons

Organic horizons		pH H_2O	pH KCl	ash	LOI	K^+ (H_2O)	K^+ HCl	Na^+ (H_2O)	Na^+ HCl
M asl		1:5		(%)		Cmol(+)kg$^+$			
Stand 2550	L	4.5	3.6	6.45	93.55	1.08	7.43	0.11	0.49
	F	4.1	2.5	7.97	92.03	0.69	26.82	0.12	0.27
Stand 1550	L	3.6	2.6	6.90	93.10	0.71	12.03	0.12	0.57
	F	3.3	1.9	14.87	85.13	0.68	16.11	0.11	0.38

Litter quality depends mainly of a few factors as soil nutrient supply, climate and tree species, although there are general differences between the groups of deciduous and coniferous trees, one difference may be their contents of hemicelluloses and lignin (2). Some key species are Na^+ and K^+, their content in soil - litter solution is also an indicator of the litter quality, therefore K^+ as a major nutrient can be used as an index cation of the litter richness (Table 3) in these sites with extremely low pH.

Soil pH values ranges are (2.8-4.3 in H_2O, and 2.0-2.8 in KCl); exchangeable bases contents are similar between both stands. TOC reserves in total soil profile, and in organic and mineral horizons ranges from 553.1-238.9 g.kg^{-1} . The density of organic C in mineral soil is almost the same as for organic layers (L and F). The trends of altitudinal distribution for C density both in mineral and organic horizons have the same shape as for total soil. At higher altitude more OC is stocked in organic layers (up to 71% at the 2550 m stand), and at the lower altitude the reserve of OC is slightly higher in mineral horizons (54%). Summarized, the soils of the studied ecosystem are extremely rich in organic carbon. OC densities range from 228 to 756 t per ha. Comparing the data with that reported for temperate, tropical, and boreal forests (96, 123 and 343 t per ha, respectively), we should conclude that the studied montane cloud forest is one of the richest in OC reserves in soil ecosystems. Humic substances (HS) showed an increase in OC from 7.7 to 9.28 g.kg^{-1} in Profile 1.1 organic horizon has 228.8 g. kg^{-1}TOC, with a C:N=25.6 and 14.3. FA are the main fraction in E and EB horizons, also in Profile 1.2, FA fraction represents more than a half of the TOC. Humins represents an important portion in their TOC of E and Bsg horizons. The main changes observed downward along the toposequence are: an increase in visible humus content with altitude. However, most of the properties considered to be important for vegetation, - acidity and exchangeable bases concentration-, show almost no difference with the altitude. We should conclude that acid, base-poor soils are characteristic for the entire ecosystem, and do not have any altitudinal gradient. We consider that complex distribution of C reserves along the toposequence reflects two competing tendencies. On one hand, the litter production should decrease with altitude (according with decreasing temperatures and, consequently, decreasing biological productivity), thus decreasing C densities. On the other hand, decreasing temperature and increasing soil moisture contents should limit microbial decomposition and mineralization of OM, thus favoring higher amounts of stocked OC. We can tentatively consider that a linear component of a square trend reflects the decrease in biological productivity with altitude, while square component reflects a consequent decrease in organic residues mineralization rate. Forest topsoils tend to produce more DOC and their leaches contains *bomb carbon* in different turnover times (3). Water transport carries DOM from the top soil into the deeper soil, and where this comprises mineral horizons, sorption of DOM generally occurs, although water by-pass processes can transfer some DOM directly to streamwater.

4. CONCLUSIONS

The studied montane cloud forest is one of the richest in organic C reserves in soil ecosystems. Acid, base-poor soils are characteristic for the entire ecosystem, and do not have any altitudinal gradient. C-stocks as humic substances are mainly FA and humin fractions in these soil ecosystems.

ACKNOWLEDGEMENTS

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Mechanisms of Soil Carbon Storage in Experimental Grasslands

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Keywords: carbon isotopes, dissolved organic carbon, soil organic matter, biodiversity

1. INTRODUCTION

It has been hypothesized and confirmed by recent experiments that plant biodiversity positively affects carbon storage in soils (1). Higher root biomass production in more diverse plots, however, has not been able to sufficiently explain these observations. It still remains unclear, what additional mechanisms driven by plant biodiversity are responsible for the increased carbon storage. Therefore, a time series of soil and soil solution samples was investigated at the field site of The Jena Experiment, a large-scale biodiversity ecosystem functioning experiment (2).

2. MATERIALS AND METHODS

In addition to the main experiment with C3 plants, a C4 species (*Amaranthus retroflexus* L.) naturally labeled with ^{13}C was grown on an extra plot. Changes in organic carbon concentration in soil and soil solution were combined with stable isotope measurements to follow the fate of plant-derived carbon into the soil and soil solution. A split plot design with plant litter removal versus double litter input simulated differences in biomass input, i.e. constant root biomass and varying litter.

3. RESULTS AND DISCUSSION

After 2 years the no litter and double litter treatment, respectively, showed that 381 g C m^{-2} and 263 g C m^{-2} were stored to a depth of 20 cm, while 71 g C m^{-2} and 393 g C m^{-2} were lost between 20 and 30 cm depth. The isotopic label in the top 5 cm indicated that 11 and 15 % of soil organic carbon were derived from plant material on the no litter and on the double litter treatment, respectively (Fig. 1).

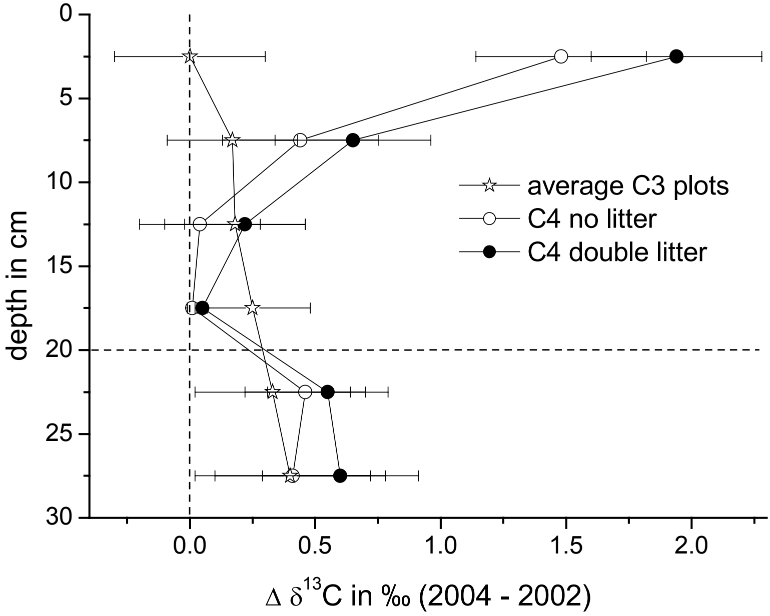

Figure 1. Shift of carbon isotope values in soil organic carbon between 2002 and 2004 averaged for C3 plots (stars) and in C4 treatments no litter and double litter (circles); data are calculated by δ13Csoil 2004 - δ13Csoil 2002. Error bars represent standard deviations between C3 plots and within the C4 plot, respectively. The dashed lines mark zero change (vertical line) and the border for observed carbon storage in the C4 treatments (horizontal line).

In the no litter treatment, this equals the total amount of carbon newly stored in soil, whereas in the double litter treatment this corresponds to twice the amount of stored carbon. Our results showed that higher litter input resulted in lower carbon storage and larger carbon losses and consequently accelerated turnover of already present soil organic carbon. Isotopic evidence indicated that present soil organic carbon was replaced by fresh plant-derived carbon near the input location, i.e. soil surface. Our results suggest that primarily carbon released from soil organic matter but not the newly introduced plant-derived organic matter was transported in the soil solution to deeper horizons and there contributed to the observed carbon storage (Fig. 2).

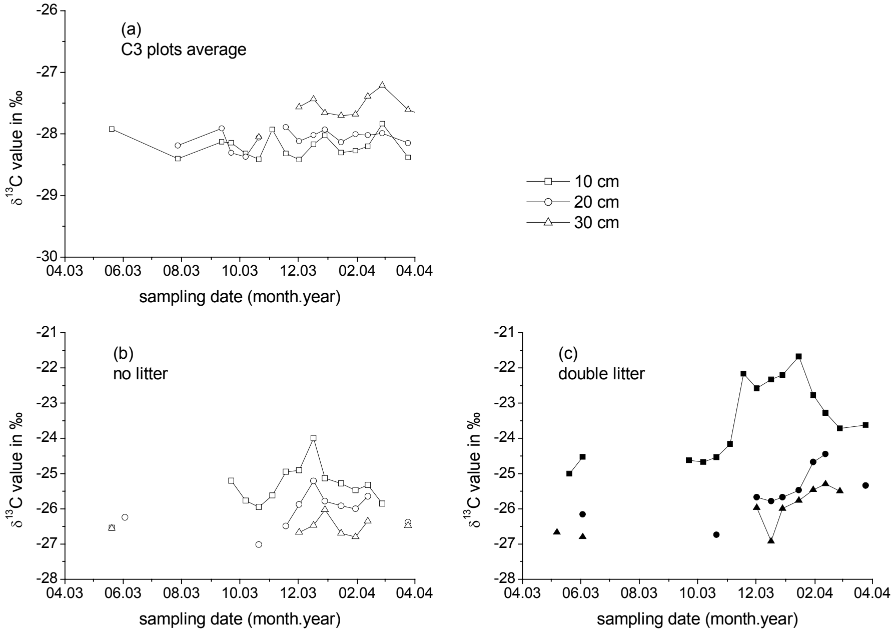

Figure 2. Seasonal variation in δ^{13}C values of DOC in different depths averaged for plots with C3 vegetation (a), in the C4 no litter treatment (b) and in the C4 double litter treatment (c).

4. CONCLUSIONS

Isotopic evidence on dissolved organic carbon proved that dissolved organic carbon collected deeper in the profile is originating from this depth and is not related to DOC collected near the surface. Consequently know changes in the chemical structure of DOV in depth profiles are caused by selective preservation or adsorption of compounds but by different sources of these molecules.

ACKNOWLEDGEMENTS

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The Content of Humic Substances in Ground Water from Agricultural Area in Opole Region, Poland

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Keywords: humic substances, organic matter, agricultural area, ground water quality

1. INTRODUCTION

The composition of surface and underground waters depends on geochemical properties of soils, the biological circulation of matter, the degree of mobility of chemical elements and compounds, velocity of biological sorption and the quantity of waters in a given area. These natural processes are modified by human activities, which result in introduction of certain components into the environment in excessive amounts. Changes in the natural proportions of ions and total quantity of elements in waters and bottom sediments very frequently are caused by inappropriate agricultural (2, 7, 8). Both components of fertilizers and compounds eluted from sorption complex of soils from rural areas are introduced into waters. Particularly dangerous are nutrients leading to eutrofization of surface waters and increased content of toxic substances (7). Biogenic substances coming from farms may be introduced into the environment from various sources: out flow of nutrients from excessively fertilized and watered fields, erosion wash-offs, where organic fertilizers where inappropriately stored, pollutants and sewage from farm households, etc.

The goal of these investigations was the determination of the influence of agriculture on the quality of surface and underground waters in the area called Opole Triassic region.

2. MATERIALS AND METHODS

Studies of water quality of ground waters in the Opole region were carried out taking samples from 20 wells (situated nearby households) in the period between 2002 and 2007 (3). Some of these wells are being used to water gardens, animals and so on. None of them is being used as a source of drinking water for humans.

The following parameters were determined in water samples: pH, conductivity, color, orthophosphorus dissolved, total phosphorus, COD-Cr, humic substances.

Conductivity and pH was determined with the use of electronic instruments: pH-meter and conductivity-meter. A spectrophotometer was used to analyze the color without prepreparation of water (wave length – 455 nm).

Total phosphorus was determined methods with acid mineralization and ammonium molibdate and zinc chloride as reductant. Orthophosphorus dissolved was determined spectrometric methods with ammonium molibdate and zinc chloride as reductant. COD-Cr was determined with ferroine as the indicator and Mohr salt as titrate. The aquatic humic substances were isolated with the XAD (XAD-8 resin) by the Aiken method (1, 6). Composition of humic substances was determined by IR spectroscopy taking the spectra in the 4000-400 cm^{-1} absorption region were taken.

In 2007 additional research was carried out to characterize the properties of humic substances dissolved in water. The proportion between humic and fulvic acids present the direction of transformation and stability level of organic pollutants put into the ground waters. These studies were conducted with the use of the method elaborated by Górniak (5). The ratio of absorption A_{260}/A_{330} gives a numerical value determining the quality of dissolved organic substances.

3. RESULTS AND DISCUSSIONS

In the analyzed territory ground water appearing near ground surface displays greater differences of temperature than deeper located water (Table 1). Despite differences in properties of water in various depths it presented pH neutral or slightly alkaline. Conductivity of this water was on average level and indicated the medium value of total dissolved solids.

Table 1.Some properties of investigated objects

Index	Unit	Deep wells to 3 m			Deep wells 3-12,5 m			Artesian well		
		up	to	average	up	to	average	up	to	average
Temperature	^0C	12.9	19.0	15.0	11.0	13.2	11.5	9.2	11.0	9.8
pH	pH	6.96	8.11	--	7.36	8.14	--	7.12	7.38	--
Conductivity	µS	415	951	699	490	884	654	720	736	730
Color	mgPt/L	7.2	169	36.3	17.0	43.3	32.3	36.0	44.0	40.0
TOC	mgC/L	0.96	22.2	5.71	2.82	5,28	3.76	1.56	1,8	1.71
A_{260}/A_{330}	--	2.14	4.25	2.77	3.16	3.60	3.44	2.60	2.67	2.65
$P_{disol.}$	$mgPO_4^{+3}/L$	0.08	19.2	2.31	1.17	3.70	2.52	0.06	0.07	0.07
Total P	$mgPO_4^{+3}/L$	0.24	19,6	2.92	0.40	1.52	2.10	0.70	0.74	0.73

The correlation between the value of water color and value of TOC is very high. The correlation indexes both for water from wells to 3m deep and water from wells above 3 m deep were 0.990 and 0.974 respectively. This permits working out a method for quick measurement of the content of TOC in ground water by the measuring color of water without complicated analytical procedures. To recalculate the color of water (presented in mgPt/L) into TOC (presented in mgC/L) the factor 7.39 must be used.

The obtained values A_{260}/A_{330} are within the range of 2.14-3.60. It means that this water lacks polymer particles and it does not include big amounts of carboxylic groups. Low values of this index indicate the low content of aromatic particles included in humic substances in this water.

The content of phosphorus in analyzed water is high. Acceptable value of phosphorus in ground waters which are being used for human activities should not exceed 5 $mgPO_4^{-3}/L$ and it was higher only in one of the investigated water wells. In other wells the quality of water was better, the content of phosphorus was lower than 2.1 $mgPO_4^{-3}/L$. In Poland it is a typical content of phosphorus in ground waters in agricultural areas. However, in surface water there should not be more than 1 $mgPO_4^{-3}$ /L (4).

4. CONCLUSIONS

1. The main threat for the quality of waters in this region are daily human activities and stock animals breeding. In the wells located on the farms the water was of lower quality.

2. Groundwater located nearby buildings not associated with farming shows more diversity in quality than water this found in farming grounds.

3. There is a high correlation between the depth of water surface and the amount of pollutants. The water in wells which have water surface less than 3m deep is more polluted than water in deeper wells.

4. The waters highly polluted by humic substances (with high value of color index) displayed the greater content of fulvic acids than humic acids.

5. The complicated analysis of TOC in groundwater can be simplified by using only the analysis of the color of water. The content of TOC is highly correlated with color of water.

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Espectroscopic Features of Humic Substances in Diagnostic Horizons from Andosols as Seen by FT-IR and ^{13}C CPMAS NMR

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Keywords: Andosols, melanic epipedons, humic acids, fulvic acids, FT-IR and ^{13}C CPMAS NMR

1. INTRODUCTION

Andosols, usually formed on a volcanic substrate, accumulates high quantities of organic carbon (5-20% weight), mainly in the form of stable humic acids. This organic matter stabilization is derived from the existence of short-range ordered minerals or noncrystalline components (allophane, imogolite and ferrihydrite) with large surface and considerable potential to form stable complexes with organic molecules (1). Therefore, andosols have large potential to sequester carbon. In order to obtain a better understanding of the processes responsible for such accumulation of organic matter, our objective was to compare the molecular features of the organic materials stored in humic acids extracted from a sequence of soils with andic properties (allophanic and nonallophanic) from the island of Tenerife (Canary Islands, Spain).

2. MATERIALS AND METODS

Three representative soils with more or less pronounced andic properties (“Ravelo”, “Siete Lomas” & “Frailes”) and a non-Andic Cambisol (“Tabaibal de Rasca”) were selected from Tenerife Island, Canary Islands. All samples were collected from the upper 10 cm of the soil A horizon. A complete description of the soils can be found in (2). Humic fractions isolation was done after Soxhlet extraction of the soil lipid fraction with petroleum ether. The soil residue was washed with water and then with 0.1 M $Na_4P_2O_7$ and 0.1 M NaOH. Total humic extract was precipitated with 6 M HCl to separate the acid-soluble FA from the acid-insoluble HA. The latter fraction was then dissolved into 0.5 M NaOH and centrifuged at 43,500 g, precipitated with HCl, dialysed in cellophane bags, and desiccated at 40°C.

FT-IR spectra were obtained in a Bruker IFS28 using KBr pellets (0,003 g in 0,20 g KBr). In order to facilitate the recognition of band patterns a mathematical conversion was applied (3). Solid-state Cross Polarization Magic Angle Spinning (CP-

MAS) ^{13}C NMR spectra were obtained on a Bruker DSX 200 using standard ramp-CP pulse program. Relative proportion of C functional groups was estimated by integration of the signal intensity of each spectrum over given chemical shift regions.

3. RESULTS AND DISCUSSION

Humic and fulvic acid improved resolution FT-IR and solid state ^{13}CNMR spectra are shown in Figure 1. The infrared spectra of the HA fraction show strong peaks at 1720 cm^{-1} (carboxyl band) and at 1610 cm^{-1} generally attributed to aromatic C=C stretching and COO$^{¯}$ symmetric stretching. After resolution enhancement the spectra showed a fairly well-defined band pattern with diagnostic peaks centred at 1510, 1420, 1340 and 1210 cm^{-1}, which is interpreted as the presence of a substantial domain of selectively preserved lignin (4). No substantial differences were found between soils but in the non-andic Cambisol ("Tabaibal") that showed a higher intensity in the alkyl stretching band (2920 cm^{-1}) and in the band attributed to O—H deformation and C—O stretching of phenolic OH (1340 cm^{-1}). The infrared spectra of the FAs show more variability between soils that that from Has. Soils with andic properties (Ravelo, Siete Lomas & Frailes) share the first section of the "fingerprint" region below 1800 cm^{-1} up to 1100 cm^{-1}; intense bands are found at about 1710 cm^{-1} (C=O stretching of COOH and other carbonyl groups), at 1610 cm^{-1} (aromatic C=C stretching and COO$^{¯}$ symmetric stretching), about 1390 cm^{-1} (O—H deformation and C—O stretching of phenolic OH and at about 1210 cm^{-1} (C—O stretching and OH deformation in acids or C—O stretching of aryl ethers). Below 1100 cm^{-1} difference in the relative intensity in the region 1080-1030 cm^{-1} are found indicating enrichment in polysaccharids or polysaccharide-like substances in the most evolutioned andic soil ("Ravelo"). The FA isolated from the non-andic Cambisol ("Tabaibal") show intense signals at 1210, 1150 and 1060 cm^{-1} indicating enrichment in aliphatic molecules, fatty acids and sugars that is in agreement with previous data obtained by thermal desorption and pyrolysis (2).

The main differences found when analyzing the soild state ^{13}C NMR spectra between HAs and FAs consists of a reduction in aromaticity (110-160 ppm) in the latter at expenses of the *O*-alkyl signal (46-110 ppm). When comparing between soils, humic and fulvic chemical composition varies with soil type. In accordance with FT-IR and Py-GC/MS results, a decrease in the alkyl region (0–46 ppm) of HA with the andic character of the soils is observed from 28.5% for the "Ravelo" soil to 39.2% for the non-andic "Tabaibal" soil. An increase in the carbonyl region (carbons in carboxylic and amide groups, 160-220 ppm) with soil andic character is observed for both AHs and AFs, with the signal for “Ravelo” showing 18.8% (AH) and 25.3% (AF) whereas that for the non-andic Cambisol (“Tabaibal”) 15.6% (AH) and 12.5% (AF).

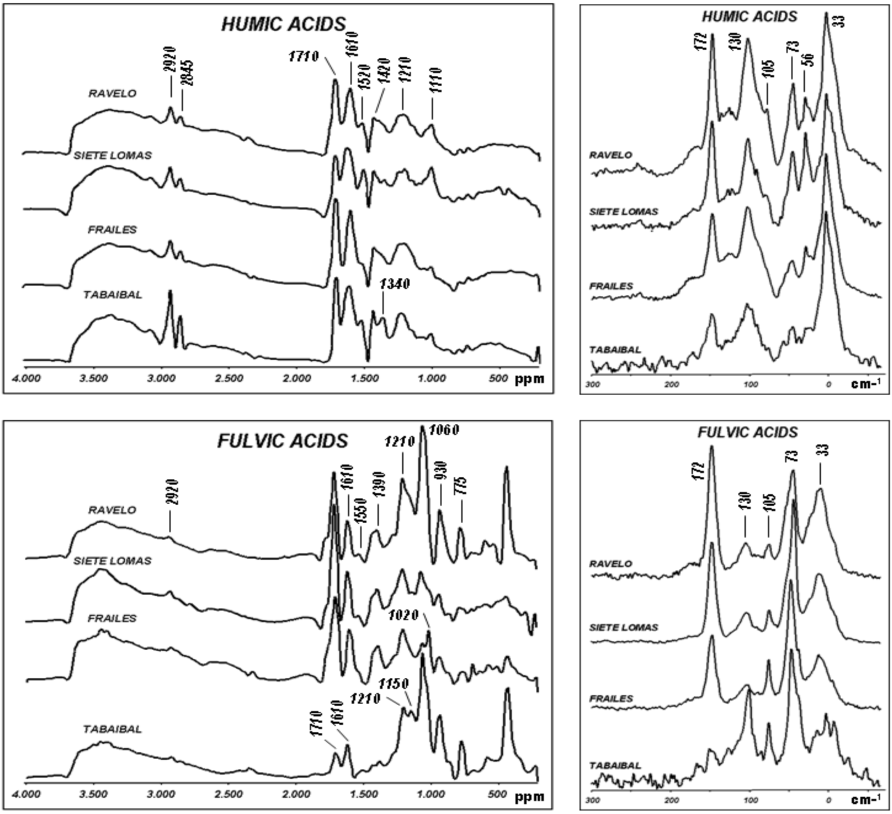

Figure 1: Improved resolution FT-IR and ^{13}CNMR spectra of humic and fulvic acids.

Spectroscopic differences observed between HAs and FAs from the studied soils may be related with the way SOM is stabilized through organo-mineral interactions (5 and references therein); the relative enrichment in carboxyl functions observed is possibly involved in stable complexes with the aluminium of allophane materials as previously suggested (6). Also electrostatic bonds can be established between reactive mineral sites and organic amide (7).

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Characterization of Humic and Fulvic Acids in Tropical Spodosols by FTIR and Fluorescence Spectroscopy

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Keywords: podzols, humic substances, fluorescence, FTIR

1. INTRODUCTION

There is a special interest among soil science researchers on studying Podzols, also called Spodosols. These are formed from quartz sandy parent materials and are characterized by a peculiar horizon sequence: an organic surface horizon O, a weathered grey eluvial E horizon, and a dark brownish–reddish illuvial B horizon, which is enriched by organic matter (OM), Al and/or Fe (can be Bh, Bhs or Bs). The OM plays an essential role in the formation of Spodosols. It has been generally accepted that the migration of humus particles from the surface and their accumulation in deeper layers are the main processes of podzolization. The transport of Al and/or Fe through the profile as organic complexes is the dominant mechanism of eluviation and podzolization. However, the genesis of the podzolization process is still being researched.

The aim of the present work is to characterize the humic (HA) and fulvic acids (FA) composition of Brazilian podzols by FTIR and fluorescence spectroscopy and their correlation with organic matter translocation in the podzolization process.

2. MATERIALS AND METHODS

Two Brazilian podzols were selected from sandy coastal soils (Restinga forests) in Cardoso Island, State of Sao Paulo, Southeast - Brazil. The profiles are part of a toposequence and are located 260 and 280 m near at the shoreline, respectively. The profile C14 has an E horizon 30 cm width and profile H13 has 8 cm. Table 1 shows some properties from those soils.

Table 1. Distribution of size fractions separated from soil samples and some of their chemical properties

Soil/ Horizon	Depth cm	Sand	Silt	Clay	pH H_2O	C/Me[1]	C	N
		-------------g kg^{-1}	----------				------g kg^{-1}	------
Profile H13 - Histic Alaquod								
Ho	0-10	n. d.	n. d.	n. d.	3.8	14.9	319.0	15.3
Hd	10-20	n. d.	n. d.	n. d.	4.0	40.5	241.0	5.4
A1	20-30	n. d.	n. d.	n. d.	4.2	40.5	68.9	1.0
E	30-38	970	0	30	4.3	0.0	4.7	0.5
Bhs1	38-55	970	0	30	3.3	13.5	30.3	0.9
Bhs2	55-75	960	0	40	3.2	11.1	15.5	0.4
Bhs3	75-130	950	10	40	3.3	6.1	8.8	0.5
Profile C14 - Typic Alorthod								
A	0-15	960	0	40	4.3	12.4	35.8	1.9
AE	15-20	980	0	20	4.3	16.0	2.2	0.3
E	20-50	980	10	10	5.0	0.0	0.3	0.3
Bhs1	50-58	920	0	80	4.0	14.8	37.4	1.4
Bhs2	58-75	900	0	100	4.0	10.4	64.2	1.9
Bhs3	75-100	920	10	70	4.8	2.5	17.5	0.8
Bs1	100-120	910	30	60	4.8	1.4	9.8	0.8

n.d: not determine

[1] $Me=Fe_p+Al_p$

Humic and fulvic acids were extracted using the IHSS method.

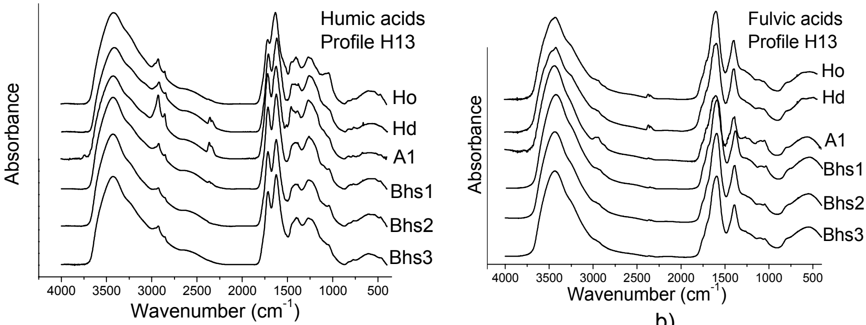
FTIR spectra were recorded on a Perkin Elmer, Paragon 1000 PC spectrophotometer using KBr pellets (1 mg HA: 100 mg KBr).

Fluorescence experiments were recorded on a Perkin Elmer LS 50B Luminescence spectrometer using $NaHCO_3$ (0.05 mg. L^{-1}) solutions of HA (20 mg. L^{-1}). Emission spectra were measured using λ_{ex} = 240 nm. The synchronous spectra were recorded using $\Delta\lambda$= 55mn. The scan speed was selected 200 nm min^{-1}.

3. RESULTS AND DISCUSSION

The soil particle size distribution of both profiles was similar. In all horizons, sand was the dominant fraction (> 90%), followed by clay and silt, corresponding with a sandy texture (Table 1). The soil pH was always very acidic, ranging from 3.2 to 4.8 in both profiles.

The FTIR spectra of the HAs are very similar along and across profiles (Fig. 1). In the FTIR spectra of HA from profiles H13 (Fig. 1a) the reduction of non-dissociated carboxylic groups and the increment of dissociated ones with depth can be observed analyzing the intensity at 1715 cm^{-1} and at 1627 cm^{-1}. The FTIR spectra of the FAs (Fig. 1b) show an increase in dissociated carboxylic groups with depth. This may be associated with an increase in metal complexation with Al^{+3}, as indicated by the C/Me ratio. This represents strong evidence of vertical drainage of OM throughout the profile, and below the E horizon that the OM therefore was formed by illuviation.

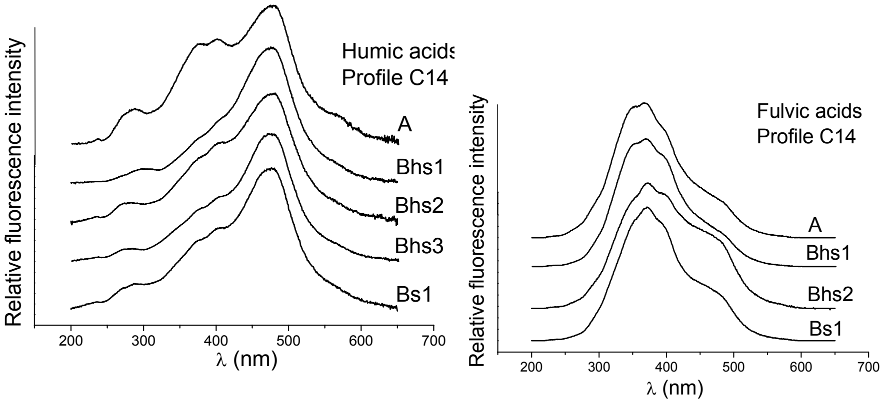


a) b)

Figure 1. FTIR spectra of HA (a) and FA (b) from Histic Alaquod (profile H13).

The content of COOH decreases with depth as observed by NMR results (1) suggesting a decrease of humification degree of OM downwards the soil profile.

Fluorescence emission spectra (λ_{em}= 240 nm) of HA from both profiles show a broad band, indicating a wider molecular heterogeneity and the presence of low and high condensated aromatic compounds. In contrast, the FA spectra show a narrow band. Figure 2 illustrates Fluorescence syncronous spectra ($\Delta\lambda$= 55 nm) of HA and FA from profile C14. The band of HA, which shows higher intensity is centered at 480 nm, in the region of more complex and humificated structures. On the other hand, FA spectra show the strongest band at 372 nm in the regions of less humificated compound. Thus, the HA show higher degree of condensation, conjugation and also humification than FA, and it decreases along the profile. The degree of humification of FAs increases with depth as can be seen by the increase of band at 475 nm. The humification degrees calculated by Kalbitz et al (2) and Zsolnay et al. (3) also support these results.



a) b)

Figure 2. Fluorescence syncronous spectra ($\Delta\lambda$= 55 nm) of humic (a) and fulvic acids (b) from Typic Alorthod (profile C14).

4. CONCLUSIONS

The HA and FA composition in both profiles were similar indicating similar OM dynamics. This is an evidence of vertical translocation throughout the profiles, with no incorporation of OM components from other sources. FTIR and fluorescence spectra showed HAs are more humificated than FAs and indicated a reduction of aromaticity degree of HA with depth. The increase in dissociated carboxylic groups with depth may be associated with an increase in metal complexation with Al^{+3}, as indicated by the C/Me ratio. This is strong evidence of vertical drainage of OM throughout the profile and below the E horizon indicating that the OM was therefore formed by illuviation.

ACKNOWLEDGEMENTS

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Distribution of Water Soluble Organic Nitrogen (WSON) in Acidic Forest Soils, Southwestern China

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Keywords: water soluble organic nitrogen (WSON), acidic forest soils, N deposition

1. INTRODUCTION

Atmospheric nitrogen deposition has been a world wide environmental problem. Accelerated N deposition could significantly alter internal soil N cycling and lead to nitrogen leaching (1). Water soluble organic nitrogen (WSON) was an important nitrogen fraction leached from forest soils (2). Though its percentage in total dissolve nitrogen (TDN) decreased with N deposition, the leaching flux of WSON seemed increase with nitrogen deposition in forest areas (2, 3). So far little is known on the geochemical processes of WSON in soils, because most focuses were put on the dynamics of inorganic nitrogen compounds in the previous studies.

In some areas southern and southwestern China, N deposition was even higher than the values reported in Europe and North America (i.e. 25–38 kg $ha^{-1}yr^{-1}$) (4). N deposition may have already brought some influences on the soil N processes in this subtropical region. However, we have little information about WSON in these acidic soils. In current study we presented the distribution of WSON in acidic forest soils, based on both field monitoring and laboratory soil extraction.

2. MATERIALS AND METHODS

Soil and water samples used in this study were collected from LuChongGuan (LCG), LeiGongShan (LGS) and TieShanPing (TSP) forest catchment, respectively. At these three catchments, located in southwestern China, long term monitoring on the chemistry of precipitation, soil water and stream water was run by Sino-Norwegian IMPACTS (Integrated Monitoring Program on Acidification of Chinese Terrestrial Systems) project since 1999. Detailed catchment information and monitoring procedures of each catchment can be found in IMPACTS annual report (5) and manuals (available on: http://folk.uio.no/rvogt/Impacts). There were two kinds of soil solution (i.e. soil water and water extract) used in this study. Equipments for soil water sampling were located in 5 - 8 soil water plots in each catchment. In each of these plots, 3 - 4 ceramic tension lysimeters (Ceramtec, Germany) were located at different soil depths according to genetic horizon (i.e. A, AB, B and BC horizon). Soil waters from all lysimeters were sampled weekly. The water extracts were obtained by adding 100 mL deionzied water to 20 g fresh soil, and then the slurries were shaken for 5 h. Water extracts were frozen after pass 0.45 μm filter until chemical analysis. For soil water and stream water samples, NO_3^- and NH_4^+ were determined by Ion Chromatography (Dionex, DX 120) in the IMPACTS qualified local laboratories. For water extracts, nitrate (NO_3^-+NO_2^-) and NH_4^+ were analyzed using international commonly used colorimetric methods after de-freezing. For all the water samples, total dissolved nitrogen (TDN) was determined by ultraviolet colorimetric method, after digestion using alkaline $K_2S_2O_8$. The difference between TDN and inorganic nitrogen was calculated as WSON.

3. RESULTS AND DISCUSSION

The distribution of nitrogen species in soil water is shown in figure 1a. Total dissolved nitrogen (TDN) at TSP site ranged from 370 μmol/L in AB horizon to 710 μmol/L in A horizon, and was significantly higher than that at LCG and LGS site receiving lower N deposition (Table 1). Percentage of WSON varied with different sites and soil horizons. At LCG and LGS sites with lower nitrogen deposition, WSON was very important with percentage higher than 30%. In the B horizon of LGS sites, WSON even reached 59% of TDN. At TSP site with higher nitrogen deposition, WSON was not so important with percentage of about 5%. In addition, percentage of WSON generally increased with soil depth, except TSP site.

Figure 1b shows the nitrogen distribution in water extracts. Compared with soil water, there were three significant differences in the nitrogen distribution. Firstly, the site variation in TDN of water extracts was not as large as soil water. TDN at LGS site was little bit higher than that at LCG and TSP sites, which may be due to higher soil nitrogen content in LGS site (Table 1). Secondly, in all horizons NH_4-N reached to about 15% of TDN. This was likely due to the dissolution of weakly bound NH_4^+ during shaking and extraction. Thirdly, WSON in water extracts increased significantly in all four TSP horizons with percentage of about 30%, compared with soil water. At LGS and LCG site, percentage of WSON did not changed so much.

WSON was also important N species in stream water (Table 1). Its percentage, decreasing with N deposition, was 67%, 37% and 13% at LCG, LGS, and TSP, respectively. Such distribution was similar with that in soil water, but different from that in water extract. Leaching mobility of WSON is usually related with its locality in soil. WSON in soil water was transported through macro-pores, whiles most WSON in water extracts was located in micro-pores or precipitated on soil surface, with lower mobility. The lower WSON percentage in soil water may be one of the reasons for lower stream water WSON at TSP site. However, WSON in water extracts may be soluble and leached in certain hydrological and physiochemical conditions. Leaching of these water extractable WSON compounds may result in significant seasonal variations in stream WSON.

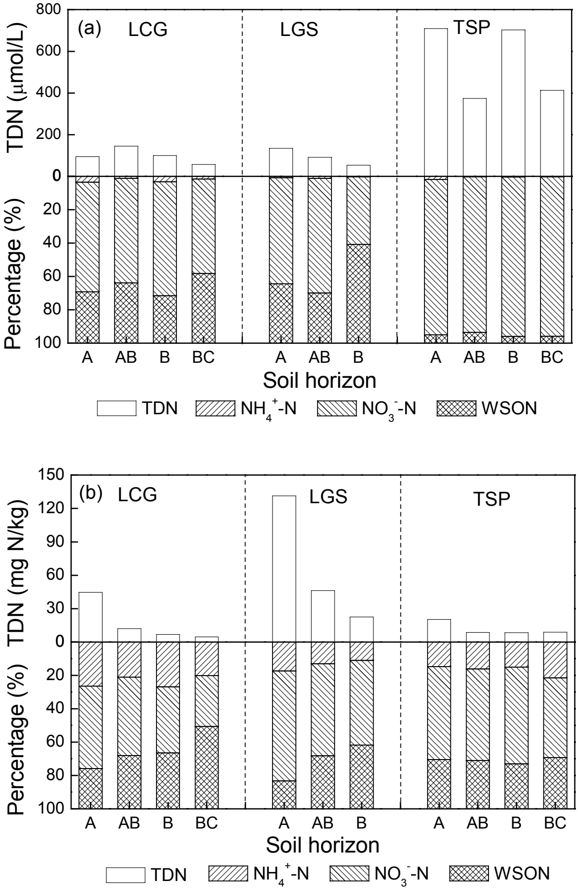

Figure 1. Distribution of water soluble nitrogen species in different soil horizons.
a): soil water; b): water extract.

Table 1. Some related information about three catchments

Site	N deposition g N/m^2/yr	Soil			Stream water	
		pH	Org. C %	TN %	Inorg. N μmol/L	Org. N μmol/L
LCG	0.70	4.27±0.24	4.40±6.17	0.25±0.33	16.87±13.07	33.73±12.51
LGS	0.93	4.81±0.49	7.07±4.95	0.54±0.25	26.60±8.31	15.88±8.41
TSP	1.93	4.22±0.23	5.30±12.92	0.20±0.43	65.18±18.52	9.73±13.82

4. CONCLUSIONS

Besides well known inorganic nitrogen, water soluble organic nitrogen (WSON) was an important nitrogen fraction in Chinese acidic forest soils. In lysimeter soil water and stream water, WSON percentage in TDN decreased with N deposition flux. In water extracts, percentage of WSON was less dependent on N deposition. WSON compounds obtained by different ways may have different roles in the WSON leaching from these acidic forest soils.

ACKNOWLEDGEMENTS

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Evolution and NOM

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Keywords: natural organic matter, humic acids, functional recalcitrance, lignin-free environment

1. INTRODUCTION

The earliest forms of life on Earth were aquatic, non-vascular plants which did contain carbohydrates but not lignin. These lignin-free, non-vascular plants have formed natural organic matter (NOM) in soils already in the Late Ordovician and Early Silurian ages.

Formation of lignin was an essential evolutionary step to support the vascular plant structure for terrestrial life. Vascular, lignin containing plants were present in the Early Devonian time but only during Carboniferous time they started dominated the landscape. In the time scale of the evolution of photosynthetic organisms lignification is a relatively recent process.

2. MATERIALS AND METHODS

This is a deductive study of the humification from the evolutionary point of view: evaluate the importances of lignin and carbohydrates as contributors to the NOM and HA while keeping in mind that carbohydrates are evolutionary older than lignin, and that humification is practically as old as carbohydrates.

3. RESULTS AND DISCUSSION

An important feature associated to HA is their recalcitrance. This is thought to be derived from lignin constituents in HA, and taken as a proof about the aromatic nature of HA. On the other hand, carbohydrate structures are thought to be non-recalcitrance, and rapidly consumed by microbes during humification.

At present, aquatic plant forms still do not contain lignin. So in lignin-free environments, humification, one of the major pedogenic factor, has been running through the eons with the same original mechanism from the very beginning. This suggests that carbohydrates have recalcitrance in humification.

Some microbes start the humification process, others continue it. These microbial populations have a succession, and new microbes are not only utilizing the original biomass, they utilize remnants of the previous populations/generations as well. This recycling through the microbial metabolism provides a kind of functional recalcitrance to the carbohydrate structures.

Actually, the carbohydrate structures have chemical resistance as well. In alkaline pulping wood polysaccharides are attacked by strong alkali solutions at elevated temperatures, and several peeling and hydrolytic reactions are taken place. Peeling reaction, which is leading to the loss of terminal polysaccharides, is actually protecting the rest of the cellulose molecule. Further reactions are stopped and so cellulose can be extracted as long fibers. Lignin, on the other hand is soluble under these conditions.

Many researches have already proved that aerobic microbes are degrading lignin. However, it is still thought that aromatic constituents are not degraded by microbes in anaerobic environments.

Recent biotechnology studies, however, have shown beyond doubt that aromatic structures are degrading in anaerobic conditions (Roman, 2004). Lignin, actually, does not have such recalcitrance in dead material that it is supposed to have. Additionally we need to observe that in humification lignin has a non-returnable, disposable nature: no microbial mechanism is producing new lignin in the system. Particularly in this aspect lignin is more fragile than carbohydrates, its only fate in humification is to be consumed.

4. CONCLUSIONS

It is relevant to suppose that when lignin was emerged, the humification process accepted is as one additional material to consume rather that lignin fundamentally would have changed the process. It seems that 1) the recalcitrance, and importance of lignin in humification has been overestimated, and 2) those of carbohydrates has been underestimated.

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Long Term Trends of DOC and Colour in Raw Water from a Forest Lake Caused by Increased Precipitation and Changed Precipitation Chemistry

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Keywords; humic, colour, climate, acid rain

1. INTRODUCTION

The forested area Østmarka near the city of Oslo is the catchments area for one of the major water utilities serving the capital. A study of raw water from the reservoir and selected minor lakes in the area shows that the water quality has changed significantly from 1983 to 2007.. We will here propose and discuss mechanisms of how precipitation quantity and quality influences coloured dissolved organic matter (DOM) species

2. MATERIALS AND METHODS

Lake Elvåga is a drinking water reservoir situated within Østmarka, a forest area neighbouring Oslo city. The lake has a surface area of 1 km^2 and theoretical retention time about two years. The waterwork's data series for raw water extracted from 40 m depth dates back to 1983.True colour and DOC have been measured with up to daily resolution . We have also sampled a selection of other lakes in Østmarka with variable hydrological characteristics Size separation of samples from the autumnal turnover are performed with Amicon Ultra Centriprep YM-10 filters. DOC in filtrates is determined with a Shimadzu TOC-V analyzer True colour of filtered samples is determined according to (4)

3. RESULTS AND DISCUSSION

Our study shows that the water quality in the reservoir an the other lakes has changed significantly with decreased concentrations of sulphate and increased consentrations of DOC and true colour (Fig. 1) From correlations of these trends with fluctuations in precipitation and long time trends in deposition of strong acid anions in precipitation (especially acid rain related sulphate) we propose mechanisms of how precipitation quantity and quality influences coloured dissolved organic matter (DOM) species.

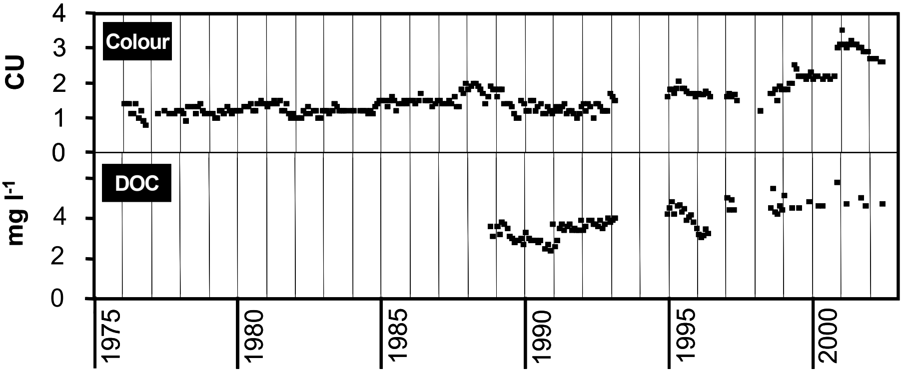

Figure 1. Temporal changes in colour and DOC concentrations in raw water at the Skullerud treatment plant (3).

First: Inter annual changes in concentration of DOC and the specific colour of DOM in the studied surface waters are basically driven and explained by variations in amount of precipitation.(3) DOM in the lakes are generally more coloured in wet than in dryer years. Size separation analyses demonstrate that the DOM with high nominal molecular weight (>10 000 Dalton) have higher specific colour (Colour/DOC) than low molecular weight compounds (Figs 2 and 3).

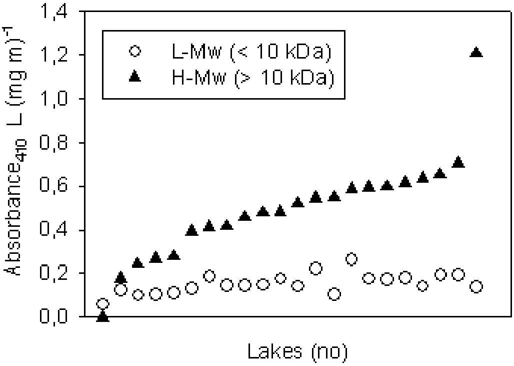

Figure 2. Specific absorption (SA_{410}) for the nominal high molecular weight fraction of DOC (> 10 kDa) and low molecular weight fraction of DOC (< 10 kDa) in lake samples from 2005. The results are arranged according to increasing specific absorption for the high molecular weight fraction.

Climatic change towards more frequent episodes with heavy rain and higher concentrations of high molecular weight DOM species leached from the upper forest floor during high precipitation episodes can, therefore, explain a development towards more coloured lake waters (2)

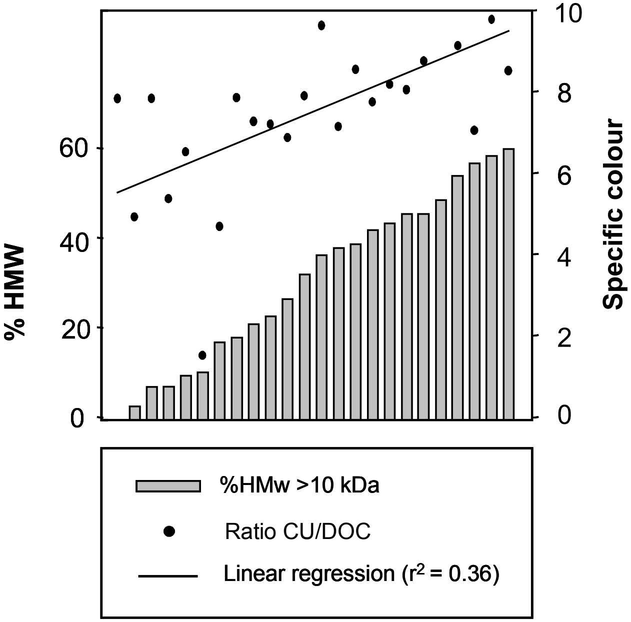

Figure 3. Percentage of nominal high molecular weight NOM (>10000 Da) and Colour/DOC ratio for in lake water samples from autumn turnover 2004 (2).

Secondly: The recent decline in acid deposition, and reduction in sulphur concentration and ionic strength in precipitation, influence important mechanisms for leaching and precipitation of DOM. This appears as a co working reason for the long term increased DOC and water colour in Østmarka.We have also studied an expanded dataset for the years 1986-2005 for 77 lakes all over Norway which were sampled and analysed annually for major ionic constituents, aluminium fractions and TOC (5). In this study we see that the lakes with a high sulphate/chloride ratio had the most substantial increase in TOC over the 20 year period. These lakes are in general the ones with the lowest ionic strength that also had a marked reduction in sulphate concentrations due to the acid rain decline. Furthermore, we see substantial yearly variations in several of the lakes, mainly in those with large natural fluctuations in chloride (seasalt) concentrations (1, 2, 4).

ACKNOWLEDGEMENT

We thank the research council of Norway for funding the project: “Effect of climate change on flux of N and C: air-land-freshwater-marine links (CLUE), no 155826” (www.climatechange.no/clue). We appreciate technical support at the laboratories of Department of Plant- and Environmental Sciences, Norwegian University of Life Sciences, Oslo Water and Sewage Works,and the Norwegian institute of Public Healt. Per Kristian Lunde and Ingvild Riise Lunde are acknowledged for pleasant assistance during fieldwork.

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Role of Aromaticity Degree in the Stability of Humic Substances

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Keywords: humic substances, aromaticity, thermogravimetry, isoconversional methods

1. INTRODUCTION

Because of global warming and other environmental problems, the reduction of greenhouse gases is of worldwide interest. Such process demands fundamental understanding of carbon (C) stabilization in soils because the amount of organic matter stored in soils represents one of the largest reservoirs of organic carbon on the global scale. The mechanisms for carbon stabilization in soils are still not well understood and thus the ultimate potential for C stabilization in soils is unknown (1).

Both soil fertility and stability are related to its organic matter presence. Many functions of soil organic matter (SOM) are due to its stable fraction, the humified materials, and to its balance with the labile fractions. Changes in SOM content are related to changes in microbial biomass turnover, because they reflect the balance between rates of microbial organic matter accumulation and degradation. However, SOM as a whole responds less quickly to changing soil conditions than microbial biomass. Therefore, the nature of the organic matter itself, rather than its concentration, may be more sensitive indicator of the changes in soil quality. Chemical and structural characteristics of humic substances (HS) are known to be better predictors of the rate of SOM turnover than SOM content itself. (2)

The main fraction of organic matter contained in soils, peats, sediments, low-rank coals (leonardites, lignites) and natural waters consists of humic substances, which comprise a complex mixture of both aromatic and aliphatic moieties, having a large number of functional groups (3).

Processes in condensed phase are extensively studied by thermoanalytical methods. Mechanisms of these processes are very often unknown or too complicated to be characterized by simple kinetic model. To describe their kinetics, the methods based on the single-step approximation are often used, either the model-free or model-fitting ones. It is generally recognized that the rate of the processes in condensed phase is a function of temperature (T) and conversion (α). The rate of the complex multi-step condensed-state process can be formally described as

$$\frac{\mathrm{d}\alpha}{\mathrm{d}t} = k(T)f(\alpha), \tag{1}$$

where $k(T)$ is temperature function and $f(\alpha)$ conversion function. Basic assumption is that these two functions are separable, independent of each other. The temperature function in Eq. (1) is mostly considered to be the rate constant (depending solely on temperature T) and the conversion function is considered to reflect the mechanism of the process (depending solely on conversion α). (4, 5)

With only for few exceptions, the temperature function is expressed by the Arrhenius equation

$$k(T) = A\exp\left[-\frac{E}{\mathrm{R}T}\right], \tag{2}$$

where A and E are considered the pre-exponential factor and the activation energy, respectively, T is the absolute temperature and R stands for the gas constant.

The evaluation is carried out at fixed conversion. The use of isoconversional predictive procedure is huge; it has already been successfully employed also in studying humic substances. (6)

2. MATERIALS AND METHODS

The samples of humic substances extracted according to IHSS procedures included 3 fulvic and 9 humic acids. Their origin varied from lignites and leonardites to soil HS (cambisols, podzols). The aromaticity of samples was determined by ^{13}C LS NMR analysis and varied from 25 to 70 %.

Thermogravimetric analyses of all samples in the dynamic air atmosphere were performed. For this purpose TA Instruments TGA Q 5000 IR was used. Samples were measured in an open crucible at 7 different heating rates up to 600 °C.

3. RESULTS AND DISCUSSION

The thermogravimetric records showed two or more steps of weight losses, the first one attributable to loss of water, whereas others to degradation of organic matter. The latter steps were used to assess the stability of examined samples. The mass losses obtained at different heating rates were recalculated to conversion intervals (0–100 %). From this interval, several conversions were selected and for those the stability for 100 °C was calculated using Arrhenius temperature function. This temperature was adopted because some problems may appear when data obtained at higher temperatures are approximated to the lower ones. The reason can be seen, among others, in morphological differences in the sample at high and low temperatures or simply in introduction of different reactions occurring exceptionally at elevated temperatures (6). Values of stabilities served for comparison between samples and helped in elucidation of role of aromaticity during the humic substances degradation.

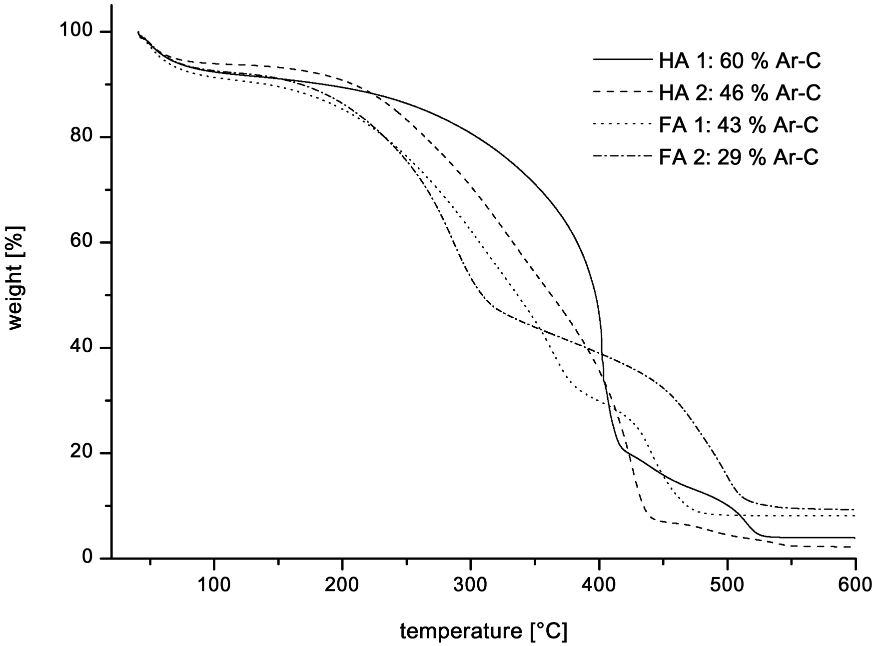

Figure 1. Degradation patterns of 4 different humic types at heating rate 10 K/min.

Figure 1 displays degradation patterns of four distinct humic substances (2 humic and 2 fulvic acids) with different degrees of aromaticity. The differences indicate that changing content of aromatic carbon profoundly affects the rate of degradation (i.e. kinetics and thus stability) in degradation progress. It is generally accepted that degradation proceeds from labile parts of humified matter to stable constituents represented by aromatic and heterocyclic cores.

Values obtained from the recalculation of temperatures from Fig. 1 imply that high degree of aromaticity plays a role mainly at the beginning of degradation. As suggested elsewhere (7), humic molecules tend to recombine and therefore the introduction of new, more stable molecules is probable. Moreover, samples with higher aromaticity degree showed a sudden increase in kinetics of degradation at higher conversions. Sample with the highest aromatic content (HA 1) shows initially slow degradation, which later accelerates. The explanation is as stated above, first the low amount of aliphatic parts and after that the aromatic structures degrade. HA 2 and FA 1 have almost the same aromatic C content and also their degradation kinetics profiles are practically similar. On the contrary, FA 2 is the least aromatic sample and its degradation in the beginning is fast but than significantly slows down and gradually continues up to high temperatures. In this case the recombination processes and thus formation of stable, originally absent molecules is most probable.

4. CONCLUSIONS

Summarizing all the results, it can be seen that the aromaticity itself is not the absolute indicator of stability of humic materials but can be helpful in assessment of soil organic matter erosion and help in development of remediation and soil restoration techniques.

ACKNOWLEDGEMENTS

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Humic Acids Structure as a Reflection of Bioclimatic Factor of Soil Formation in Boreal Ecosystems

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Keywords: climatic belts, humic acids, elemental composition, optical density

1. INTRODUCTION

The given paper is a part of joint work conducted in the frame of bilateral scientific exchange between CCMA CSIC and ISSA SB RAS.

At present the fact is beyond of any doubt that factors involved on humus formation and soil formation are absolutely the same. As a product of soil formation soil humus is known to be as a system of macromolecular, polydisperse heterogeneous and heterochronous substances. Its accumulation in soils, its nature and properties and the structure of its specific constituents such as humic acids (HA) and fulvic acids (FA) which are closely related to the type of soil formation, in particular to bioclimatic conditions (1). Thus, the changes in climate and humus-forming material should be reflected in humus accumulation and its nature. Such a regularity is especially clearly pronounced by the example of automorphic soils of the northern hemisphere where in the direction from the north to the south the increase in aridization degree is rather sharply revealed.

2. MATERIALS AND METHODS

The experimental design was based on sampling from soil sequence ranging within the limits of various bioclimatic belts of the Western Siberia. The soils under study (Table) develop under contrasting bioclimatic conditions of the 8-th belt of continentality (2). While determining humus reserve in 1-m soil layer the soils of semiarid area of Central Spain (Vertic Luvisols) have been also taken into consideration as a typical object of an extreme arid link of the same 8-th belt of continentality (2). The isolation and qualitative analysis of humus fraction such as carbon ratio of HA and FA, elemental analysis of HA were carried out in ISSA SB RAS by generally accepted procedures described elsewhere (3). The values of spectroscopic characteristics of HA in the visible range (E4 and E6)/E6) were determined in laboratories of CCMA CSIC and ISSA SB RAS from solutions of 200 mg

CL^{-1} in 0,02 M $NaHCO_3$. Degree of aromaticity (DA) was calculated based on data of elemental analysis of HA by modified graphical statistical Van-Krevelen method (4). The moistening coefficient (C_m) was taken from the literature (5, 6). It is generally agreed that this coefficient being as a ratio of precipitation and evaporation is a convenient and representative one in comparative geographical studies of humus formation although, as it was shown later (6), it is presumably of theoretical significance.

3. RESULTS AND DISCUSSION

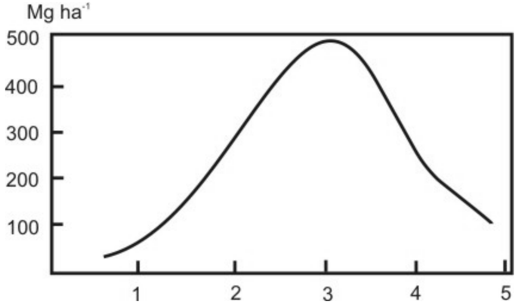

Figure. Distribution of humus reserve in 1-m soil layer of the Northern Hemisphere. 1- Tundra, 2 - Boreal forests (taiga), 3 - Forest steppe, 4 – Steppe, 5 – Dry steppes and semideserts.

As it is evidenced by Figure, humus accumulation is definitely dependent on bioclimatic conditions. As for the Western Siberia, the maximum values are typical for the belt with optimal bioclimatic situation (Cm around 1), i.e. for forest steppe. High humus accumulation is here favored by concentration of grass phytomass in soil layer (up to 90% of total), over-ground part is of subordinate significance. It is significant to note that excessive moisture and deficiency of heat (tundra, taiga) as well as excessive heat and deficiency of moisture (steppe, semideserts) produce practically the same effect.

As it is evidenced by the data of the Table, the increase in aridity within the limits of the same belt of continentality is a main reason of the change of humus nature as a whole and its constituents, HA in particular. Qualitative composition of humus, as a rule, does not depend greatly on the magnitude of humus accumulation. Specifically, one of the main characteristics of humus quality, for example carbon ratio of HA and FA (HA/FA) increases with increase in aridity that is quite natural. It is well known that nonpercolative water regime and calcium deficiency in biological cycle are an important factor to favor HA formation. Other indices of HA structure proved to be rather informative as well as to show its dependence on bioclimatic situation.

Table. HA/FA and basic structural indices of HA of A soil horizon

Soil, location	HA/FA	Atomic H/C	E4/E6	DA,%	Cm
Epigenic Albeluvisols, taiga (soils of Podzol type)	0,4-0,6	1,2-1,4	4,3-5,0	≤20	≥2,0
Albic Luvisols, forest steppe (soils of Grey forest type)	0,8-1,5	1,0-1,1	3,6-4,0	20-25	1,0-1,2
Luvic Chernozem, forest steppe (soils of Chernozem type)	1,8-2,5	0,8-0,9	3,3-3,5	25-30	8,8-1,1
Kastanozems/Calcisols (steppe Chestnut soils)	1,5-1,8	0,6-0,8	3,3-3,5	30-35	0,4-0,6

For example, the decrease of the H/C values in the direction from the north to the south indicates the decrease of the role of H in the structure of HA and respectively to increase in degree of HA polymerization (polycondensation). This fact is in accordance with the increase in climate aridity in soil sequence under study. Such a regularity conforms to other structural indices of HA such as values of E4/E6 and degree of aromaticity (DA HA).

According to universal scientific experience, the lowering of E4/E6 can formally witness to the fact that on increasing aridization the concentration of aromatic nuclei, molecule size and molecular mass also increase to some extent.

DA HA is a representative index on soil type level in natural virgin soils where the processes of decomposition and new formation of organic matter are practically in equilibrium (4). It is also representative index for HA of agricultural soils affected by human impact, for example as a result of prolonged practical activity (4). Corresponding data given in the Table testify that increase in the role of thermal factor favors to increase in DA HA.

In environmental respect the increase in DA can not be considered as being favorable phenomenon. It has been shown by laboratory and field (fire effect) experiments that heating leads to an increase in aromaticity of humus acids and removal of oxygen-containing groups (7-9). In fact, even FA are transformed into acid insoluble organic matter similar to HA, and HA undergo further dehydratation and decarboxylation, becoming as humin-like fraction resembling kerogen or the so-called black carbon material.

4. CONCLUSIONS

Humus is known to be for the most part a product of soil-forming process, therefore the bioclimatic conditions of especially contrasting belts leave a mark on its accumulation as a whole and on the nature of its principal constituents, HA in particular. Some structural indices of HA are greatly influenced by thermal factor as it was shown by our example with increase in aridity. The data obtained permit us to arrive to an idea that under condition of increasing global warming HA because of increase in DA can lose bioprotective function and can be converted into inert material.

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Bioluminescent Monitoring of Detoxification Processes

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Keywords: detoxification, bioluminescent assay, quinones, radionuclides

1. INTRODUCTION

It is known that bioluminescent bacteria and enzymes isolated from these bacteria are used as ecological assay systems. They are convenient objects to study remediation processes. Luminous bacteria and their bioluminescent enzymatic reactions are considered as the simplest models of living organisms. This is a reason of their application as bioassay, mainly in ecological investigations. Advantages of this assay are simplicity, fast response, and no requirement for highly organized organisms.

Generally, the main feature of all bioassays is integral response, hence, they could be applied as alarm tests, indicating habitat unfavourable for life. The results of bioassay reveal the necessity of a detailed chemical assay, which is more expensive and time-consuming than the biological one (1).

Microorganisms, luminous bacteria included, are currently preferable in ecological monitoring as bioassays (1-2). The main principle of the bioluminescent assay is to correlate toxicity of samples and the changes in bioluminescent intensity.

Advantages of bioluminescent assays are simplicity fast response (1-3 min), sensitivity; bioluminescent assays are not expensive; they do not use highly-organized organisms. There exists a possibility to use bioluminescent assay systems of different organization level, i.e. *in vivo* or *in vitro*. The last position is very important because it provides possibility to compare toxic effects on the levels of whole cells and enzymatic reaction. This means that we can discuss mechanism of a toxic effect.

One more important point deals with the fact that bioluminescent assay systems are a basis of bioluminescent biosensors which appeared to be very promising now.

In paper (3) the bioluminescent assay *in vivo* was applied to evaluate detoxification efficiency under combination of the detoxification factors – UV and VIS irradiation, humic substances' and microorganism' treatment. Proper detoxification factors and conditions were found. In (4) the bioluminescent assay systems were shown to be sensitive to the solutions of alpha-radioactive elements (with Americium-241 taken as an example) of low and mean activity.

The aim of the paper is to demonstrate a potential of bioluminescent assay systems in study of detoxification processes. Organic oxidizers (quinones) and alpha-radioactive compounds were taken as examples of toxic compounds.

2. MATERIALS AND METHODS

A series of quinones with different redox characteristics: 1,4-benzoquinone, tetrafluoro-1,4-benzoquinone, methyl-1,4-benzoquinone, tetramethyl-1,4-benzoquinone, and 1,4-naphtoquinone, was used.

The toxicity of quinone solutions was assessed using the bioluminescent assay *in vivo* - Microbiosensor 677F, based on the lyophilized luminous bacterium *Photobacterium phosphoreum* (Krasnoyarsk, Russia). The influence of 50μl of the quinones on the bacteria was studied in 0.5 ml of 3% NaCl solution.

Solutions of $^{241}Am(NO_3)_3$ and $(UO_2)(NO_3)_2$ were used as sources of α-radiation.

Cell suspension of 16-h *P.phosphoreum* 1883 IBSO culture from the Collection of Luminous Bacteria CCIBSO was applied as a bioassay system to study toxicity of the radioactive solutions. Additionally, the intact bacterial cells were used for electronic microscopy studies.

The Gumat-80 preparation (“Gumat”, Irkutsk) was used as a source of humic substances (HS). It was produced by non-extracting treatment of coal. Concentrations of HS that inhibit bioluminescence by less than 20% were $5 \cdot 10^{-4} \div 0.3$ g/L.

The measurements were carried out with a BLM-8802 bioluminometer (Nauka Special Design Bureau, Krasnoyarsk, Russia).

3. RESULTS AND DISCUSSION

The bioluminescent assay in vivo were used to monitor detoxification of solutions of quinones, phenols, and radionuclides of low activity by humic HS.

Correlation between redox-potentials of 1,4-quinones and detoxification efficiency of HS in their solutions was found. Conclusion was made on reduction activity of HS in these solutions. Electron microscopy images revealed the bioluminescent bacteria to intensify synthesis of extracellular slime layers under detoxifying action of HS as a response to unfavorable influence of the quinones (5).

Detoxification of the radioactive solutions of $^{241}Am(NO_3)_3$ and $(UO_2)(NO_3)_2$ by HS was under study. It was shown that HS change bioluminescence kinetics in ^{241}Am solutions approaching it to control, thus, decreasing radiotoxicity of the solutions. (Figure 1). Probably, humic acids form complexes with americium, and hence, protect bacterial cells from alpha-irradiation.

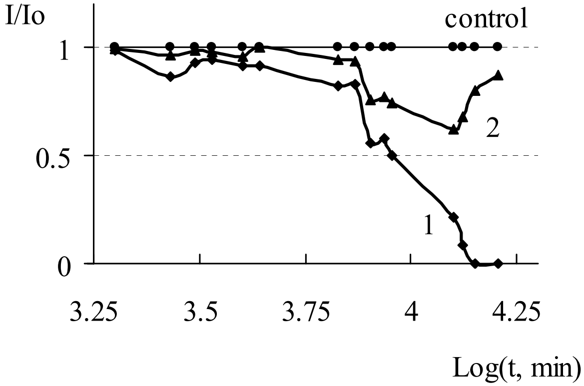

Figure 1. Bioluminescent intensity vs time of exposure (t, min) in solutions ^{241}Am(N0$_3$)$_3$ (10^{-10} M, 3000 Bq/l) in the absence (1) and in the presence (2) of HS (C=0,25 mg/ml). Control – bioluminescent intensity in the absence of ^{241}Am(N0$_3$)$_3$.

Electron-microscopy study confirmed that HS decrease damage of the bacterial cells in Am-241 solutions, and do not affect the cells in Uranium solutions.

4. CONCLUSIONS

The study demonstrated a high potential of bioluminescent assay systems to monitor detoxification efficiency of HS in solutions of organic oxidizers and radioactive compounds. Detoxification of quinones and radioactive salts was found in the solutions.

Conclusion was made on reduction activity of HS in solutions of 1,4-quinones. Electron microscopy study showed that bioluminescent bacteria could intensify synthesis of extracellular slime layers under detoxifying action of HS as a response to unfavorable influence of the quinones

Humic substances (C = 0.25 mg/ml) detoxified solutions of Americium and did not detoxify solutions of Uranium.

ACKNOWLEDGEMENTS

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Humus Substances in Natural and Technogenic Polluted Waters in Priamurje

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Keywords: humic, fulvic, pollution, Amur, Songhua

1. INTRODUCTION

Humic substances (HS) are complex poly-functional organic substances that form a link in the evolution of living and nonliving matter (1). Soil is the most important source of HS in natural waters depending on their water migration capacity. Humic and fulvic acids are present in all the areas, where biotransformation of organic matter takes place (in soil, water, bottom sediments) and participate in processes of migration and concentration of elements in natural waters. (2, 3)

Data on genesis and distribution regulations of humic and fulvic acids in water are important for assessing many hydrochemical, landscape and geochemical aspects, as well as water treatment purposes. This is a very important task for drinking water treatment facilities in Khabarovsk and other Amur River cities, as the river highly colored water is rich in organic matter (OM) in general and humic (HA) and fulvic (FA) acids in particular (42). They sediment during water treatment. Meanwhile colorless organic substances (non-coagulated low-molecular fractions) most often form chlorine organic compounds which worsen organoleptic qualities of water, but have a cancerous active character (4).

The paper presents the results of research of HAs and FAs and their distribution regularities in the Amur and Songhua Rivers after the accident in the Jilin city (China), where over 100 tons of benzene, toluol and other pollutants were discharged into the river.

2. MATERIALS AND METHODS

Sampling was carried out from the surface and bottom layers at hydrological cross sections in the following period: 2006 – in the Songhua River upper Harbin, Tsyamusi and Tuntsyan, in 2006-2007 in the Amur River near Amurzet (a control station upper the Songhua juncture), Leninskoe (lower the Songhua juncture), Khabarovsk, Komsomolsk, Bogorodskoe and Nickolaevsk.

Humus acids (HFA), i.e. humic (HA) and fulvic (FA) acids were determined with concentration and extraction of humic substances with DEAE–method. For organic matter analysis standard methods were used and total (TOC) and dissolved organic carbons (DOC), color index (CI) and suspended matter (SM) were calculated.

3. RESULTS AND DISCUSSION

In March 2006, it was revealed that water in the Amur upper the Songhua (Amurzet) was highly colored (up to 130 degrees) with high a concentration of humic acids (4 mg C L^{-1} or 55% of DOC). Mountain taiga soil formation specifics had a high impact on the composition and content of humus acids in the river run off. One of the specifics in the Lower and Middle Amur Basin is the formation of acid humus rich in migration-capable HA and FA fractions (5, 6). It is also important that in brown taiga soils most of them, especially FA, are not attached to the mineral base and freely migrate in the soil profile and out of it, as the soil layer is not thick and highly rocky. Maximal content of HFA was registered in the Bureya River water (~ 6.5 mg C L^{-1}) and its upper tributaries Urgal, Chegdomyn and Dublican (7, 8). In Zeya, the other tributary, HFA content equaled ~ 5.0 mg C L^{-1} a little lower due to cryogenic processes in the drainage area. Soils specifics in these areas include Al-Fe-humus migration of mobile humus formation products including water-soluble products. FA content in Zeya and Bureya was a degree lower than the FA content.

Songhua water compared to Amur water was observed to be less colored (70 degrees), but revealed high concentrations of OM and SM (up to 200 mg L^{-1}). Organic matter values here constantly exceeded the permissible level. HFA concentration was not high (~ 1.5 mg C L^{-1} or ~ 20% DOC) and reduced towards the river mouth. Such low concentrations seem to be associated with soil specifics in the North-East China plains and humus transformations.

Lower the Songhua near Leninskoe OM concentration was much higher (Figure 1). At the left (Russian) bank OM values were close to background normal with a high humus content, but at the right (Chinese) bank the share of HA and FA did not exceed 25% DOC and the values were 2 times lower than at the control station at Amurzet. Total increase of OM values in water along the right bank of the river and towards its middle resulted from Songhua discharge of technogenic organic compounds. Songhua water comes up to Khabarovsk following the right bank whereas water with a high content of natural HA and FA form Zeya and Bureya tends to the left bank. It is evident from hydrochemical data and satellite images.

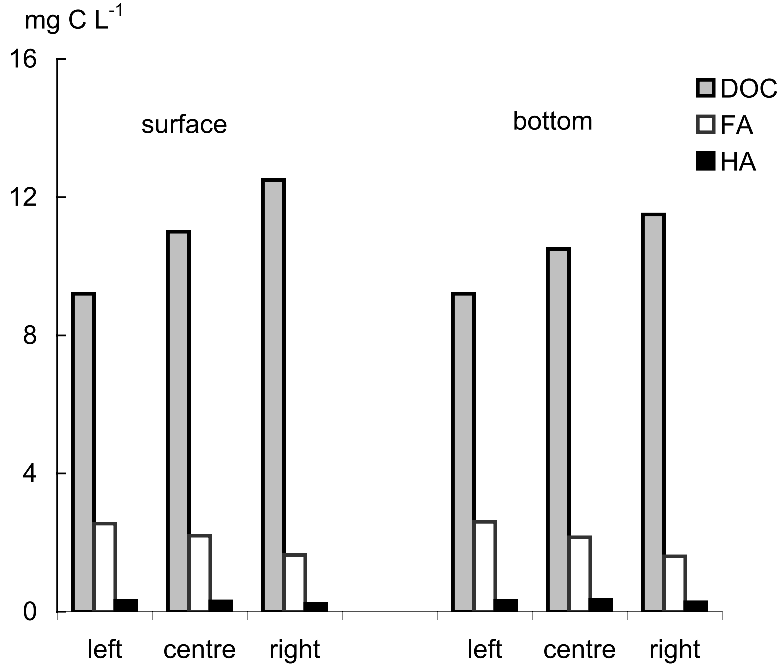

Figure 1. Concentrations of dissolved organic carbon, humic and fulvic acids in the waters of the Amur near Leninskoe (lower the Songhua juncture) in March 2006.

In June 2007 OM dynamics and content as well as OM values in the Amur water (lower the Songhua) were the same as in 2006 but in August 2007 they sharply increased. Both TOC (16 mg L^{-1}) and suspended matter (250 mg L^{-1}) increased. HFA concentration was not high (1.6 mg L^{-1}, i.e. 20% DOC). FA values exceeded HA values 7 times at the middle of the river and at its right bank, whereas at the left river bank (lower the Songhua) there was 8 times difference. Upper the Songhua the difference in FA and HA values was 12 times.

4. CONCLUSIONS

Thus, irrespective of the measures undertaken by China to improve the environmental situation in the Songhua Basin, significantly high concentrations of OM in the water, mostly of technogenic origin and low content of humus acids indicate that those measures are not effective enough. Soils, which provide humic substances, constantly degrade under intensive pressure in cities and agricultural areas. The Amur Basin environment can be improved only with measures preventing soil OM degradation, with contsruction of sewage complete-treatment facilities and industrial buffer zones in the Songhua Basin.

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Analysis of Pore Water Dissolved Organic Matter by UV-spectroscopy and Spectral Fluorescence Signatures Technology

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Keywords: dissolved organic matter, sediments, pore water, UV-spectroscopy, spectral fluorescent signature

1. INTRODUCTION

UV and fluorescence spectroscopy can be used to analyse unaltered samples, which is important in DOM studies. Only carbon that is in aromatic structures has been shown to have an effect on the UV absorbance spectrum of DOM. Compared to absorption spectra, the fluorescence spectra contain more detailed information and they can be used for DOM composition and source investigations.

The technique of Spectral Fluorescent Signatures (SFS) is based on a direct measurement of a 3-dimensional fluorescent pattern of the sample. Three dimensions are excitation wavelength, emission wavelength and the fluorescence intensity. By comparing SFS image to the fingerprints of known compounds, qualitative and quantitative analysis is possible. SFS method can distinguish between carbon fractions and differentiate environmental organic pollutants. Technique is considered rapid, requiring small amount of samples and enabling low concentrations to be measured.

2. MATERIALS AND METHODS

The Lake Võrtsjärv sediment samples were taken in 2003. The frozen sediment core (120-cm long, dated from year 500 to present) was sliced into 1-cm thick sub-samples and packed into plastic bags. Pore water was extracted from thawed sediments by centrifugation at 3500 rpm for 30 minutes, filtered through 0.45 μm filter (Millex® GP, Millipore) and stored at 4 ^{o}C in the dark.

Table 1. Characteristics of Lake Võrtsjärv, South-Estonia

Location	58°17´N 26°02´E
Area, km^2	270
Average depth, m	2.7
Maximum depth	6
Catchment area, km^2	3100
Trophic status	eutrophic

Absorbance spectra of the samples were recorded with the Jasco V-530 UV/VIS Spectrophotometer (Japan), with 1-cm-pathlength fused silica cells and MilliQ water as the blank. Spectra were measured over the range of 200-500 nm with 2.0 nm bandwidth.

Absorption coefficients were calculated using formula $a_{CDOM}(\lambda) = 2.303\ A(\lambda)/L$, where $A(\lambda)$ was the absorbance at selected wavelength and L the pathlength of the cell in meters.

Fluorescence spectra were measured with spectrofluorimeter Fluo-Imager M53 (Skalar, Netherlands) in excitation range of 240-360 nm and corresponding emission range of 250-570 nm. Fluorescence effectivity (F_{eff}) was calculated as ratio between fluorescence emission maximum intensity and absorbance at 254 nm and is considered proportional to fluorophores quantum efficiency (1).

3. RESULTS AND DISCUSSION

The fluorescent DOM components have been identified in Fig.1. Protein-like compounds have been separated into tyrosine- and tryptophan-like, having excitation – emission maxima in wavelength regions 280-290/305-320 nm and 285-290/340-350 nm, respectively. Tyrosine-like fluorescence was detected in all studied sediment pore water samples while tryptophan-like was detected in older samples and only in some samples from 20th century. Both originate from proteins and thus may indicate autochthonous material leached into pore water after protein denaturation or by biological production (bacterial activity). Humic-like fluorophores had characteristic excitation at 315-350 nm and emission at 400-440 nm. Additionally, in recent sediments from depths 5-13, 26-28 and 32-36 cm (dated to 1997-2000, 1983, 1963-72) a group of fluorescing compounds was detected with excitation – emission maxima in range 325-335/365-380 nm and was identified as PAHs, possibly pyrene and phenanthrene with characteristic emission at 350-380 nm at excitation of 340 nm (2).

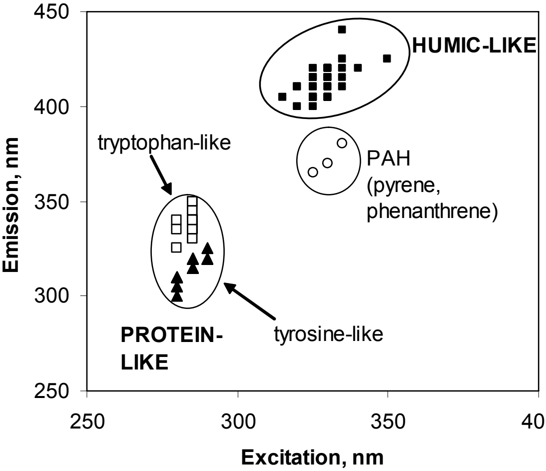

Figure 1. Positions of the fluorescence maxima of Lake Võrtsjärv pore water detected by SFS method.

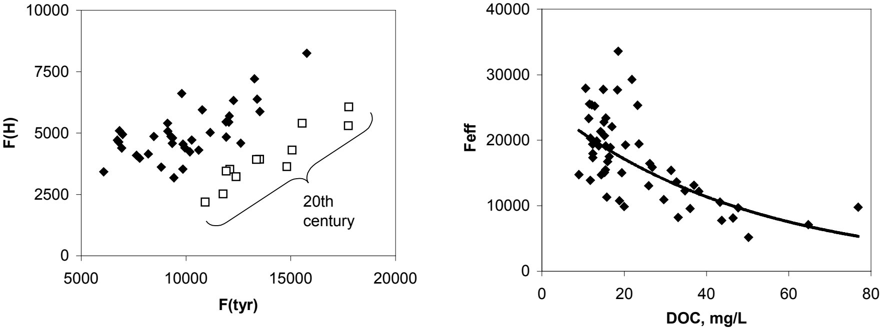

Figure 2. Relations between humic F(H) and tyrosine F(tyr) fluorescence intensity and fluorescence effectivity F_{eff} and dissolved organic matter (DOC).

Differentiation of pore water by fluorescence characteristics was possible as shown in Fig.2. Samples from 20th century had lower humic-like fluorescence intensity than older samples (up to year 500). Fluorophores quantum efficiency was without temporal differences decreasing exponentially with increasing DOC concentration in pore waters.

Studied UV- and fluorescence characteristics varied mostly in 20th century samples as shown in Fig.3. DOC and absorbance were slightly decreasing in older pore waters. Fluorescence ratio as indicator of biological activity was unusually low (below 1).

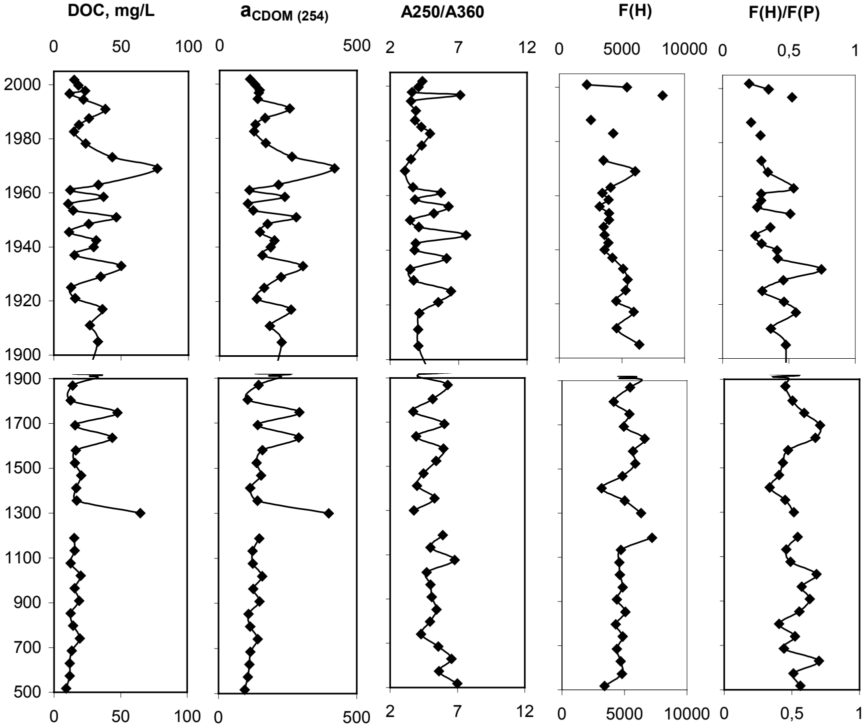

Figure 3. Temporal changes in DOC, absorption coefficient a, absorbance ratio A250/A360, humic-like fluorescence intensity F(H) and humic- and protein-like fluorescence intensity ratio F(H)/F(P); upper panel 20th century, lower panel years 500 to 1900.

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Risk Assessment of Soil Degradation and Possible Soil Recultivation in Mining in Priokhotje Region

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Keywords: soil cover, degradation, recultivation, mining

1. INTRODUCTION

An increasing attention is given in Priokhotje region to studies of big mining developments with a high technogenic impact on soil cover, as they cause both direct and indirect damage of environment. Degradation and decomposition of soil cover as a uniform system due to soil mechanical removal and marked chemical contamination create a situation, when most of fertile forest soils cannot be rehabilitated to generate forest vegetation. Thus developing technologies of soil recultivation and conservation to mitigate a negative impact of mining operations on soils seems most urgent.

Ecological damage (ED) of soils is determined as a reverse value of soil cover structure sustainability (SCSS): ED = 1/SCSS. The more sustainable is the system the less is the damage of soils. That is why it is important to make quantitative and probability assessment of soil cover sustainability within the river valleys and on the mountain slopes where the mechanical disturbance of soil cover is coupled with intensive melting of permafrost and sliding of mellow mass down the slopes. The situation is even more complicated on the steep slopes, where soil cover degradation rate is higher and causes rapid plant extinction, changes of mechanical composition of soils, intensive physical weathering and other negative phenomena, which disturb natural development of environment complexes as a whole (1).

The main tasks for the analysis of natural and technogenic degradation of soils and the selection of recultivation technologies are the following: 1) assessing soil degradation risks and possible recultivation conditions; 2) examining ecological state of degraded soil cover structures and estimating time for reaching a set-up value of the determining parameter of soil cover degradation; 3) defining irreversibility points of soil functioning mechanisms and rehabilitation limits.

2. MATERIALS AND METHODS

The research was conducted in the basins of the rivers Ariavkan, Tukchi, Muktana and Taryng-Lata (Ayano-Maisky District of Khabarovsky Krai).

1. The main characteristics of the territory and mining specifics (intensity and area of uncovering, down gradient, temperature parameters, shape and steepness of slopes, thickness of mellow cover, rate and thickness of soil profile melting on the open surface, speed of mellow cover sliding down the mountain slope, etc.) have been investigated.

2. Soil horizon thickness and deformations have been measured every 20 meters within a radius of 200 meters down the slope from the uncovering. For control and experiment purity purposes soil samples were also taken in areas free from the impact of mining. Previously collected results of chemical composition and specifics of soils were also analyzed.

R.P.C. Morgan (2) methods were used in field works. Soil degradation in a moment of time (t) was characterized as intensity of processes, contributing to soil cover degradation under anthropogenic impact (through changeability index – C_t) at probable condition of a soil ecosystem $dS(t)$.

3. RESULTS AND DISCUSSION

If we consider the formation of technogenic soils as a scenario of a sequence of possible events that take place at mining, than the prospected outcomes at a particular moment (t) may be characterized as the extreme values of the permissible interval. Mathematical prediction of the possible condition of soil ecosystems at any time moment (t) can be described with the equation

$$dS(t) = k \cdot 1/dZ(S_r) \cdot C_0(t) \cdot dt \text{, where } Z = 1/H$$

k – an observed correction factor for physical and chemical properties of soil (sorption, resistance); r, t – space and time coordinates; Z – estimated resistance reserve (soils), which determine the probability of soil system functioning within the limits of its sustainability; $C_0(t) \cdot dt$ – a changing technogenic load on the area unit.

Possible scenarios for the development of the particular soil ecosystem and its transition from state S_i into state S_j at the moment of time (t) is determined with

$$\lambda_{ij}(t) = \lim_{\Delta t \to 0+0} \frac{P_{ij}(t, \Delta t)}{\Delta t} \text{, where}$$

P_{ij} – density of possible events during t, Δt interval, Dt – the time needed for the system to transmit from one state to the other.

The interaction of natural and technogenic soil systems within ecological and geographic regularities is determined with tension (H).

$$H = G / \underset{i=1}{J_i} \cdot E \cdot Y_{pi}$$

H – an integral parameter of the main soil cover specifics that reflect the tension of the soil cover state. In physical sense the soil ecosystem tension is the density of system resistance to external factors; G / J_i – correlation of similarities and differences in natural and anthropogenic soil ecosystems; Y_{pi} – soil and ecological index of a relative natural soil stability (or an elementary soil areal). Coefficient (G) reflects the rate of disturbance of elementary soil areals. Localization index (J_i) determines the concentration of the disturbed soils within the limits of the sustainable structure of soil cover.

Our studies showed that a) uncovering operations in existing conditions without recultivation create unfavorable prerequisites, sharpen other risk factors and intensify natural and technogenic degradation processes of soil cover transformations; b) mechanical disturbance of 1/5 of soil area on the slopes of 25^{o} steepness during uncovering disrupts soil functioning mechanisms (Table 1) (3).

Table 1. Risk Assessment of Soil Degradation and Possible Soil Recultivation

Soils, recultivation and predictions for soil cover rehabilitation	Soil degradation assessment and Recultivation results					Forming techno-genic structure of soil cover	Class of eco-logical damage
	Mechanical disturbance (% /100 ha)		Pollution with trace metals (g/m^3)		Trace metal impact on biomass growth		
	Removal of SC	Degradation of horizons	Cu	Zn			
Brown soils	>30	shifting, flashing, washing away, erosion, compression	<5	5-10	Growth decrease, needles yellowing, weak regeneration	1. techno-genic; 2. deg-raded	П
Podzolilluvial humus soils	>25		>5	5-6			П-Ш
Types of recultivation	Surface smoothing	Phytomelioration	Chemical melioration, liming		Larch planting	-	I
Predictions for soil cover rehabilitation	Impos-sible	Degradation decrease	Decreasing negative after effects by more than 50%			Tolerant	I

A soil system passes the irreversibility point and transfers to a new-quality level of adapted functioning. As the results degraded natural and technogenic or industrial landscapes are formed and many of them cannot be rehabilitated as fertile forest soils suitable for vegetation (4). Industrial landscapes need surface smoothing. Slag heaps and tailing pits are rich in copper and zinc and also contaminated with trace metals (5).

Water and air easily penetrate through the loose structure of uncovered ground masses and intensify mineral oxidation, formation of acid solution and their geochemical mobility (5). Chemical contamination of soil exceeding 5 g/m^3 decreases coniferous biomass growth, needles yellowing and larch expansion (Table 1). Liming is most effective recultivation method in case of toxic contamination of soils.

4. CONCLUSIONS

Soil cover degradation in Priokhotje causes irreversible physical and chemical changes in soils. Mining impact on the soil cover, when exceeding the permissible level, disturbs soil formation processes. Accumulating transformations misbalance soil structure sustainability and may lead to the complete degradation and losses of fertile forest soils. Existing norms for permissible soil losses at mining are derived from average observation data at some mining developments. The real situation is more complicated. The method to calculate the permissible soil degradation rate (annual per area unit) or quantitative assessment of soil self-regulation processes have not been worked out yet.

The results revealed that a disturbed soil localization index is of the chief importance for soil degradation assessment. Soil degradation rate in the investigated territory appeared to be different. Chemically contaminated soils degrade slower due to self-regulation processes, whereas mechanically disturbed soils have higher rate risks and need ecological and engineering measures (different types of recultivation and melioration, decreasing areas of uncovering, etc.).

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Variation in Lipid Relative Abundance and Composition among Different Peat Bog Profiles

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Keywords: crude lipid fraction (CLF), peat humic substances

1. INTRODUCTION

Humic substances are comprised of three operationally defined fractions, water soluble fulvic acids, acid precipitated humic acids and water insoluble humin. Soil humic acids are mainly derived from the biochemical degradation of plant residues and from microbial synthetic activity and constitute a significant fraction of the soil organic matter (2). Lipids in humic acids originate from aliphatic components of macromolecules and are important for conformational flexibility (3). Free and bound lipids, carbohydrates and amino acids form the major fractions of analytically recognizable compounds in many organic matters of various origin. The structural diversity of the lipid moieties encountered can offer valuable information an organic matter sources and diagenetic processes. Deposits of peat in mires (especially of ombrotrophic peat bogs) provide special archives of climate changes (1). A record of plant remains living in the bog can be inferred from the geochemical paleoclimate proxies available in the organic matter constituents of peat layers.

In the present study, peat humic substances (humic and fulvic acids) of different origin were examined with respect to their bound lipid compositions.

2. MATERIALS AND METHODS

Peat samples were collected in six peat-bog complexes: which are situated in the north-east, south and south-west of Poland, in areas created by the Baltic glaciations. The selected wetlands varied in terms of plant communities, as well as the type of peat deposit (fen, transitional and ombrothrophic bogs). Before lipid analysis the species of peat was determined.

Air-dried peat samples were extracted with the mixture chloroform – methanol (2:1) yielding after evaporation the crude lipid fraction (CLF). The CLF from each sample was saponified yielding the unsaponifiable fraction (USF). Also plant sterols as the important secondary metabolites were determined. CLF and USF were determined by weight method, whereas sterols were purified by TLC as usual and GLC determination was carried out in typical condition.

3. RESULTS AND DISCUSSION

The CLF content in different peat species was between 32.17 and 163.34 mg/g whereas the USF content was lower varying from 2,12 to 59.66 mg/g. Some peat species are reach in CLF (more than 100 mg/g). There is no simple correlation between CLF and USF content. The content of main plant sterols (campesterol, stigmasterol and sitosterol) was much lower, varying within broad limits from 0.55 to 59.66 µg/g. The is a good correlation between USF content and sterol content.

4. CONCLUSIONS

The lipid content, both CLF and USF, can be used as a good marker of peat species differentiation, although some variations in CLF and USF content occurring in the same peat species located in different depth were established. Variations in the composition of lipids indicated that the participation of lipids in the formation of humic acids is markedly different. Diterpenoid and unsaturated fatty acids appear to be more readily incorporated into humic acid structure than triterpenoids, saturated fatty acids or sterols. Sitosterol is the dominant sterol in all investigated peat samples. It is concluded that peat lipids are potential structural units of peat humic acids.

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Characterization of Humic Substances Isolated from Various Peat Bog Profiles

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Keywords: humic substances, peat profiles, Poland

1. INTRODUCTION

Humic substances are present in all natural waters, soils, sediments and peats. In wetlands, due to lack of oxygen, the decomposition process is very slow and hence contributes to the accumulation of huge amounts of organic matter. The elemental composition and chemical features of peat humic substances are believed to be similar to those of humic acids in mineral soils. Though it has been reported that humic matter in peat is composed mostly of fulvic acids, most information indicates that peats contain large amounts of humic acids (3). At the present stage of knowledge, it is not known if the properties of the humic substances are different in different genus of peat. The properties and the structure of the humic substances depend on their origin (5).

In the present study, we characterized humic and fulvic acids fractions isolated from various peat cores from 6 peatlands with the aims of (i) comparing the chemical properties of peat humic substances formed in different geological origin, hydrology and botanical composition (ii) furthering knowledge of the humic substances in peatlands.

2. MATERIALS AND METHODS

The peat cores were collected from 5 areas in Poland - the Sudeten Mountains, the Orava Basin, the Silesia and the Masurien District. Investigated peatlands represent ombrotrophic and transitionall bogs and fens. The peat cores differ in their degree of decomposition, botanical composition and age. The peat samples were collected from each horizon of the stratigraphical profile of peat deposits. Horizonts were determined according to their subfossil communities and assigned to genetic types of peat according to Polish Standard PN-85/G-02500.

Before analyses, the samples were air dried, and the fraction > 2 mm was removed by dry sievieng. The following features were determined: (i) peat genus; (ii) ash content and acidity (pH H_2O; KCl); (iii) percentage of soil organic carbon (C_{org},) humic acid and fulvic acid content (by Kononova method).

3. RESULTS AND DISCUSSION

For the majority of peatlands studied here a general tendency occurres – fen peats contain more humic substances than raised bog peats. Moreover, it was noticed that the increase in the rate of decomposition usually raises humic substances content. Therefore the highest percentage humic substances content was observed in one of the fen profiles, while the lowest in the bog peat profile (Mechacz). The degree of decompositon itself increases with the depth of the profile. These results correspond with Duan and Ma, who stated that the concentration of humic acids increases with age, depth and decomposition rate of peat (2). Furthermore, large amounts of humic and fulvic acids can be found in highly decomposed peat that formed in an oxygen-rich habitats (1).

In the studied samples content of humic acids was higher than content of fulvic acids and Ch/Cf ratio was mostly above 1. Such correlation was observed before by Kononova (4). However, other authors reported that humic matter in peat is composed mostly of fulvic acids (6).

4. CONCLUSIONS

Humic substances content is higher in the fen peat than in the raised bog peat. Humic acids constitute a bigger part of peat humic substances than fulvic acids, for both fen and raised bog peats. Additionally, it was observed that the chemical features of peats differ distinctly, among the peats belonging to the same botanical class. Thus, indicating limited usage of purely botanical classifications.

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The work was performed within proposal No N 305 023 31/0930 financed by the State Committee for Scientific Research.

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Characterization of Soil and Humic Acids Extracted from Native Forest and Oil Shale Industrialization Rehabilitated Areas

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Keywords: rehabilitated area, humic acid, FTIR, DRUV-VIS and EPR spectroscopies

1. INTRODUCTION

Reserves of oil shale occur in several Brazilian locations. An expressive emerging of the Irati Formation occurs in the region of São Mateus do Sul (PR), where the oil shale Industrialization unit from Petrobras, the Brazilian Government Oil Company, is installed (PETROBRAS/SIX). The oil shale mining happen in open skies with plowing, in parallel strips and after the mined recovering process is made. There is a systematic way recovering proceed in the plowing, using the disposal of the mining process byproducts to restore the relief followed by the recuperating with clay and soil (1). The recovery of the degraded area is considered effective when the physical, chemical and mineralogical properties of the soil present conditions adjusted for the plants development (2). The goal of this work is to characterize by the elemental composition and FTIR, DRUV-VIS and EPR spectroscopy, and to compare the native forest soil (NFS) and oil shale industrialization rehabilitated forest soil (RFS), and the humic acid (HA) sequentially extracted with $Na_4P_2O_7$ + NaOH and urea + NaOH from these soils.

2. MATERIALS AND METHODS

Samples from NFS and RFS were taken at 0 – 5 cm depth from PETROBRAS/SIX area, São Mateus do Sul, PR, Brazil. Humic acids (HA) were sequentially extracted with [$Na_4P_2O_7$ (1 mol L^{-1}) + NaOH (0.25 mol L^{-1}) (HAP)] and [Urea (6 mol L^{-1}) + NaOH (0.1 mol L^{-1}) (HAU)] solutions, adapted from references 3, 4 and 5. The first extract was done in 200 g of soil, adding 500 mL of NaOH solution and 500 mL of $Na_4P_2O_7$ solution. For the following extraction, it was used the humin (HU) resulting from the first extraction were added 500 mL of NaOH solution and 500 mL of urea solution (4). The samples were dried in the oven (50°C), finely grounded, and analyzed by FTIR, DRUV-VIS and EPR espectroscopy in the solid state.

3. RESULTS AND DISCUSSION

In a general way, the NFS and RFS soils presented similar FTIR spectra with bands in 3700, 3620 and 3530 cm^{-1}, attributed to stretching absorption of internal O-H groups (ν) from clays (Figure 1). The HA samples from both soils, extracted with the same extractor type (pyrophosphate or urea), also presented similar FTIR spectra. The samples NFHAP and RFHAP showed band at 1250, 1720 and 2500 cm^{-1} due to COOH groups. There are bands in the region of 1100 and 1030 cm^{-1} due to ν of Si-O and C-O (carbohydrate), respectively. These bands are less intense in NFHAP and RFHAP showing the greater capacity of this extractor in eliminating those kinds of groups in both soil samples. All samples, except for NFHAP and RFHAP, presented bands below to 910 cm^{-1} due to inorganic structures (5, 6). As a result of the FTIR analysis it was concluded that there are few differences between the studied soils and the extracted HA.

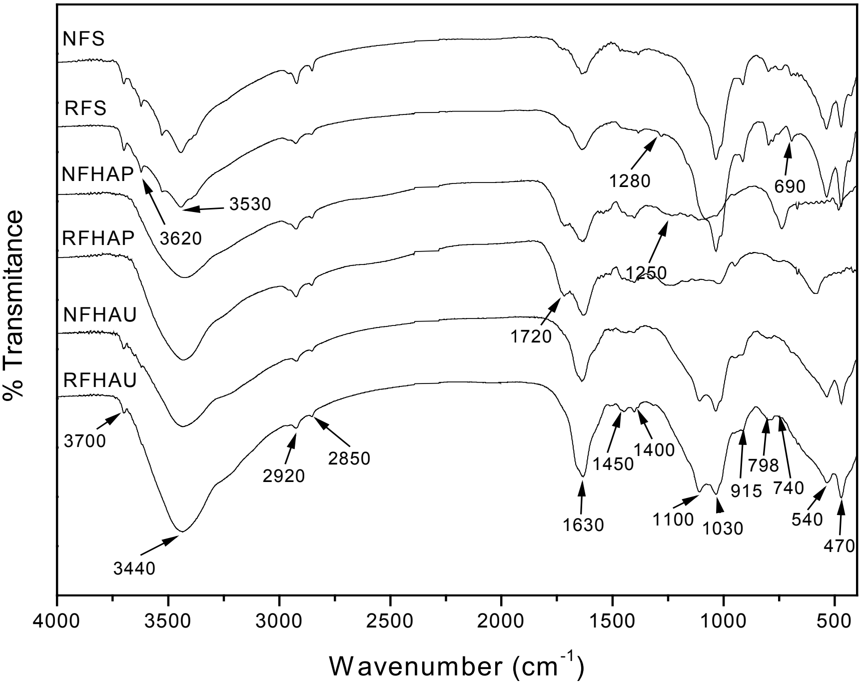

Figure 1. FTIR spectra of the NFS and RFS soils and NFHAP, RFHAP, NFHAU and RFHAU extracted samples.

DRUV-VIS spectra of the NFS and RFS soils, as well as NFHAU and RFHAU presented similar features with a notorious absorption band around 500 nm attributed to the $^6A_1 + {}^6A_1 \rightarrow {}^4T_1({}^4G) + {}^4T_1({}^4G)$ electronic d-d transition due to Fe-Fe pair of hematite structure (Figure 2) (7). The HA samples from both soils, extracted with pyrophosphate, presented typical spectra of organic aromatic structure with the E_4/E_6 ratio relation quite similar, NFHAP = 1.70, RFHAP = 1.76 (6). Once again also the DRUV-VIS technique showed the similarity between the two soil samples.

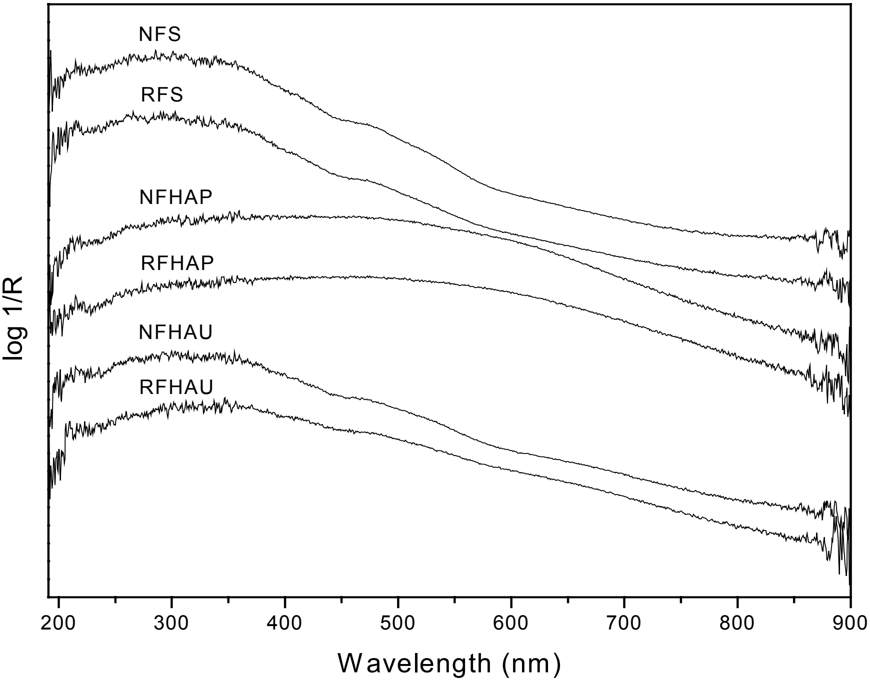

Figure 2. DRUV-VIS spectra of the NFS and RFS soils and NFHAP, RFHAP, NFHAU and RFHAU extracted samples.

In the EPR spectra at room (~300 K) and liquid nitrogen temperatures (77 K) it was observed a broad absorption line in g ~ 2 .0 attributed to Fe^{3+} ions in concentrated domain in all the samples. All the spectra at 77 K showed an absorption line of low intensity in g ~ 4.3 indicating the presence of Fe^{3+} ions complexed into the organic and inorganic matter, in diluted domains. This absorption line is more intense in the NFAHP and RFAHP than the others. The absorption line due to the organic free radical (OFR) in g ≈ 2.0 is observed just in the NFHAP and RFHAP where the g values are typical of semiquinone free radicals (NFHAP = 2.0032 and RFHAP = 2.0034) and the number of OFR are quite similar for NFHAP (4.67×10^{17} spin g^{-1}) than for RFHAP (2.03×10^{17} spin g^{-1}) and typical of soil HA (8). Again, by EPR spectroscopy the studied soil samples are comparable.

4. CONCLUSIONS

All the FTIR, DRUV-VIS and EPR analyses showed that the soil and HA from the rehabilitated area are reasonably humificated and presented slightly higher aromatic structures than the native soil and their respective HA.

On the base of the analytical techniques applied in this work, both soils were quite similar showing that the soil rehabilitation process utilized by the PETROBRAS/SIX is effective in terms of the agroforest proposal when compared with the native forest.

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Diversity of Fractional Composition of Humic Substances and the Content of Total and Availability Forms of Some Metals in Selected Soils of the Opole Region, Poland

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Keywords: humic substances, heavy metals, organo-mineral complexes

1. INTRODUCTION

In Opole region there is high diversity of parent material of soils characterized by varied chemical properties, which are modified by agriculture and other human activity. Chemical properties developed under this impact can influence the formation of humic complexes with mineral substances (2, 4, 5). Among inorganic contaminants heavy metals require special attention because of they create potential environmental hazard (1-3). The solubility and the bioaccumulation of metals depends on physico-chemical properties of soil solution (mainly pH) and the concentration of inorganic ligands and soil's organic matter. At the same time their accumulation at the smallest fractions is one of the contamination indeces of the soil environment (3). The aim of this investigation was to determine various humic substances created at various soils and their potential for making complexes with some metals, especially with their availability forms.

2. TREATMENTS AND METHODS

The objects of these investigations were five soil profiles localized in the Opole region. Analyzed soils represent the following systematic units: Eutric Cambisols (profiles 1 and 5), Stagnic Humic Gleysols (profile 2), Cambic Rendzinas (profile 3) and Haplic Podzol (profile 4). The granulometric composition was determined in each soil horizon by the PN-R-04033 method. The following parameters were determined in soil samples (in particles <1 mm): pH in KCl, salinity by conductivity of water solution (5:1), carbon, according to the oxymetric method by Tiurin, total nitrogen by the Kjeldahl method, total content of heavy metals and their availability forms.

In soil samples originating from A_p, AB_{br}, A, A_d, A_hE_{es} horizons the fractional composition were determined by the Kononowa–Bielczikowa method (4).

3. RESULTS AND DISCUSSION

Variability of the parent rocks and intensive farming influenced the development of the physic-chemical and chemical properties of studied soils (pH, salinity, content of analysed forms of heavy metals – Table 1.).

Table 1. Some properties of investigated soils

Systematic units	Genetic horizon	pH_{KCl}	Salinity gKCl·kg^{-1}	C g·kg^{-1}
1. Eutric Cambisols	A_p	5.2	1.560	7.99
	B_{br}	7.2	3.150	1.30
	$B_{br}C_{Ca}$	7.4	1.950	0.47
2. Stagnic - Humic Gleysols	A_d	5.9	1.360	12.47
	A	6.0	1.320	7.07
	C	6.3	0.870	2.15
	$C_{gg}1$	6.5	0.690	0.90
	$C_{gg}2$	6.2	0.750	0.40
	CG	6.3	0.720	0.20
3. Cambic Rendzinas	A_p	6.0	2.520	12.01
	B_{br}	6.7	1.350	1.96
	$B_{br}C_{Ca}$	7.6	2.220	1.00
4. Haplic Podzol	A_hE_{es}	2.7	0.326	8.10
	E_{es}	3.5	0.190	1.91
	B_{hfe}	3.8	0.247	4.61
	C	4.8	0.185	---
5. Eutric Cambisols	A_p	6.4	0.415	14.50
	AB_{br}	6.2	0.326	8.70
	B_{br}	6.0	0.247	2.15
	C	6.1	0.159	0.9

Formation of the soil humic substances depends on the basic physico-chemical and chemical properties of the soil and of the undergoing soil processes (1, 3). In studied soils we observed varying accumulation of total organic carbon (Table1), and quantitative differences in humic fractions (Figure 1).

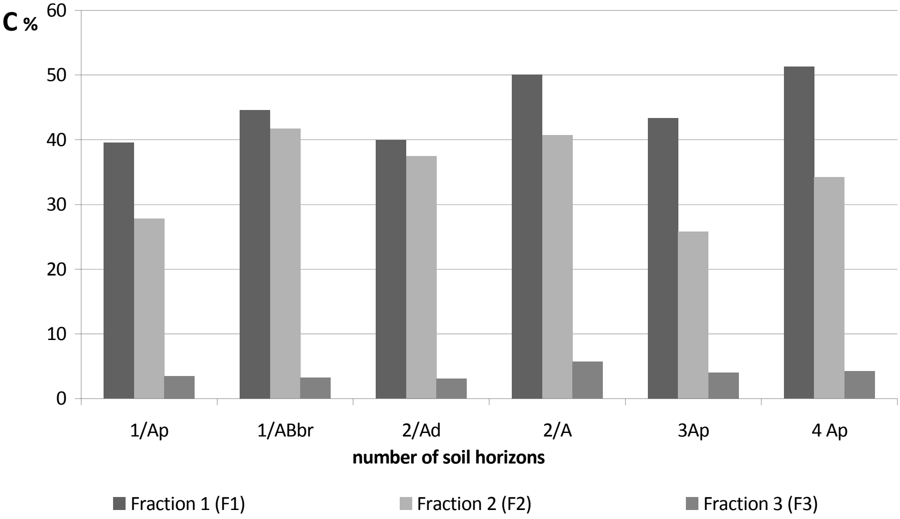

Figure 1. Fractional composition of humus in investigated soils.

Organo-mineral complexes take part in many soil processes and can influence the properties of the soil environment. With high content of organic matter and in the presence of mineral colloids the metals can associate with mineral-humus complexes. Such a possibility is indicated by the correlation between the carbon content, floating and colloidal particles and heavy metals and their available forms (Table 3).

Table 3. Correlation coefficients between some parameters of the investigated soils.

Feature (X)	Feature (Y)	r
Particle <0,02	Zn_{total}	0.5782*
	Cu_{total}	0.7281*
	Cu_{av}	-0.4425**
	Fe_{av}	0.6889*
	Mn_{total}	0.4826**
	Mn_{av}	
Particle <0,002	Cu_{total}	0.6702*
	Cu_{av}	0.7908*
	Fe_{av}	-0.4841**
	Mn_{total}	0.6553*
	Mn_{av}	0.4854**
C	Zn_{total}	0.8495*
	Zn_{av}	0.7669*
	Cu_{total}	0.6395*
	Cu_{av}	0.7467*
	Mn_{total}	0.7588*
	Mn_{av}	0.8564*

n = 20, *significantly p = 0.001, ** significantly p = 0.05

4. CONCLUSIONS

1. The diverse granulometric composition and the use of the investigated lands influence the development of their physic-chemical and chemical properties, which was shown in salinity, carbon accumulation and the content of analysed metals.

2. Qualitative and quantitative differences in formed of humic substances were demonstrated in analysed soils.

3. Significant influence of inorganic ligands and organic matter in the studied soils on the immobilization of analysed metal forms was noticed, which was demonstrated by correlation coefficients.

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Characterization of Microbiological Effects on the Composition and Photochemical Properties of DOM in Coastal Sands Using Ultrahigh Resolution Mass Spectrometry and 3-D Excitation/Emission Fluorescence Spectroscopy

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Keywords: dissolved organic matter, FT-ICR mass spectrometry, fluorescence spectroscopy, estuaries, coastal sands

1. INTRODUCTION

The combination of electrospray ionization and ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) has revolutionized the characterization of humic substances. High-field (9.4 Tesla) FT-ICR MS can now fully resolve individual compounds in complex humic mixtures (1), providing a level of molecular detail thought impossible just a decade ago. In this presentation we will highlight the molecular information that ultrahigh resolution ESI-MS can provide about terrestrially-derived humic materials as they move through estuaries and permeable coastal sands along the Gulf coast of Florida. Permeable sands filter large volumes of water along the Northern Gulf of Mexico coastline, but there is very limited data on the biogeochemical and microbial processes affecting DOM turnover during sand filtration. We also evaluated the impacts microbes had on the photochemical properties of DOM using excitation-emission matrix spectroscopy (EEMS).

2. MATERIALS AND METHODS

Sand cores were obtained from the Apalachicola Bay near the mouth of the Apalachicola River in Northern Florida. These sand cores have microbial densities several orders of magnitude higher than in the overlying water column and thus act as mini bio-reactors. Humic-rich river water and water spiked with “fresh” DOM was then passed through sand columns at flow rates comparable to those in the sandy sediments. DOM was extracted from these water samples before and after passage through the columns using polymer C-18 solid phase extraction (SPE) cartridges. DOM was eluted from the SPE cartridges with methanol and then freeze-dried.

ESI FT-ICR mass spectra were acquired on a home-built 9.4 T FT-ICR mass spectrometer at the National High Magnetic Field Laboratory in Tallahassee, FL. The use of this instrument to acquire ultrahigh resolution mass spectra of humic materials has been described previously (1). Excitation-emission matrix (EEM) fluorescence spectra were obtained with a Jobin Yvon SPEX FluoroMax-4 scanning fluorometer. For details of the procedures used to acquire 3-D spectra, see Kowalczuk et al. (2).

3. RESULTS AND DISCUSSION

High resolution negative-ion ESI FT-ICR mass spectra of DOM before and after passage through the microbially-dense sand columns revealed that “fresh” DOM isolated from algal growth chambers was much more reactive than older DOM flowing into the bay. Molecular analysis using three-dimensional van Krevelen diagrams and Kendrick plots, and double bond equivalency (DBE) frequencies, reveal much about the molecular changes that occur to DOM in coastal sands. The microbial changes had little impact on the photochemical properties of “old” DOM, since it appeared to be reasonably photobleached by the time it reached the estuary. The photochemical properties of “fresh” DOM were substantially altered, however.

4. CONCLUSIONS

High resolution FT-ICR mass spectrometry of DOM before and after filtration through microbially-dense sand columns reveals much about the molecular changes that accompany biological utilization of humic materials. “Old” riverine DOM appears largely refractory, although some non-specific loss of certain compounds was observed. “Fresh” algal-derived DOM was much more reactive, with substantial changes in photochemical properties that can be explained based on the changes in molecular composition.

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Reactive Polyphenols and Dissolved Nutrients in a Nitrogen-Limited Headwater Catchment in Western Oregon, USA

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Keywords: DOC, DON, reactive polyphenol, SUVA

1. INTRODUCTION

Both the quantity and quality of dissolved organic matter (DOM) change dramatically as it moves from the soil to the stream. In N limited systems, inorganic N losses are very low, but dissolved organic nitrogen (DON) losses may still be substantial (1, 2). The relationship between DON and polyphenols in soil solutions has been established (3, 4), but little work has been done linking the reactive polyphenolic and nutrient chemistry of soil water to stream water. Reactive polyphenols (RPP), which include but are not limited to tannins, sequester nitrogenous compounds into resistant protein-polyphenol complexes (5). DOM in N limited systems may contain a high proportion RPP which bind to DON, thus perpetuating the N limitation of the system. In this preliminary study conducted in an N-limited headwater catchment in the Western Cascades, an approach is presented to quantify and compare reactive polyphenols and nutrient dynamics over the first storm event in autumn 2007.

2. MATERIALS AND METHODS

The study site is in the H. J. Andrews Experimental Forest in the Western Cascades of Oregon, USA. Watershed 10 (WS10) is a 10.2 ha catchment with elevation ranging from 425 – 700 m. The climate is Mediterranean, with average annual rainfall of 2220 mm. Atmospheric N deposition is low, averaging 1.6-2 kg ha^{-1} yr^{-1}.(1) Vegetation is mixed conifer (Douglas fir, western hemlock), with an understory including rhododendron and salal; all known to produce tannins.

A 10 m. long impervious barrier installed to bedrock at the base of the hillslope on one side of WS10 collects subsurface flow into a calibrated weir. The stream is gauged 91 m. below the hillslope weir.The drainage area above the barrier contains 25 Prenart lysimeters at depths of 20 cm, 30-40 cm, and 70-110 cm.

The first autumn storm, beginning 15 October 2007, deposited over 190 mm of rainfall in 6 days. During and after the storm, stream and hillslope water was collected at 2 to 4 hour intervals. Lysimeter samples were collected twice daily for 3 days, and then intermittently. Samples were bulked according to depth. All water was filtered with GFF filters.

Samples from lysimeters, hillslope, and stream were analyzed for DOC, total nitrogen, nitrate, ammonium, and DON. Specific UV absorbance (SUVA) is UV_{254} normalized by DOC (mg/L), and is a measure of aromaticity. The percent of reactive polyphenols in each sample was determined by performing Folin Ciocalteu assay on samples before and after passing them through a polyamide solid phase extraction tube (Supelco DPA-6S) to remove any polyphenols that would bind to the substrate. The total (TPP) and reactive polyphenols (RPP) were measured in mg/L water-soluble tannin equivalents (wsTE).

Tannins standards were made from purified salal tannins and balsam fir tannins contributed by Dr. Caroline Preston, Pacific Forestry Centre, Vancouver, BC. The salal and balsam fir tannins were 97.6% and 70.3% water soluble by weight, respectively, and the plot of absorbance versus concentration for both tannins were essentially the same.

3. RESULTS AND DISCUSSION

The plot of DOC over the hydrograph of the storm (Figure 1) demonstrates the flushing response, with similar pattern in DON, SUVA, and TPP values. Changes in DOC, DON, SUVA, and TPP from baseflow values to the average value over the rising limb of the hydrograph are summarized in Table 1. The changes in DOC and SUVA are similar to those found in WS 1, 2, and 10 streamwater by Hood et al over a 6 day storm in 2003 (6). During the storm event, nitrate and ammonium values were below detetection limits in 76% and 93%, respectively, of samples, and DON accounted for 98% of all N in the stream.

When levels of TPP in samples were above 0.5 mg/L wsTE, the reactive polyphenols averaged 78% of the total; otherwise, the results were too close to detection limits to be meaningful. In contrast, the tannin standards were 98% reactive. There was no significant change in the proportion of RPP over the storm event, regardless of water source.

The tannin standards contained 64% DOC. On average, 16% of the DOC of samples was polyphenolic. Assuming the proportion of RPP:TPP in the watershed is constant, and the DOC concentration of TPP in the watershed is similar to that of the standards, approximately 12% of the DOC in WS10 may be composed of RPP.

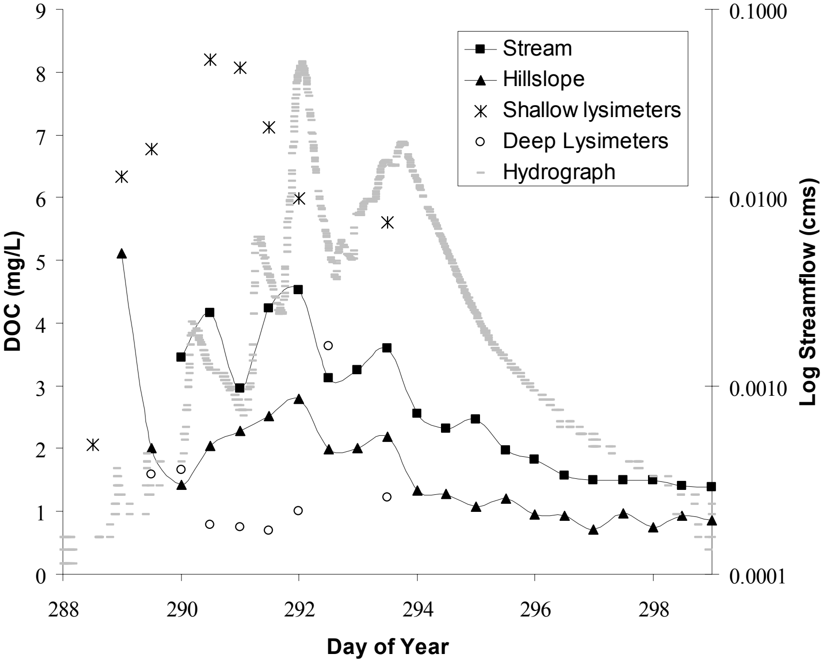

Figure 1. Dissolved organic carbon from different water sources over hydrograph of storm event October 15-25, 2007.

Table 1. Change in DOC, DON, SUVA and TPP from average pre-storm values to average values over rising limb of hydrograph

Source	DOC(mg/L)			DON(mg/L)		
	Baseflow	Rising limb	% Change	Baseflow	Rising limb	% Change
Lys shal.	3.673	6.427	75	0.183	0.207	13
Lys deep	1.153	1.078	-6	0.063	0.081	29
Hillslope	0.839	2.113	152	0.068	0.086	26
Stream	1.071	3.878	262	0.050	0.122	144
	SUVA (Abs/mg/L)			TPP (mg/L wsTE)		
	Baseflow	Rising limb	Baseflow	Rising limb	Baseflow	Rising limb
Lys shal.	0.025	0.035	29	0.81	1.05	29
Lys deep	0.017	0.024	41	0.03	0.11	266
Hillslope	0.014	0.022	57	0.068	0.68	900
Stream	0.025	0.036	31	0.26	1.35	419

4. CONCLUSIONS

DOC, DON, SUVA and TPP values consistently followed the pattern: shallow lysimeters > stream > hillslope > deep lysimeters; suggesting that the source of streamwater during the storm derives from shallow subsurface flow. The increase in polyphenols with the rising limb of the hydrograph was quite pronounced. The proportion of RPP:TPP was constant over time and sources at approximately 0.78. Reactive polyphenols may account for about 12% of the total DOC in the system. DON accounted for 98% of the total N in the stream during the storm.

Future work would explore other methods of partitioning reactive water soluble polyphenolics, including protein precipitation assays. The study will examine seasonal changes as well as at least one other storm sequence in spring 2008. Comparison of systems with varying degrees of N-limitation would be helpful in future studies.

ACKNOWLEDGEMENTS

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Density Fractions of SOM in Mediterranean Forest Soils: Characterization by TMAH-Thermochemolysis-GC-MS

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Keywords: Density fractionation, TMAH-GC-MS, Lipids, Lignin

1. INTRODUCTION

Physical fractionation methods are used to isolate soil organic matter (SOM) fractions of contrasting dynamics: i.e., to separate fresh *versus* stabilized SOM, free *versus* protected SOM (1). These methods are applied to obtain a 'light' fraction, little or not associated to mineral matter, apparently responsible for the most of microbial activity in soils. The fractions extracted with dense liquids are heterogeneous mixtures, but a relationship has been observed between SOM density and resistance to decomposition (2, 3). This suggests that density is related to the biochemical composition of the fractions (4).

The molecular identification of these differences is the aim of this study. We focus on the study of biomarkers in density fractions obtained from forest soils. To this end, we apply thermochemolysis with tetramethylammonium hydroxide (TMAH), coupled to GC-MS. This method is widely used to identify and quantify biomarkers released from complex organic matrices, useful to estimate the origin and the decomposition state of the studied organic material. With a preparative apparatus (5), it is possible to carry out the analysis on samples with small amounts of organic carbon (several milligrams).

2. MATERIALS AND METHODS

The studied soil samples were taken from H horizons of forest soils, all over carbonatic materials (marl, limestone, calcareous sandstone), all below holm oak stands (*Quercus rotundifolia*). After sampling, the soil samples were air-dried and sieved (2 mm).

The fractionation of soil samples was done as described in Fig. 1. The method is inspired in that proposed by the Adelaide's group (4). The result is a set of five fractions of increasing density. After isolation, each fraction was rinsed with distilled water, dried at 60ºC, and finely ground. The fractions were pyrolyzed in the presence of TMAH (5). The products of the pyrolysis were characterized and identified by GC-MS.

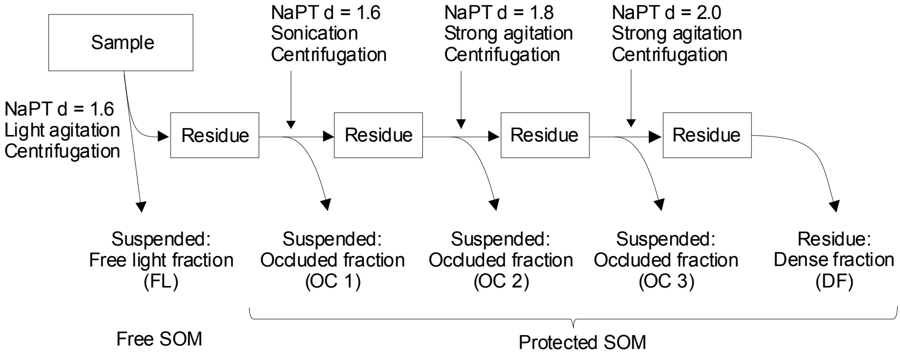

Figure 1. Density fractionation method (NaPT: sodium polytungstate).

3. RESULTS AND DISCUSSION

Results from one of the studied samples (H horizon over limestone) are given here as an example of the data obtained. In this sample, the distribution of total SOM among the density fractions is as follows: FL: 66.8%, OC1: 2.1%, OC2: 5.5%, OC3: 9.2%, DF: 16.4%. I.e., the two extreme fractions (FL and DF) are by far the most of SOM, the occluded fractions being a very minor part.

We focus on molecular families able to inform us about the degree of microbial transformation of the SOM, relative to its origin (mostly plant-derived): fatty acids (FAs), aromatics and carbohydrates. For many of these groups it is possible to obtain significant information from the identified compounds and the relative proportion between them.

The identified fatty acids (FA) reflect the origin of the SOM fractions: both microbial-derived (branched compounds, iso and ante-iso C_{15} and C_{17}) and plant-derived (long chain compounds, C_{20}-C_{36}, with even carbon number). While short-chain FAs are typical of microbial organisms, plant-derived FAs usually show a bi-modal distribution, with two maxima, one at C_{16} or C_{18}, the next at either C_{22} or C_{24}. Long-chain FAs are largely found in cuticular waxes. The presence of both short- and long-chain FAs in both the FL and the DF fractions suggests the dominance in these fractions of plant-derived lipids; in contrast, the very low proportion of long-chain FAs in the occluded fractions (OC1, OC2 and OC3) suggest a dominance of microbial-derived FAs in these fractions.

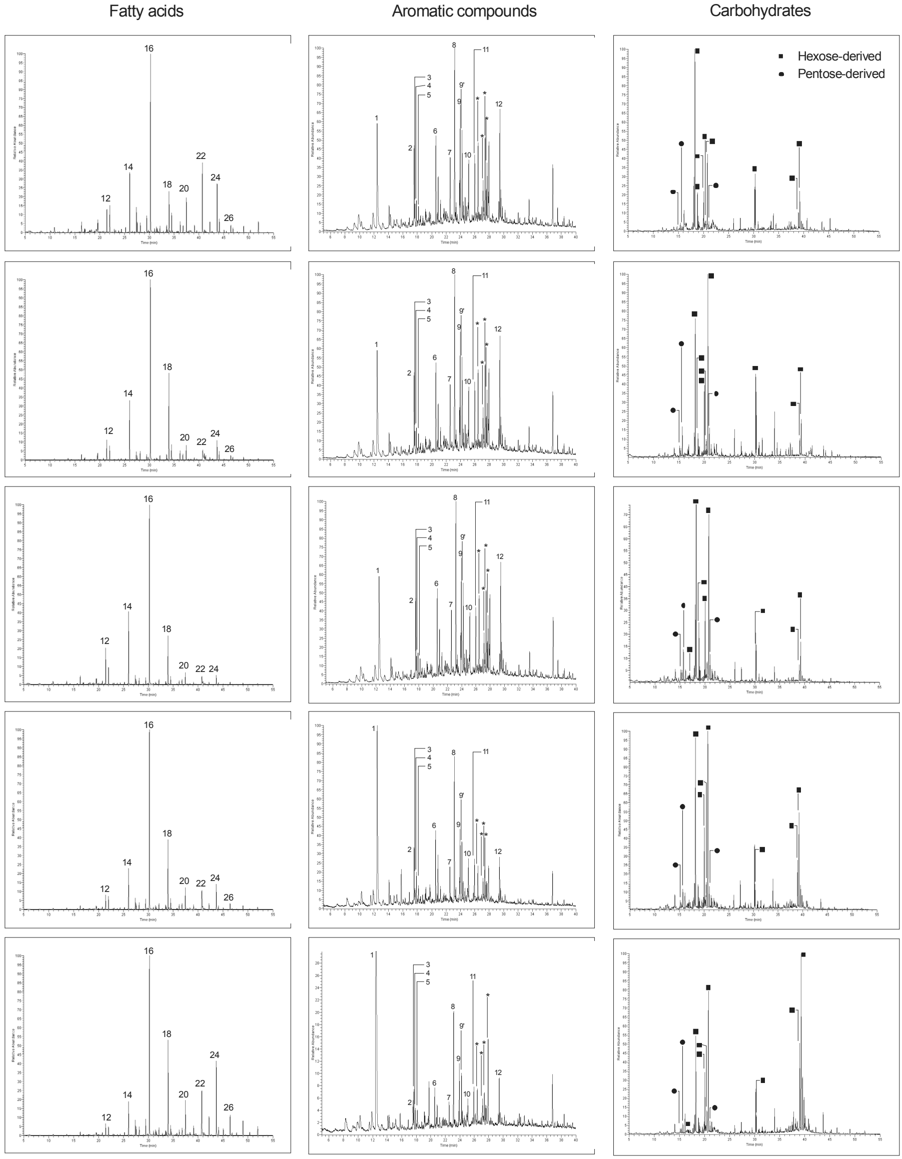

Figure 2. Ion chromatograms showing fatty acids (m/z = 74), aromatic compounds (m/z = 77 and 91), and carbohydrates (m/z = 88 and 101) as their methylated forms.
*: unidentified. See text for more details.

A number of aromatic compounds have been identified (Fig. 2, middle column): 1, benzeneacetic acid. 2, dimethoxystyrene. 3, trimethoxybenzene. 4, methoxybenzoic acid. 5, cinnamic acid. 6, dimethoxybenzaldehyde. 7, dimethoxy-acetophenone. 8, dimethoxy-benzoic acid. 9, dimethoxyterephtalic acid. 9', unknown. 10, p-methoxycinnamic acid. 11, timethoxybenzoic acid. 12, dimethoxycinnamic acid. The identified compounds reveal a vegetal origin; the most abundant ones are known lignin monomers. However, some aromatic compounds (e.g., trimethoxybenzene) have been described as the result of TMAH treatment on polysaccharides (6).

TMAH-thermochimiolysis is not too often applied for polysaccharide characterization, but sugar derivatives are abundant in the chromatograms. An accurate identification of the original sugar compound is not possible, but it is possible to reliably separate pentose-derived from hexose-derived compounds. Pentose-derived products have been found in low amounts: hexose-derived products are widely dominant (Fig. 2, right column). Pentoses are found in low amounts in microbial polysaccharides; they are rather typical of non-cellulosic, plant-derived structural polysaccharides (hemicelluloses in a broad sense). Since hemicelluloses decompose faster than cellulose (which is made of glucose, an hexose), this suggest the co-dominance of resistant plant-derived polysaccharides (cellulose, more or less microbially-degraded) and polysaccharides of microbial origin and a lack of fresh plant-derived polysaccharides.

4. CONCLUSIONS

The fatty acids and the aromatic products (lignin monomers) show the importance of plant-derived materials in the OM of all density fractions. However, the presence of ramified FAs and the poor signs of pentoses in the chromatograms suggest that this OM has suffered microbial transformation. The occluded fractions (OC1, OC2, OC3) seem more microbially transformed than the free (FL) and the dense (DF) fractions, whose composition is closer to the vegetal origin of the SOM.

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Use of Bioluminescent Assay Systems to Monitor Detoxification Processes in Radionuclide' Solutions

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Keywords: detoxification, bioluminescent assay, alpha-radionuclides

1. INTRODUCTION

Increase of radioactive contamination in the environment makes the effects of low-level radiation on living organisms very important. Detailed investigations of the effect of low dose radiation on living organisms were conducted using simple assay systems such as microorganisms, bacteria, plant and mammal cells, tissues of laboratory mice, drosophila flies, freshwater crustaceans, etc (1-4). Assay systems based on luminous bacteria are good candidates for such investigations. Bacterial bioluminescent assays are widely used to monitor environmental toxicity (5). The tested parameter is luminescent intensity of bacteria, which can be easily measured instrumentally. The advantages of the bioluminescent assays are their rapidity, sensitivity, simplicity, and availability of the devices for toxicity registration (5-6).

Bioluminescent bioassays have been used to monitor the effect of low-level alpha-ray irradiation in solutions of $^{241}Am(NO_3)_3$ (7). In paper (8) effects of two radionuclides (Am-241 and U(235+238)) on bioluminescent bacteria were compared.

It is known that Humic Substances (HS) detoxify solutions of radioactive elements due to their complexiation ability, thus protect the bacterial cells from alpha-irradiation. The purpose of this study was to monitor the detoxification ability of HS in solutions of the two radionuclides using bioluminescent bacteria as assay system.

2. MATERIALS AND METHODS

Cell suspension of 16-h *P.phosphoreum* 1883 IBSO culture from the Collection of Luminous Bacteria CCIBSO was applied as a bioassay system to study toxicity of the radioactive solutions. Additionally, the intact bacterial cells were used for electronic microscopy studies.

The Gumat-80 preparation (“Gumat”, Irkutsk) was used as a source of HS. It was produced by non-extracting treatment of coal. Concentrations of HS that inhibit bioluminescence by less than 20% were $5 \cdot 10^{-4} \div 0.3$ g/L.

Solutions of $^{241}Am(NO_3)_3$ and $(UO_2)(NO_3)_2$ were used as sources of α-radiation.

The measurements were carried out with a BLM-8802 bioluminometer (Nauka Special Design Bureau, Krasnoyarsk, Russia).

3. RESULTS AND DISCUSSION

The dependence of bioluminescence intensity on time of exposure to radioactive solutions in the presence and in the absence of HS was monitored. It was shown that HS change bioluminescence kinetics in ^{241}Am solutions approaching it to control, thus decreasing radiotoxicity of the solutions (Figure 1).

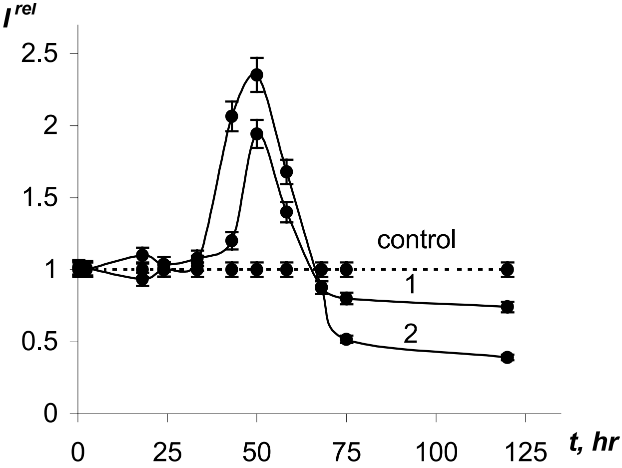

Figure 1. Bioluminescent relative intensity (Irel) vs time of exposure (t, hr) in solutions of $^{241}Am(N0_3)_3$ (10-10 M, 3000 Bq/l) in the absence (1)
and in the presence (2) of HS (C=0,25 mg/ml).
Control – bioluminescent relative intensity in the absence of $^{241}Am(N0_3)_3$.

Redistribution of the radionuclides inside the bacterial cells was studied in the presence of HS (Figure 2).

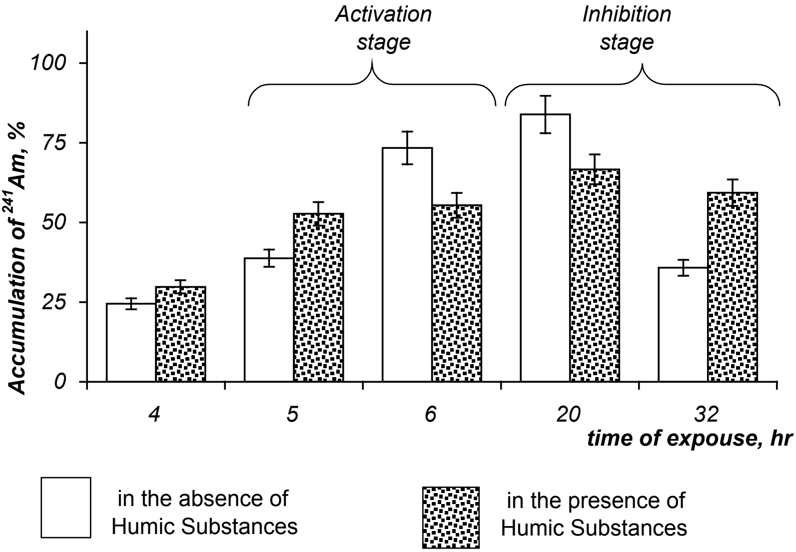

Figure 2. Accumulation of ^{241}Am by bacterial cells vs time of exposure (t, hr) in solutions of ^{241}Am(N0$_3$)$_3$ (10^{-10} M, 3000 Bq/l) in the absence and in the presence of HS (C=0,25 mg/ml).

The electron microscopy study of bacterial cells was carried out in the presence and absence of HS. HS were shown to decrease the damage of the bacteria in the americium solutions.

We found that HS (C=0,25 mg/ml) do not change time-decay of bioluminescent intensity under all concentrations of $(UO_2)(NO_3)_2$ ($C=10^{-3} \div 2{\cdot}10^{-7}$M). Microscopy study showed that HS do not change bacterial images. Hence, these solutions are not detoxified by HS at the conditions of the experiment.

4. CONCLUSION

The study demonstrated a high potential of the bioluminescent assay to monitor detoxification efficiency of HS in solutions of radioactive compounds. HS (C = 0.25 mg/ml) detoxified solutions of Americium and did not detoxify solutions of Uranium.

ACKNOWLEDGEMENTS

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Humification of Peat and Characteristics of its Humic Substances Depending on their Origin and Age

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Keywords: Peat; Humification degree; Decomposition degree

1. INTRODUCTION

Major part of peat organic matter is composed from humic substances. To characterize humification process, the development of humification indexes that link the rate of transformation of living organic matter and the development of humic substances with parameters describing the properties of the formed materials can be an important indicator (1). Usually humification process is evaluated by making indirect measurements that describe structural changes that occur during the humification process. Several methods have been suggested for description of humification, such as the measurement of E_4/E_6 ratio that indicates development of condensed macromolecules and the amount of organic /aliphatic carbon estimated by ^{13}C CP MAS NMR.

The aim of this article is to study the humification process of peat and to identify links between peat age (its decomposition degree) and the basic properties of peat and the peat humic substances.

2. MATERIALS AND METHODS

28 peat samples were collected from the profiles of five high type bogs of industrial importance in Latvia. The profiles were obtained in varying depths from 0 to 145 cm. The peat samples were characterized by their decomposition degree, ^{14}C dating, elemental analysis (C, H, N, S, and O) and UV/Vis spectra and organic carbon content. Ratios E_2/E_6, E_2/E_3, E_3/E_4, E_4/E_6 were determined for the solution of the humic or fulvic in $NaHCO_3$ solution. Organic carbon concentration of peat extracts was determined using Shimadzu TOC – VCSN.

To study humification process following indicators has been used:

1. Humification index (HI) (2)
2. Pyrophosphate index (PyI) (3)
3. Humus quality K value (4)

3. RESULTS AND DISCUSSION

In this study peat from 5 raised bogs in Latvia has been used and samples from different depths have been obtained, reflecting bog development conditions in Northern Europe. The age of the samples varies from 400 to 2260 years, and their decomposition degree (van Post scale) varies from H2 to H6.

Basic parameters of peat do not display a similar increasing trend for all the studied samples. Carbon and nitrogen content of peat samples from the bog Mazais Veiķenieks increases with increasing age (the depth and decomposition degree), while the content of sulphur is decreasing. However, for the rest of the studied bogs the variability of elemental composition is not so random and most probably depends on the botanical composition and the specific peat formation conditions (first of all, the hydrological regime in the corresponding bog).

The peat humification process has been analysed using van Krevelen graphs frequently applied for the analysis of carbon biogeochemical cycle and genesis of fossil fuel (5). The index of atomic ratios O/C, H/C, and N/C is useful in the identification of structural changes and the degree of maturity of peat in different depositional environments. The graph of H/C atomic ratio against O/C atomic ratio reveals changes in the properties associated with coalification reactions.

The relation between H/C atomic ratio and O/C atomic ratio of organic material of a differing decomposition degree – beginning with bog plants up to brown coal, lignite, and coal. The graph in Fig. 1 could be considered as a graphical statistical representation of humification process, indicating the degree of maturity and intensity of degradation processes such as dehydrogenation (reduction of H/C ratio), decarboxylation (reduction of O/C ratio), demethylation occurring during the decay of peat forming plants, and peat maturation continuing up to coal. From the point of view of chemistry, humification can be considered as a process in which more labile structures (carbohydrates, amino acids, etc) are destroyed, but thermodynamically more stable aromatic and polyaromatic structures emerge. It follows that the analysis of atomic ratios confirms the background of processes during peat humification. As shown in Figure 1, H/C ratio decreases with increasing decomposition degree of the original living matter, starting with peat forming plants, cellulose, and proteins, and up to bituminous coal. Comparatively, the studied peats are at the start of the transformation process of living organic matter. In order to provide reliable and quantity information about the early diagenesis, we carried out further studies of the dependence of the elemental composition of the peat samples on their age (depth and decomposition degree). The trends of dependence between H/C values and the depth of the peat samples mostly show negative relation; Kaigu and Mazais Veiķenieks peat bogs have the highest negative correlations, while Dižais Veiķenieks bog shows a strong positive relation indicating the general trends of peat transformation, however, at the same time demonstrating that atomic ratio cannot be correctly used to study the humification process due to the significant impact of the original plant composition and peat formation conditions.

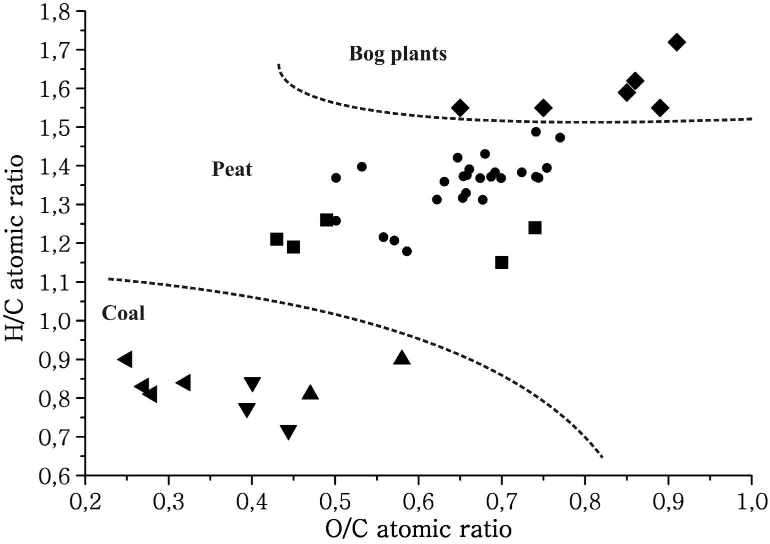

Figure 1. Van Krevelen (H/C *vs*. O/C atomic ratio) graph of bog plants (◆) peat samples from studied bogs in Latvia (●), reference peat samples (IHSS) and peat samples from common peat bogs (■), brown coal (▲), coal (◀), lignite (▼).

The first question to be resolved includes the definition of the concepts "decomposition degree" and "humification degree". The decomposition degree describes the extent to which original (living) organic matter is transformed. Decomposition process thus includes: a) transformation processes of living organisms and their tissues; b) degradation of molecules forming a living organism; c) mineralization (transformation of organic carbon compounds containing organic nitrogen, phosphorous, and sulphur compounds into their inorganic species); d) formation of refractory organic substances – humic substances. Decomposition can also be described as the breakdown of plant material accomplished by microorganisms that use decaying organic matter as a source of energy and a building material. Besides the chemical decomposition, an important indicator of decomposition is mechanical friction. In case the decomposition degree is one of the key parameters describing possibilities to use peat for agricultural purposes, several schemes of the characterization of decomposition are suggested, such as von Post scale, r value, and the peat classification of International Peat Society.

For analysis of humification degree (HD) we have suggested to use the ratio of the total amount of organic matter to the amount of formed humic substances:

$$HD = \frac{C_{HS}}{C_{peat}}.$$

In our study for a large number of well characterized peat samples we have found that this parameter strongly correlate with the peat depth and age as well as with other humification parameters and it can also be used to study the transformation processes of organic matter and peat development.

4. CONCLUSIONS

The humification degree as an indicator of the living organic matter transformation degree into new, refractory organic matter could be based on the monitoring of humic substances (HS), isolated by extraction in alkaline solution. Thus humification is narrower concept than decomposition degree and it can be estimated, analyzing the development of humus (humic substances). As humification degree we suggest to use the ratio of the total amount of organic matter to the amount of formed humic substances:

$$HD = \frac{C_{HS}}{C_{peat}}.$$

The humification degree values found for a large number of peat samples show, that this parameter strongly correlate with the peat depth and age and it can also be used to study the transformation processes of organic matter and peat development.

ACKNOWLEDGEMENTS

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The Impact of Land Use Conversion on Carbon and Humic Substances

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Keywords: carbon, humic acids, gel chromatography, E_4/E_6, IR-spectroscopy

1. INTRODUCTION

Land use is considered to be an important factor influencing soil carbon variation and properties of humic substances. Soils under different land use classes can be ranked according to their average Corg contents (0-20cm): cropland (41.3 t C ha^{-1}) < orchards/ vineyards (ca. 48 t C/ha) < intensive grassland (60.5 t C ha^{-1}) < extensive grassland (91.8 C t ha^{-1}). In extensively used grassland (incl. alpine meadows) 42% of Austria's total soil Corg pool is stored. Sustainable land use practises and landscape conservation seem necessary to maintain this high storage capacity of montane grassland soils (Gerzabek et al., 2001). There is little experimental evidence about molecular weight and chemical structure of humic acids in differently used agricultural and natural soils, especially describing changes influenced by land use conversion. As a result, one of the main objectives of this work was to study the effect of land use on carbon changes and properties of humic substances.

2. MATERIALS AND METHODS

Soil samples from the 0-30 cm layer were taken in 2007 in Silute and Kedainiai regions of Lithuania. Part of samples (6-10) represented *Albeluvisols* and the other part (15-17, 20-21) represented *Cambisols*. Carbon content in all samples was determined by a dry combustion method using an automatic analyzer Vario EL III (Germany). Humic substances were extracted using alkaline sodium pyrophosphate solution. Humic acids (HA) were investigated by the methods of gel permeation chromatography, absorption spectroscopy in visible region, and IR-spectroscopy. The molecular weight distribution of HA was determined by using gel permeation chromatography on Sephadex G-100 (Pharmacia, Sweden) with the application of 0.05 M Tris-HCl-buffer at pH 9.0 as eluent.

The formula of Determan (Determan, 1970) was used for the estimation of an average apparent molecular weight of fractions. The relative contents of the molecular weight fractions of HA were estimated from the areas under corresponding peaks on the curves of their molecular weight distribution. Optical densities of HA at 465 nm (D_{465}) and 665 nm (D_{665}) in 0.1 M NaOH were measured and E_4/E_6 ratios calculated. IR-spectra were run on KBr pellets.

3. RESULTS AND DISCUSSION

The content of carbon in Albeluvisols varied in range 1.40–2.83% and in Cambisols in range 1.98–2.22% in agricultural soils; 1.33%– in natural meadows and was much higher (6.81%) in flower garden fertilized by peat litter (Table 1).

Table 1. Carbon content in differently used soils

Sample number	Site	Soil description	C%	±
6	Silute region	Nikelai fallow	2.77	0.17
7	„	Nikelai grassland	2.83	0.19
8	„	Kalniske – Jurgaiciai forest	1.98	0.24
9	„	Palendriai fallow	1.40	0.06
10	„	Palendriai pastured grassland	1.42	0.17
15	Kedainiai region	Long term pasture (since1946)	2.22	0.06
16	„	Arable land converted from pasture	1.98	0.13
17	„	Natural meadow	1.51	0.06
20	„	Abandoned apple-tree orchard	1.33	0.02
21	„	Flower garden fertilised by peat litter	6.81	0.93

It was found that land use conversion had both positive and negative effects on soil carbon sequestration. We determined that in agricultural soils carbon sequestration occurred in longevous sown grass swards (1.16 t C ha-1 year -1). Negative C changes occurred due to conversion of swards into fallows as well as in unfertilised abandoned apple-tree orchards.

The results of the humic acids analyses by gel chromatography show high molecular weight and polydispersity of the humic acids (Table 2). All humic acids made up two major fractions. A higher molecular weight fraction 1 with an average molecular weight (MW) ≥ 100, 000 Da was found to vary from 16 to 40% for different HA under study. The content of a lower molecular weight fraction 2 characterized by MW from 15,240 to 36,980 Da varied within 60–80% range.

Table 2 . MW, D_{465} and E_4/E_6 ratios of humic acids from differently used soils

Sample	Fraction 1		Fraction 2		D_{465}	E_4/E_6
	MW, Da	%	MW, Da	%		
HA-6	≥100,000	20	22,340	80	0.671	4.53
HA-7	≥ 100,000	16	24,430	84	0.632	4.51
HA-8	≥ 100,000	23	26,180	77	0.459	4.88
HA-9	≥ 100,000	20	15,240	80	0.618	4.04
HA-10	≥ 100,000	20	20,650	80	0.626	4.06
HA-15	≥ 100,000	29	18,280	71	0.474	4.47
HA-16	≥ 100,000	25	18,790	75	0.559	4.27
HA-17	≥100,000	23	23,600	77	0.585	3.95
HA-20	≥100,000	27	28,180	73	0.488	4.24
HA-21	≥100,000	40	36,980	60	0.441	5.73

Note: Humic acids numbered according to sample number shown in Table 1

Optical density D_{465} has been considered to characterize the degree of polyconjugation in the humic molecules. A higher value of D_{465} usually corresponds to a higher degree of polyconjugation and, consequently, to a higher degree of chemical maturity of HA (Orlov, 1990). The E_4/E_6 ratio supposedly decreases with progressive humification and increase in the aromatic condensation (Kononova, 1966).

The values of D_{465} for HA under study varied in a wide range of 0.441–0.671 and the E_4/E_6 ratios in a range of 3.95–5.73 (Table 2) which shows the difference in the degrees of polyconjugation and aromatic condensation in their macromolecules.

The analysis of the data shows that conversion of grasslands and pastured grasslands into fallows and arable lands leads to the transformation of HA in the direction of decreasing MW, amide groups and carbohydrates, increasing the aromatic structures, carboxylic groups and polyconjugated systems and, consequently, in the direction of increasing the degrees of their humification and chemical maturity. This is due to decreasing the input of fresh organic residues into the soil. Conversion of swards in unfertilised abandoned apple-tree orchard leads to the transformation of HA in the same direction.

The HA-21 from the soil of the flower garden fertilised by peat litter contains macromolecules with the highest MW and is characterized by the least developed systems of polyconjugation, a low content of carboxylic functionalities and a high proportion of amide groups. The HA-21 is the youngest among all the HA under study. This indicates that the process of humification with the formation of the young immature humic molecules and accumulation of soil organic matter in this soil is the most intensive.

The HA-8 from the forest soil has a relatively low degree of polyconjugation. It is poor in carboxylic groups and rich in amide and phenolic groups. The HA-17 from the soil of natural meadow can be regarded as mature HA with the large amount of carboxylic groups and the high degrees of aromatic condensation and polyconjugation.

Significant relationships were identified between carbon and soil elements related to it. Significant linear correlation (*- significant at $P \leq 0.05$; ** - at $P \leq 0.01$) was determined between total phosphorus and organic matter contents ($r = 0.470^*$), carbon ($r = 0.539^{**}$), nitrogen ($r = 0.595^{**}$) contents and HA concentration ($r = 0.429^*$).

CONCLUSIONS

Land use conversion had both positive and negative effects on soil carbon sequestration. Conversion of grasslands and pastured grasslands into fallows and arable lands, as well as conversion of swards in unfertilised abandoned apple-tree orchard leads to the transformation of HA in the direction of increasing the degree of their chemical maturity.

The input of organic fertilisers into the soil of the flower garden increases the process of humification with the formation of young immature HA and accumulation of soil organic matter.

ACKNOWLEDGEMENTS

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Char and Humin Fractions in Amazonian Dark Earths

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Keywords: Amazonian dark earth, humin, Black carbon, char, urea, dimethyl sulfoxide, NMR

1. INTRODUCTION

The high and specific SOM contents of the Amazonian Dark Earths (ADE, Terra Preta de Índios), and their high contents of P and Ca provide high natural fertility to the soils. Char or black carbon (BC) plays a major role for the SOM stability in ADE soils (1) and it also provides a significant potential for carbon sequestration and sustainable ecosystems (2). There are several studies of the humic acids (HAs) of ADE soils (e.g., 3 and 4). However, because of the difficulties in isolation, there is less awareness of humin, the most abundant SOM component, and of the interaction mechanisms of recalcitrant BC with SOM (especially humin) and clays. In this study, humic materials were extracted using the IHSS procedure and by exhaustive sequential extraction procedures. Isotopic ^{13}C analyses, and solid-state ^{13}C NMR spectroscopy were used for characterizations.

2. MATERIALS AND METHODS

ADE sample 1 (500 g) was extracted by the exhaustive sequential extraction procedure using NaOH at pH 7, 10.6 and 12.6, and then with 0.1M NaOH + 6M urea (base/urea) following the procedure outlined in Song *et al* (5). Soil extracts were fractionated by the XAD-8 and XAD-4 resin-in-tandem procedure. ADE sample 2 (140 g) was treated and extracted following the procedure recommended by IHSS. Then the soil residue was extracted using 0.1M NaOH + 6M urea. HAs from 1 and from 2 were treated with 0.3M HF/0.1M HCl. Then the coarse char was carefully recovered by sieving (passing through 63 µm sieve) and sedimentation. The dry light fractions (<63 µm) from 1 and 2 were extracted with DMSO + 6% (v/v) H_2SO_4 (DMSO/H_2SO_4). The DMSO extract was adjusted pH to 2 with distilled water and further fractionated into DMSO humin (precipitate) and soluble DMSO FA (recovered by the XAD-8 resin). The humin associated with the light fraction (silt + clay) before and after DMSO/H_2SO_4 extraction was de-ashed with 10%

HF. The humic fractions were characterized by VACP-TOSS ^{13}C NMR spectroscopy (refer to Novonty *et al*. in this Conference Proceedings, and to 5).

3. RESULTS AND DISCUSSION

In the case of sample 1, the yields of HAs and FAs decreased significantly with increasing extractant pH (Table 1). The humic material isolated in base/urea was part of the humin fraction according to the classical definition. However, the isolates displayed characteristics of the alkaline humic fractions once released by base/urea. These are referred to as base/urea HAs or FAs. The HAs isolated with base and base/urea were the most abundant of the fractions (*ca*. 87%), with the FAs isolated with base and base/urea accounting for the remainder. The HAs and FAs isolated at pH 7 were the major fractions (*ca*. 57%) in the total HS isolated from base and base/urea solvents. That indicates that the ADE SOM is highly humified and oxidized. Using the urea enhanced base solvent from 8% to 19% additional humic materials were isolated. The data could suggest the humin is a mixture of HAs and FAs components that probably were trapped in, or associated with, through non-covalent bonds (e.g. H-bonds), the humic/humin core. These results support the supramolecular theory proposed by Piccolo (6), in which the HS may be regarded as associations of self-assembling heterogeneous and relatively small molecules derived from the degradation and decomposition of biomass debris. The aggregates will likely be 'held together' through a complex combination of hydrophobic associations, charge interactions, hydrogen bonds, and metal bridging (7). However, the base/urea humic fractions isolated and the DMSO humin contained relatively larger sized molecules than the conventional alkaline HAs or FAs. These are in 'stable aggregates' or 'macromolecules' (8).

Table 1. Yields of HAs, FAs, and humin isolated using base/urea and DMSO/H_2SO_4

	ADE Sample 1				ADE Sample 2	
	pH 7	pH 10.6	pH 12.6	Base/Urea	0.1M NaOH	Base/Urea
HA	3.38	1.13	0.07	1.04	17.55	1.61
FA	0.32	0.27	0.1	0.17	2.32	0.17
DMSO Humin	3.85				5.69	
DMSO FA	0.55					

The most recalcitrant SOM components associated intimately with silt and clay was isolated from DMSO/H_2SO_4. About 19 to 32% (calculated in terms of the total organic C contents of soil samples) of humin material can be extracted. With this sequence of extraction procedure, up to 74% of SOM was isolated.

The $\delta^{13}C$ values of the humic fractions indicated the humic material was mainly derived from C3 plants, with an average $\delta^{13}C$ value of *ca* -28.1‰. The $\delta^{13}C$ values of the HA fractions were slightly enriched with ^{13}C. The $\delta^{13}C$ decreased from -28.1‰ to -30.0‰ for DMSO humin and from -30.8 to -31.7‰ for the DMSO extracted residue, respectively. This might be explained selective utilization of organic compounds by microbes.

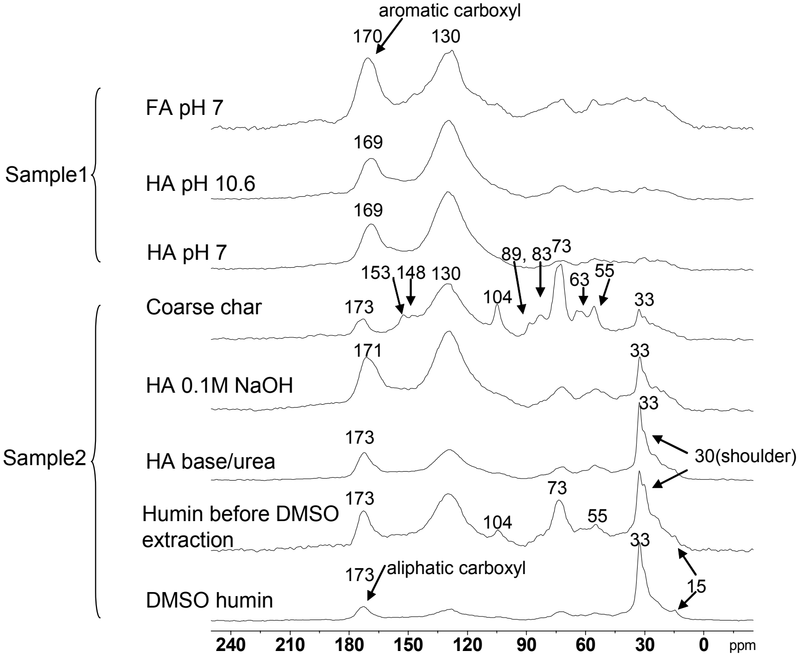

Figure 1. VACP-TOSS ^{13}C NMR spectra of HAs and FAs isolated with base, base/urea HA, humin before DMSO/H_2SO_4 extraction from light fraction, and DMSO humin.

The predominant resonances for the HAs, FAs and coarse char in 110 to 156 ppm region (Figure 1) showed hydrogen-deficient condensed aromatic C, mainly derived from char. The dipolar dephasing spectra (not shown) of the HAs and FAs isolated from 0.1M NaOH and the coarse char presented significant unprotonated C in 110 to 156 ppm region, *ca* 74%, 80% and 62%, respectively, compared to the corresponding total C area. The alkaline HAs and FAs were enriched with carboxylic groups attached to the condensed aromatic 'core', as evidenced by the relatively lower resonances of COO at *ca.* 169 to 171 ppm. The coarse char was mixed with components from lignin units (55, 148 and 153 ppm resonances), cellulose (resonances at 63, 73, 83, 89, 104 ppm) and BC (prominent aromatic C at 130 ppm). In another word, the coarse char material presents signals of unaltered plant debris, BC and aliphatic components. The base/urea HA and DMSO humin had prominent aliphatic groups (crystalline polymethylene C at 33 ppm and amorphous polymethylene C at 30 ppm), and relatively low contents of aromatic C and carboxyl groups, suggesting significant contributions from long-chain aliphatic moieties (e.g., lipids, waxes, cutin, etc.). Compared to DMSO humin, humins before and after DMSO/H_2SO_4 extraction were very similar. It is likely that condensed BC, peptides and carbohydrate (e.g. cellulose) components were trapped in the humic matrix or physically protected by intimate association with clay, whereby both base/urea and DMSO/H_2SO_4 solvent could only partially cleave the weak bonds between BC, humin and clay minerals.

4. CONCLUSIONS

The well humified and oxidised char derived from incomplete combustion of biomass contribute the main components in ADE SOM components. The most recalcitrant BC in the humin matrix is probably embedded within the silt and clay minerals forming organic-mineral complexes, or protected by steric constraints which are difficult to cleave by base/urea and DMSO solvents. The primary recalcitrant SOM (DMSO humin) is from non-BC sources, such as long-chain fatty acids/ester, lipids, cutins, etc. containing derivatives of microbial origins.

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Diverse Microbial Carbon Turnover and Dynamics in Temperate and Tropical Forest Soils

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Keywords: microbial turnover, humus, soil organic matter, tropical, temperate, forest soils

1. INTRODUCTION

Microbial metabolism is widely accepted as the driving force in humification processes; however, turnover models wholly overlook the importance of microbial biochemistry despite the fact that most plant residues likely pass through microbial biomass prior to conversion to humic substances (Kogel-Knabner, 2002). This research investigates the importance of microbial biochemistry to humification pathways in two climatically different forest ecosystems; Blodgett forest (BF), a temperate forest in the Sierra Nevada and Luquillo forest (LF), a tropical forest in Puerto Rico. 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 humification.

2. MATERIAL AND METHODS

Temperate and tropical microorganisms from four biochemically contrasting groups (fungi, actinomycetes, bacteria gram (+), and bacteria gram (-)) were isolated from BF and LF and grown in 99-atom-percent ^{13}C media. Enriched microorganisms were autoclaved and lysed microbial cells were added to soil at a depth of 3 to 4 cm at both sites in a reciprocal design. Treated soils are being excavated at 5 time points over a span of 3 years at BF and 2 years at LF. Excavated soils are separated into two depth fractions (0-7.5 and 7.5-15 cm below the surface) and analyzed for total C and ^{13}C using combustion gas chromatography-isotope ratio mass spectrometry (GC-IRMS); microbial biomass carbon (MBC) is determined using the chloroform fumigation method; dissolved organic carbon (DOC) is determined by K_2SO_4 extraction and analyzed with a UV-persulfate DOC analyzer coupled to an IRMS. Soils are incubated at 55% of field capacity and analyzed for CO_2 using infrared absorption spectroscopy and GC-IRMS.

3. RESULTS AND DISCUSSIONS

Decomposition rates were substantially greater at LF than at BF for all treatments (fig. 1 and 2). While a substantial amount of new C had been lost by the third excavation for BF and LF (66 and 82% respectively), less than 5% of new C was recovered in the 7.5 to 15 cm depth from both sites, indicating that little new C was lost to leaching at either site. The microbial biomass pool and overall CO_2 flux was greater for LF than for BF soils, which corresponds to greater decomposition rates for new C at LF than at BF. While there were initial differences in overall decomposition rates between microbial groups at both sites, over time the recoveries of new microbial C became more similar for different groups (fig. 1 and 2). Within microbial groups, there were little to no differences in overall decomposition rates when comparing temperate and tropical additions (fig. 1 and 2). However, there were differences between temperate and tropical additions as new C recovery within the microbial biomass pool for BF and LF (fig. 3 and 4).

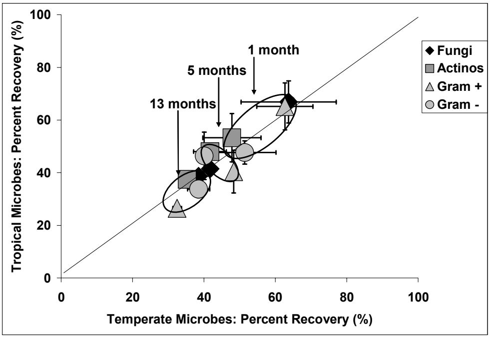

Figure 1. Percent recovery of new soil C remaining from different microbial groups at BF at 1, 5, and 13 months.

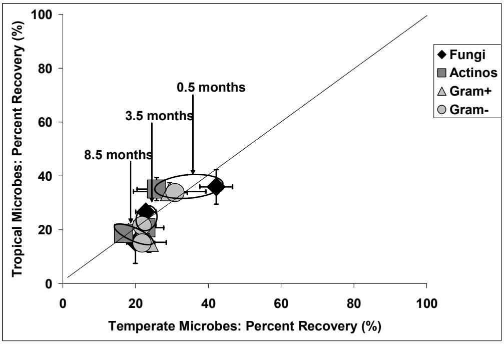

Figure 2. Percent recovery of new soil C remaining from different microbial groups at LF at 0.5, 3.5 and 8.5 months.

After one month, recoveries of tropical additions from BF as MBC were significantly greater for all groups except gram (-) bacteria. After 5 months, these trends reversed and recovery of temperate additions were significantly greater for all groups except gram (-) bacteria. After 13 months, tropical and temperate C recovered as MBC converge for all groups (fig. 3).

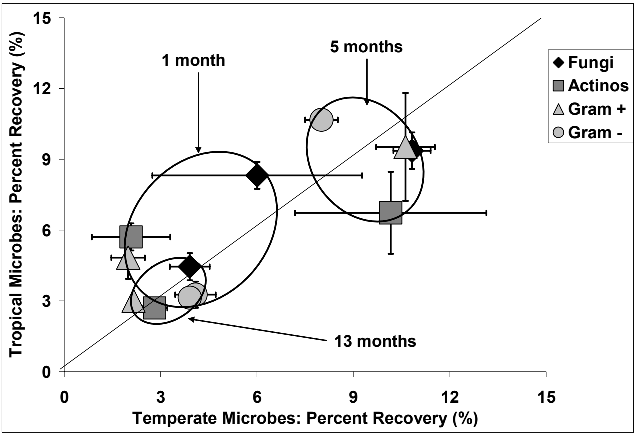

Figure 3. Percent recovery of new soil C as MBC from different microbial groups at BF at 1, 5, and 13 months.

Similarly to BF, differences between temperate and tropical additions recovered as MBC were initially observed at LF at the first excavation (at 0.5 months). After 3.5 months, temperate and tropical recoveries converge for all microbial groups (fig. 4).

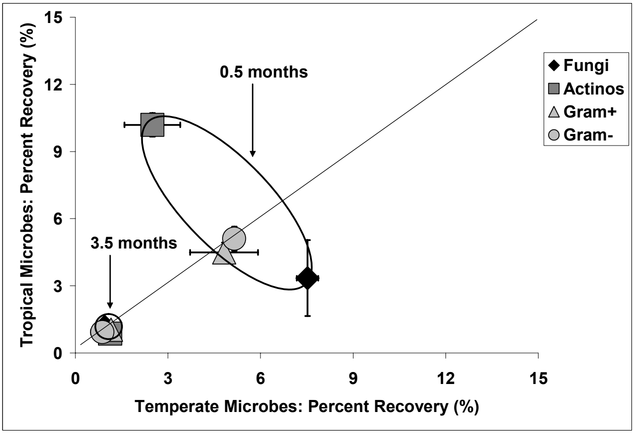

Figure 4. Percent recovery of new C as MBC from different microbial groups at LF at 0.5 and 3.5 months.

Initial recoveries of new C within the DOC pool at BF were significantly different between most treatments. After 5 and 13 months, new C recoveries as DOC converge and stabilize at ±0.4% of initial input C at BF. After 0.5 months, new C recoveries as DOC at LF were less than 0.5% of input C with some significant differences between treatments. After several months, new C recoveries as DOC declined to <0.003% of input C for all treatments.

4. CONCLUSIONS

Microbial C decomposition occurs much more rapidly in tropical than temperate forest soils due to differences in metabolic capacities of the soils. While the microbial biomass and DOC pool of temperate and tropical soils may initially respond differently to unique sources of organismal C, over time the amount of input C maintained within these pools is less influenced by the quality of input microbial C. Although the origin of microbial C (temperate vs. tropical-derived) has no strong correlation to the overall rate of decomposition in either temperate or tropical forest soils, differences observed between microbial groups demonstrates that the quality or biochemistry of microbial substrates may initially affect the rate of decomposition or turnover of microbial C in both temperate and tropical forest systems, but throughout later stages of humification the initial source or quality of microbial C becomes less important to the amount of C that is stabilized in these systems. This research provides insights into the dynamics and turnover of unique organismal C in temperate and tropical forest systems. Ongoing work to characterize specific microbial C precursors to humification utilizes curie-point pyrolysis-gas chromatography coupled to analytical mass spectrometry and isotope ratio mass spectrometry (Py-GC/MS-IRMS), and will further elucidate stabilization mechanisms for microbial C and pathways for humification.

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The Comparison between Humus Structure of 5 to 1 μm Particle-Size Fractions and Fine Clay Fractions in Soils of Locally Hydromorphic Landscapes

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Keywords: soil organic matter, chernozem, locally hydromorphic soils, humus structure, humic substances, particle-size fraction

1. INTRODUCTION

One of the factors of agricultural soil degradation in the chernozemic zone is the wide spread appearance of local hydromorphic areas. Their appearance within the structure of agrolandscape is evidence of local changes in hydrological regime. The change to hydromorphic soils last decades and affected areas are evident within the Russian Federation, Ukraine and Moldova.

Research is needed that defines the laws that govern distribution and properties of soil humus of different soil fractions. In addition we need to acquire knowledge no humus accumulation processes and the extent to which those process are affected by various mechanical elements, interrelationships between organic and mineral parts of soil and their role in humus structure formation etc. It is known (7, 8, 9) that the 5–1 μm particle-size fraction contributes in humus accumulation in to an equal or greater extent than the $<$ 1 μm fraction. The degree of humus accumulation by 5–1 μm and $<$ 1 μm particle-size fractions varies with soil type, but basically depends on the mineralogical structure (2, 3) and the degree of dispersiveness that characterizes soil organic matter (4).

2. MATERIALS AND METHODS

The soil sample used in this study was collected from an agricultural field located in the South of Rostov region, Russia. The soil fraction of the mechanical elements was obtained by sedimentation as described by Gorbunov (1). The definition of group and fractional structure of soil humus was determined according to the method shown in (6).

3. RESULTS AND DISCUSSION

The humus structure of clay fractions appreciably differs from humus structure of soil as a whole. Fulvoacids prevail in the humus structure, and the appreciable reduction of the non-hydrolyzed part of humus is also evident. In the clay fraction its content constitute only

25-30 % from the contents of the non-hydrolyzed part of humus in soils (tab. 1). Probably, it is connected that during separation of clay fraction the non-hydrolyzed part of humus really passes in group of humic acids. These results speak about the large concentration гумуса in the clay fraction of soils of the locally hydromorphic landscapes. There is it, apparently, because of changes in mineralogy of hydromorphic soils.

Table 1: Humus structure of the clay fraction of locally hydromorphic soils

Horizon	C total %	HA			FA			N-G	$\frac{C_{HA}}{C_{FA}}$
		1+2	3	∑ HA	1+1a+2	3	∑ FA		
Meadow chernozemic super-power soil									
A_1	2.86	26.57	17.13	43.70	17.48	5.24	22.72	33.58	1.9
AB	3.30	26.36	16.96	43.32	13.63	4.24	17.87	38.81	2.4
B_1	2.86	24.48	18.53	43.01	20.63	5.94	26.57	30.33	1.6
B_2	2.30	25.65	13.04	38.69	16.96	8.26	25.22	36.09	1.5
BC	2.52	18.25	19.05	37.30	16.27	7.54	23.81	38.89	1.56
C	1.85	18.37	15.14	33.51	22.70	13.51	36.21	30.28	0.93
Meadow gleyed power soil									
A_1	2.63	28.89	24.33	53.22	9.13	6.46	15.59	31.19	3.41
A^S	2.63	33.08	18.25	51.33	3.04	8.37	11.41	37.26	4.5
AB	3.19	33.23	15.67	48.90	3.76	4.39	8.15	42.95	6.0
AB_g	2.63	29.66	7.0	37.26	7.60	3.80	11.40	51.34	3.27
B_g	2.07	25.60	7.25	32.85	8.21	4.83	13.04	54.11	2.52

The research conducted earlier by O.G. Nazarenko (5) showed the significant humus accumulation in 5 to 1 μm particle-size fraction as compared with coarse and fine fractions in soils of locally hydromorphic landscapes. This study showed the structure of 5 to 1 μm particle-size fraction includes a prevalence humic acids coupled with calcium. The fraction humic acids strongly coupled to clay minerals decreases slightly with clay fraction. Experiments determined that the sum total of fulvoacids was minor (tab. 2). Appreciable differences in the humus structure of the clay fraction were observed with the contents of the non-hydrolyzed portion of humus. For all researched soils, the quantity of the non-hydrolyzed portion varies within small limits. Other research effort (3, 9) found the amount of humus increased with particles size; for this to be true, it would require an assumption that humic acids were strongly associated with the mineral fraction of soils.

Table 2: Humus structure of 5 to 1 µm particle-size fraction of locally hydromorphic soils

Horizon	C total, %	HA			FA			N-G	$\frac{C_{HA}}{C_{FA}}$
		1+2	3	∑ HA	1+1a+2	3	∑ FA		
Meadow chernozemic super-power soil									
A_1	5.0	32.00	14.0	46.0	9.0	2.2	11.2	42.8	4.1
AB	4.56	37.50	15.35	52.85	7.46	1.32	8.78	38.37	6.0
B_1	4.23	45.63	13.95	59.58	5.44	1.18	6.62	33.8	9.0
B_2	3.16	45.25	15.51	60.76	5.38	3.16	8.54	30.7	7.1
BC	4.0	35.75	14.75	50.5	4.25	2.75	7.0	42.5	7.2
Meadow gleyed power soil									
A_1	5.43	32.41	12.89	45.30	7.37	3.13	10.50	44.2	4.3
A_S	4.54	35.24	15.42	50.66	6.17	2.42	8.59	40.75	5.9
AB	5.43	39.59	11.79	51.38	3.13	2.21	5.34	43.28	9.7
AB_g	4.42	33.48	13.35	46.83	6.33	2.49	8.82	44.35	5.3
B_g	4.09	26.65	17.11	43.76	8.31	2.69	11.0	45.24	4.0

4. CONCLUSIONS

The following tendencies in changes of humus structure of the clay fraction of the hydromorphic soils in comparison with chernozems are carry out. A reduction of the humic acids:fulvoacids ratio and reduction of the share non-hydrolyzed part of humus are observed. In addition, for the soils studied, a substantial fraction humic acids were observed to be strongly associated with clay minerals. These tendencies increase with soils exposed to increasing durations of water saturated condition.

As a whole, differences existed in the humic structure associated with particle-sizes fractions ranging from of 5–1 µm and particles $<$ 1 µm. The structure of humus from particles sizes ranging from 5–1 µm was typically coupled with calcium. The content of humic acids in soils was strongly correlated to clay minerals and decreased slightly as the fraction of clay decreased. Appreciable differences in humus structure were observed with respect to the content of non-hydrolyzed humus. For the 5–1 µm particle-size fraction small variations were observed in the ratio of non-hydrolyzed:total humus; whereas for the $<$ 1 µm particle-size-fraction, this ratio decreased.

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Properties of Humic Acids in Forest Soils: Influence of Different Plant Covering

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Keywords: Forest litter, humic acids, infrared spectroscopy, lignin-derived phenols

1. INTRODUCTION

The chemical composition and morphology of plant litter is among the main factors that affect the dynamics and properties of organic matter in forest soils (1). In particular, the transformations of the humic acid (HA) fraction with depth generally show a progressive loss of carbohydrates and, to a lesser extent, of lignin, and an increase of carboxylic functional groups. Lignin is considered a relatively recalcitrant biopolymer (2) and a precursor of more complex compounds such as humic substances (3). The use of soil biomarkers such as lignin-derived phenols could improve our understanding of how plant residues are transformed into stabilized soil organic matter.

This work aims to evaluate the influence of different plant covering on soil HA properties in temperate forest floor layers.

2. MATERIALS AND METHODS

Forest litters and soil surface layers (0-2, 2-5 and 5-20 cm) were sampled from four forested sites of the "Bosco delle Pianelle", (Taranto, Southern Italy), which is characterized by the presence of the following species: (i) *Quercus ilex* L. (L); (ii) *Carpinus betulus* L. and *Carpinus orientalis* Miller (CC); (iii) *Pinus halepensis* Miller (P); (iv) *Quercus trojana* Webb. and *Quercus ilex* L. (FL).

The HAs were isolated from litters (humic acid-like, HAL) and soil samples (HA) according to the procedure suggested by the IHSS (4) and characterized by elemental analysis, ratio of absorbances at 465 and 665 nm (E_4/E_6), carboxylic group content (calcium acetate method) and Fourier transform infrared (FT IR) spectroscopy. The contents of lignin- derived phenols and their degree of oxidation in the bulk samples and HA fractions were estimated using alkaline CuO oxidation followed by GC-FID quantification (5, 6).

3. RESULTS AND DISCUSSION

Elemental analysis (data not shown) shows significant similarities between the composition of organic matter in the bulk soil samples and the corresponding HAs, suggesting that the composition of HAs is related to that of the plant debris from which they originate. The C/N ratio decreases with soil depth, whereas the C/H ratio tends to increase with depth for both bulk soil samples and HAs.

Table 1. Chemical characteristics of forest floor layers
and their respective humic acid fractions

Site / Horizon	Bulk litter and bulk soil samples			Humic acids				
	VSC	S/V	$(ac:al)_v$	VSC	S/V	$(ac:al)_v$	E_4/E_6	COOH
Quercus ilex forest (L)								
Litter layer	48.32	1.28	0.19	28.98	1.41	0.44	8.2	2.87
0 – 2 cm	32.63	0.90	0.25	25.94	1.40	0.47	6.9	2.88
2 – 5 cm	23.43	0.67	0.37	19.40	0.90	0.57	5.8	3.55
5 – 20 cm	11.78	0.90	0.32	4.95	0.67	0.60	5.0	3.98
Carpinus betulus and Carpinus orientalis forest (CC)								
Litter layer	67.54	1.27	0.24	26.71	1.52	0.33	8.3	2.98
0 – 2 cm	14.21	0.96	0.23	12.10	1.19	0.63	5.2	3.63
2 – 5 cm	10.03	0.84	0.32	7.79	0.97	0.72	5.0	4.12
5 – 20 cm	5.24	0.66	0.32	4.85	0.81	0.69	5.0	4.12
Pinus halepensis forest (P)								
Litter layer	37.37	0.08	0.20	40.44	0.10	0.36	11.0	2.88
0 – 2 cm	27.68	0.15	0.27	31.97	0.15	0.44	7.2	3.42
2 – 5 cm	31.05	0.23	0.33	19.33	0.28	0.52	6.5	3.30
5 – 20 cm	20.56	0.35	0.24	17.07	0.40	0.54	6.0	3.48
Quercus trojana and Quercus ilex forest (FL)								
Litter layer	72.22	0.68	0.23	33.48	0.93	0.34	8.0	2.66
0 – 2 cm	34.74	0.64	0.30	39.50	0.75	0.78	6.5	3.46
2 – 5 cm	31.33	0.59	0.34	21.17	0.70	0.66	7.1	3.12
5 – 20 cm	24.66	0.55	0.36	18.32	0.70	0.68	6.8	3.30

The sum of the C-normalised yields of the major lignin-derived phenols (VSC, vanillyl, syringyl and cinnamyl phenols; Table 1) decreases with depth for bulk samples as well as for HAs in all soil profiles, indicative of extensive degradation of lignin derived from plant debris in the litter layers. As expected, all soil profiles are characterised by low values for the ratio of cinnamyl to vanillyl phenols (C/V; data not shown), typical of forest floor layers under woody coverage. The ratios of syringyl to vanillyl phenols (S/V; Table 1) are higher for soil profiles under angiosperm plant coverage (L, CC, FL) with respect to those from the coniferous forest (P). The similarity in S/V values obtained for bulk litter and soil samples, and their corresponding HAs (*r* = 0.94) confirms the direct influence that plant coverage exerts on the HAs composition. With the exception of samples from the coniferous P series, the S/V ratio tends to decrease with depth for both bulk soil and HA fractions, which indicates the occurrence of lignin degradation. During the degradation of lignin by white-rot fungi, syringyl units are preferentially degraded over vanillyl structures, resulting in the lowering of the S/V ratio (7). The ratio of vanillic acid to vanillin, $(ac{:}al)_v$ increases with depth for both the bulk soil samples and their corresponding HAs (Table 1). In particular, the HAs yielded consistently more acidic phenols than the bulk soil samples, which reflects a degree of microbial alteration of the residual lignin. This result, together with the observed enhancement of carboxylic groups with depth (Table 1), suggests an increase of the degree of oxidation along the soil profile. Moreover, the decrease of the E_4/E_6 ratio (Table 1) and the increase of the C/H ratio (data not shown) with depth suggest the increase of aromaticity and degree of polycondensation of the HAs possibly due to the microbial degradation of more labile constituents.

The FT IR spectra of HAL (Figure 1) feature common bands with different relative intensities. The HAL samples from the L, CC and FL series also show typical bands attributable to syringil and vanillyl rings of lignin-derived phenols (1152 and 1126 cm^{-1}),

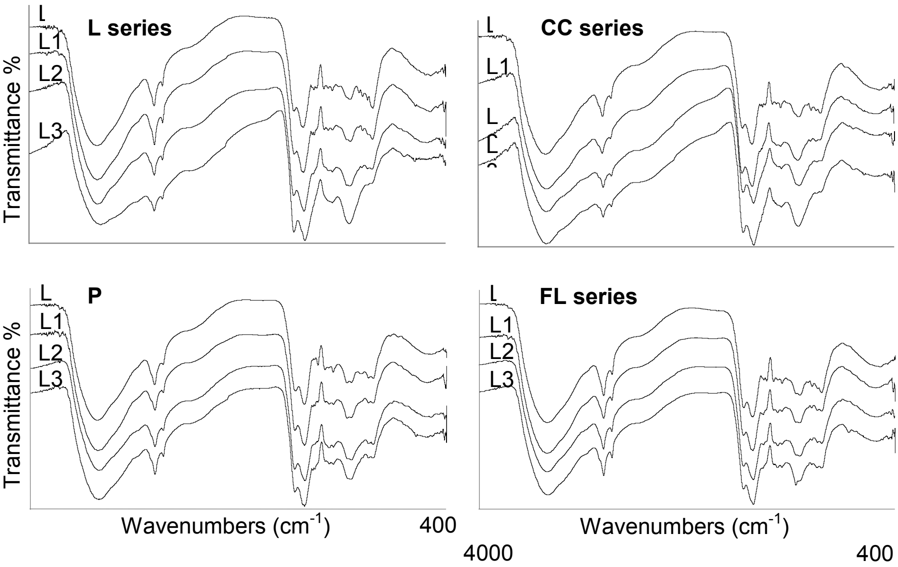

Figure 1. FT-IR spectra of HAL (L) and Has (L1= 0-2, L2= 2-5, L3= 5-20 cm) series.

whereas the HAL from the P series exhibits only the band typical of vanillyl phenols, which is possibly due to the small quantity of syringil units in the gymnosperm lignin. With depth, all HAs exhibit a decrease of aliphatic components (2922 and 2852 cm^{-1}), a slight decrease of phenols (1418 cm^{-1}), a sharp decrease of polysaccharides (1078 and 1040 cm^{-1}), and an increase of carboxylic groups (1235 cm^{-1}), which are especially evident in the L and CC series. Moreover, the FT IR spectra of HAs isolated from the deepest soil layers appear to be very similar one to another, irrespective of the plant debris from which they derive.

4. CONCLUSIONS

The properties of the litter originating from different plant covering appear to influence directly the chemical composition of HAs in the underlying soils. Lignin appears to be present in the HA in structural units similar to those found in plant debris from the overlying organic layers. Furthermore, the lignin composition is reminiscent of the lignin signature obtained for the plant covering, appearing relatively intact but somewhat oxidised. The HAs isolated from the deeper soil horizons, which have been subjected to more intensive diagenetic modifications, tend to be highly homogenous and relatively deprived of the more labile constituents such as polysaccharides. Finally, the lower aromatic polycondensation and oxidation degree of the HAs from the P and FL series, with respect to those from L and CC series, would indicate a partial incorporation of lignin-derived products into native soils HAs.

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Fluorescence Spectroscopy of Humic Acids Isolated from Different Litters and Corresponding Underlying Soils

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Keywords: Forest litter, humic acids, fluorescence spectroscopy

1. INTRODUCTION

Humic acids (HAs) represent an important fraction of soil organic matter, and are characterized by a wide chemical and structural complexity that is yet not well-known. In the last decades numerous studies have been carried out using fluorescence spectroscopy to characterize humic substances also in relation to their origin (1). Obviously, a series of factors such as the occurrence of aromatic units and extended electron π systems, heteroatom substitution, type and number of substituent groups on the aromatic rings, etc., are affecting fluorescence properties of these macromolecules. Therefore, the fluorescence behaviour of humic substances depends on their various structural components, and each fluorescence spectrum is due to the contribution of several individual fluorophores present in the molecule.

The aim of this paper was to investigate by means of fluorescence spectroscopy the properties of HAs isolated from various forest litters and the corresponding underlying soils as affected by different covering plants.

2. MATERIALS AND METHODS

Forest litters and the corresponding soil surface layers at three different depths (0-2 cm, s1; 2-5 cm, s2; and 5-20 cm, s3) were sampled in the "Bosco delle Pianelle", Taranto province, Southern Italy, under four different covering species: (a) *Quercus ilex* L. (L); (b) *Carpinus betulus* L. and *Carpinus orientalis* Miller (CC); (c) *Pinus halepensis* Miller (P); (d) *Quercus trojana* Webb. and *Quercus ilex* L. (FL). The HAs were isolated from litters (humic acid-like, HAL) and soil samples (HA) according to the procedure suggested by the IHSS (2). Fluorescence spectra were recorded on aqueous solutions of HALs and HAs, at a concentration of 100 mg L^{-1}, equilibrated overnight at room temperature, and adjusted to pH 8 with NaOH, by using a Perkin-Elmer (Norwalk, CT) LS 55 luminescence spectrophotometer equipped with the WinLab 4.00.02 software for data processing.

Emission spectra were recorded over the range 380–600 nm (λ_{exc} fixed at 360 nm); excitation spectra were recorded over the range 300–500 nm (λ_{em} fixed at 520 nm); and synchronous-scan excitation spectra were measured by scanning simultaneously both the excitation and the emission wavelengths (from 300 to 550 nm), while maintaining a constant, optimized wavelength difference $\Delta\lambda$ (λ_{em} – λ_{exc}) = 18 nm. Total luminescence spectra, in the form of excitation-emission matrices (EEMs, contour maps), were recorded over the emission wavelength range from 300 to 600 nm, increasing sequentially by 5 mm steps the excitation wavelength from 250 to 500 nm. The EEM plots were generated as contour maps from spectral data by using Surfer 8.0 software (Golden Software, Inc., 2002, Golden, CO).

3. RESULTS AND DISCUSSION

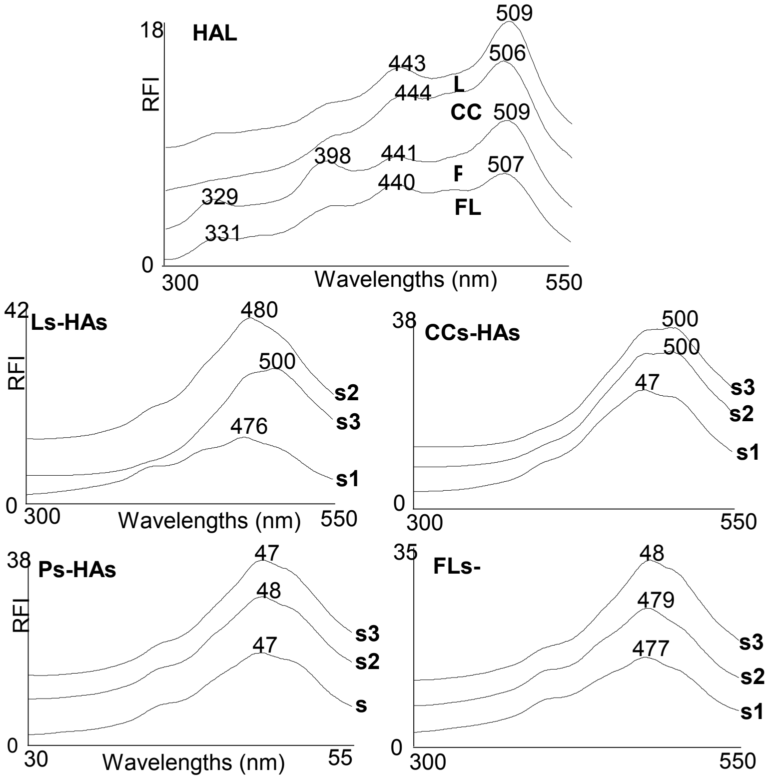

Figure 1. Synchronous-scan fluorescence spectra of HALs and HAs.

Synchronous-scan fluorescence spectra of HALs (Figure 1) are characterized by a main peak at longer wavelengths (about 509 nm) and a shoulder at shorter wavelengths (about 440 nm). Additional shoulders appear at about 330 nm for P-HAL and FL-HAL and at 398 nm for P-HAL. In all HALs the relative fluorescence intensity (RFI) values are similar each to the other, and are slightly lower in P- HAL and FL-HAL. Synchronous-scan fluorescence spectra of HAs show a main peak in the range from 476 to 500 nm (Figure 1), with the maximum slightly shifting towards longer wavelengths with increasing depth. Further, RFI values show a consistent increase with depth, with the exception of L-HAs2, which is characterized by a RFI value greater than that of L-HAs3. Emission fluorescence spectra of HALs and HAs (not shown) feature a unique broad band having the maximum in the range, respectively, between 454 and 476 nm, and 484 and 520 nm. Excitation fluorescence spectra of HALs (not shown) show a simple pattern, with a peak at about 439 nm and a shoulder at about 398 nm, whereas the HAs excitation spectra exhibit a peak between 441 and 456 nm.

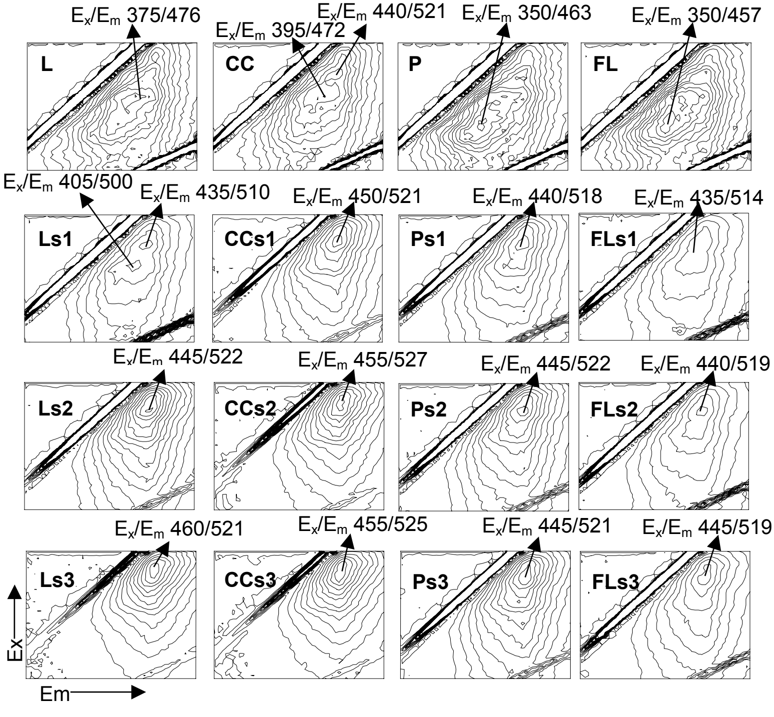

Figure 2. Total Luminescence spectra of HALs and HAs.

The EEM spectra of HALs (Figure 2) from L and CC litters are characterized by the occurrence of a main peak centered at an excitation/emission wavelength pair (EEWP) of375-395$_{ex}$/472-476$_{em}$, which suggests the occurrence of similar fluorophores, probably structural components like esculetin and/or scopoletin. Further, the EEM spectra of CC-HAL shows an additional main peak at an EEWP of 440$_{ex}$/521$_{em}$, ascribed to conjugated chinons and phenols with a high polycondensation degree.

The EEMs spectra of P-HAL and FL-HAL are characterized by numerous fluorescent units with EEWPs in the short and medium wavelength regions. The highest RFI values for both HALs are associated with the peak at about 350$_{ex}$/457-463$_{em}$, likely due to structural methoxy-coumarins, and/or hydroxyquinolines (3).

The EEMs spectra of HAs (Figure 2) show a common peak at an EEWP 435-460$_{ex}$/510-527$_{em}$, which may be associated with the occurrence of aromatic units at a high polycondensation degree. All HAs show, with increasing depth, a shift of EEWPs towards longer wavelengths and an increase of the corresponding RFI values (except sample HA-Ls2).

4. CONCLUSIONS

In general, the fluorescent properties of HALs indicate the occurrence of simple structural moieties, with slight differences due both to the parent litter and to its degradation. In particular, P- and FL-HAL would suggest a greater litter decomposition with respect to L- and CC-HAL samples.

The comparative analysis of HAs from the various series shows the occurrence of smaller structural components in the HAs from the s1 layers, which suggests the incorporation of litter degradation products into native soil HAs, especially in the P- and FL-HAs1. The HAs from the subsurface and deepest layers show an increasing humification degree with depth, likely related to a different mobility and/or adsorption of organic colloids on the mineral phase.

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In Situ Humification and Humics Preservation in Phosphatic Pellets, under Sulfate Reducing Conditions

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Keywords: phosphatic pellets, phytoplankton-derived humics, sulfate reduction, physical preservation of humus

INTRODUCTION AND RATIONALE

The phosphate-bearing particles in the Ypresian phosphatic ore of Ras-Draâ, Tunisia, dated ca. 50 My, are rounded grains, some hundreds of micrometers in mean diameter, called «pellets» by petrographers. These pellets are embedded in a sedimentary, more or less carbonated, shaly matrix. The pellets studied in this ore contained between 28.4 and 38.3 % of P_2O_5 and between 0.3 and 1.6 % of TOC. Surprisingly – with regards to the age of the pellets – their organic matter is very rich in extractable (NaOH N/10) humics, namely up to 75.6 % of humic C (% of TOC, in C V layer).

Surprisingly also – with regards to this data – the organic matter present in the matrix of the pellets is by far not as rich in humic compounds as are the pellets (up to 21.4 % of matricial humic C, % of TOC in C V layer). The composition of the organic matter of the matrix is quite usual for a 50 My old, epicontinental, never buried (over a maximum of 200-250 m depth), sedimentary layer. It is a low-rank kerogen. Pellets appear therefore as chemically singular bodies, whose diagenetic story has been quite different, and independent, of that of their embedding matricial sediment.

These bodies have been demonstrated to have derived from fish feces (1).

INTERPRETATIONS AND CONCLUSIONS

The humification of the pellets organic matter and the remarkable preservation of the resulting humics, during 50 My, address the following questions and lead to the following answers:

1 – The origin of the organic matter is marine planktonic, as deduced from elemental analysis, Rock-Eval pyrolysis and photonic microscopy. This organic matter has been ingested as food by planktivorous fishes.

2 – The early diagenesis of the organic matter excreted by fishes was carried in sulfate-reducing conditions, as shown by the following data: (1) Humic acids contain large

amounts of organic sulfur, up to 14%, by weight (2) if the gas chromatographic spectra of n-alkanes and iso-alkanes in the pellets witness generally to mixed phytoplanktonic and microbial sources for the organic matter, they can also display a purely microbial spectrum, suggesting that the original phytoplanktonic constituents were decomposed by bacteria, inside the pellets. Conversely, the alkanes present in the matrix sediments surrounding the pellets are typically phytoplanktonic in origin.

The humic constituents present in the pellets can therefore be considered as the microbially oxidized counterpart of the reduced SO_4-S.

3 – How could these humics be preserved as such during 50My?

They owed their preservation to the early physical properties prevailing in the pellets (1) as early as their egestion by the fish, the feces are embedded in a phosphatized external membrane which protects them from tearing during their transfer through the water column and during their reworking by bottom currents (2) the growth of densely-packed apatite crystals inside the pellets - P being issued from the non-digested fish bones ingested by carnivorous fishes, last in food chains – develops pressure forces on the organic particles inside the pellets and isolates these particles from any circulating fluid This pressure can be observed when dissolving the apatite crystals with diluted Hcl. The humic particles expand suddenly when the frame of the apatite crystals disappears. This can be easily observed under the binocular lens.

Finally, the transformations underwent by the phytoplanktonic-derived excreted organic matter in the fecal pellets of carnivorous fishes are early oxidation reactions which transform the organic matter present in the pellets into humics. The abundance of S in the organic matter and the easy Maillard reactions between abundant carbohydrates and amino compounds were both favorable to the stabilization of these humics. In the absence of heat input and of fluid circulation at their contact, the humic particles were protected from any further diagenesis and evolution.

The presence of humics was already acknowledged in Australian phosphorites pellets dated 510 My (Cambrian), by Mark Sandstrom (2).

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Comparison of ^{13}C NMR Spectra of Fulvic Acids from Neighboring Two Clear Water Lakes in Japan

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Keywords: fulvic acid (FA), clear water, Lake Biwa, Lake Tankai, liquid-state ^{13}C NMR

1. INTRODUCTION

Most of rivers and lakes in Japan are clear waters that exhibit low concentrations of fulvic acids (FAs) (1). FAs complex or sorb major cations, trace metals, trace anions, and hydrophobic pollutants, and thereby change both bioavailability and geochemical cycling of these substances (2). Therefore, it is important to reveal chemical properties of FAs for understanding the above functions even if their concentrations are low. One of the most promising techniques for analyzing chemical properties of FAs is ^{13}C NMR spectroscopy (3). There are however, few studies on ^{13}C NMR spectral properties of FAs in clear waters because it is difficult to collect the sufficient amounts of FA samples from clear waters. Recently, we made up a large-scale preparative isolation apparatus of aquatic FAs (4).

In this study, we collected FAs from neighboring two clear water lakes in Japan—Lake Biwa and Lake Tankai, using our isolation apparatus, and compared their ^{13}C NMR spectra. Additionally, the spectral variations of FAs from these waters in different seasons were investigated.

2. MATERIALS AND METHODS

Lake Biwa is the largest lake in Japan; the area is 670.25 km^2 and the maximum water depth is 104 m. Lake Tankai is a small lake nearby Lake Biwa; the area is 0.12 km^2 and the maximum water depth is 20 m. Lake Biwa is more affected by human activities than Lake Tankai. FA samples were collected in spring, summer, autumn and winter. These samples are named BiwaSpr, BiwaAut, BiwaWin, TankaiSpr, TankaiSum, TankaiAut and TankaiWin. Biwa Sum could not be collected for some technical failure.

The FA samples were isolated and purified by the DAX-8 procedure (5) with a large-scale preparative isolation apparatus (4). Liquid-state ^{13}C NMR spectra were recorded at 125.77 MHz on a Bruker Avance 500 spectrometer.

3. RESULTS AND DISCUSSION

Some chemical properties of FAs from both lakes are listed in Table 1. ^{13}C NMR spectra of FAs from the two clear water lakes are shown in Fig. 1. All of spectra exhibited major broad peaks assigned from Alkyl and a part of O-Alkyl carbons in ranging from 10 to 66 ppm. The spectra of BiwaSpr exhibited many distinct signals overlapping with the broad peaks. Intensities of the distinct signals in the BiwaSpr were stronger than those in the BiwaAut. Those signals were not observed in the BiwaWin. No distinct signals in the Lake Tankai FAs, which were collected from nearby Lake Biwa and are less affected by human activities, were also observed. The appearances of the aliphatic signals would be attributed to seasonal and/or regional phenomena. In early spring, more anthropogenic compounds such as nitrogen or phosphate fertilizers flow into Lake Biwa that might stimulate the growth of lake algae. Therefore, algal metabolites or detritus may influence on such phenomena.

Carbon species distributions of the FAs (6, 7) are listed in Table 2. Aromaticity was calculated by the expressing of the amount of Aryl-+O-Aryl-C (110-165 ppm) as percentage of Alkyl-+O-Alkyl-+Aryl+O-Aryl-C (5-165 ppm) (8).

Table 1. Elemental composition, Mw and E_{600}* of FAs from the two clear water lakes

Name	Weight % on ash-free basis				Atomic ratios				(HPSEC)MW	E_{600}*
	C	H	N	O	H/C	O/H	O/C	N/C		
BiwaSpr	55.4	6.42	1.79	36.4	1.39	0.35	0.49	0.03	929	0.52
BiwaSum**										
BiwaAut	56.1	6.06	2.31	35.5	1.29	0.37	0.47	0.04	884	0.71
BiwaWin	58.9	6.15	1.54	33.5	1.25	0.34	0.43	0.02	1043	1.44
TankaiSpr	55.2	5.22	0.84	38.7	1.14	0.46	0.53	0.01	874	0.91
TankaiSum	53.0	5.09	1.31	40.6	1.15	0.50	0.57	0.02	983	1.18
TankaiAut	55.0	5.34	1.07	38.6	1.17	0.45	0.53	0.02	920	1.36
TankaiWin	53.9	4.98	0.86	40.3	1.11	0.51	0.56	0.01	924	1.29

* Absorbance of 1% solution at 600 nm

**Not collected

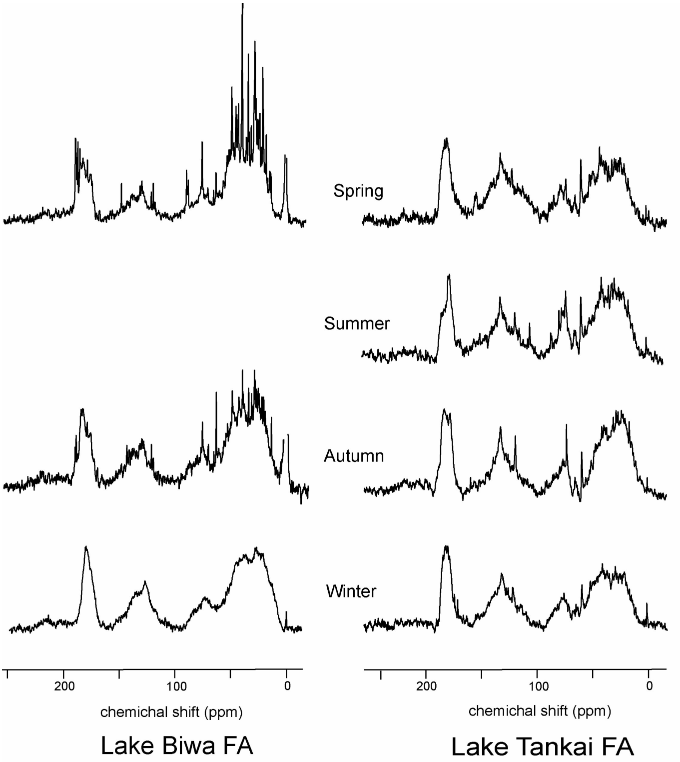

Figure 1. Liquid-state ^{13}C NMR spectra of FAs from the two clear water lakes.

Table 2. Carbon species distribution of FAs from two clear water lakes

Name	% of carbon species (δ, ppm)								AR*
	(5-33)	(33-54)	(54-66)	(66-110)	(110-145)	(145-165)	165-190	190-230	
	Alkyl-C(1)	Alkyl-C(2)	O-Alkyl-C	O-Alkyl-C	Aryl-C	O-Aryl-C	COO-C	CO-C	
BiwaSpr	24.8	27.1	5.3	11.3	10.3	2.3	14.5	4.4	0.16
BiwaSum**									
BiwaAut	22.9	21.9	5.9	12.2	13.9	3.2	14.4	5.6	0.21
BiwaWin	26.4	21.7	4.8	9.6	15.9	2.1	15.6	3.9	0.22
TankaiSpr	17.6	16.4	4.4	13.6	22.8	5.2	16.6	3.4	0.35
TankaiSum	23.0	18.7	4.8	13.5	18.5	4.4	14.9	2.2	0.28
TankaiAut	26.0	15.7	2.0	10.6	18.5	3.1	17.1	7.0	0.28
TankaiWin	19.5	19.1	4.8	10.9	20.5	3.6	17.6	4.0	0.31

* Not collected

**Aromaticity

Alkyl-C (1), (2) proportions in the Lake Biwa FAs were much higher than those in the Lake Tankai FAs. On the other hand, Aryl-C proportions in the Lake Biwa FAs were lower than those in the Lake Tankai FAs. The BiwaSpr exhibited the lowest aromaticity (0.16) whereas the TankaiSpr exhibited the highest aromaticity (0.35). These results suggest that the Lake Biwa FAs would be derived mainly from microbial biomass whereas the Lake Tankai FAs would be derived mainly from degradation of plant material and leaching of soil (9), and these derivatives reach the largest amount in spring.

4. CONCLUSIONS

There were distinct dissimilarities between ^{13}C NMR spectral properties of FAs from the two clear water lakes. Lake Tankai FAs were more aromatic than Lake Biwa FAs. Seasonal changes of the spectral properties of Lake Biwa FAs were more remarkable than those of Lake Tankai FAs. As shown by intensities of distinct signals in the spectra (BiwaSpr > BiwaAut >> BiwaWin, Lake Tankai FAs), these changes would be attributed to seasonal and/or regional phenomena.

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Correlation between Optical and Chemical Properties of DNOM

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Keywords: proxies, DNOM characteristics, spectroscopic measurements

1. INTRODUCTION

Dissolved natural organic matter (DNOM) governs important processes in the environment. These processes need to be understood and accounted for in order to predict effects of anthropogenic pressure on the environmental. The main challenges dealing with humic substances (HS) is its heterogeneous nature and the spatial and temporal variation in its physiochemical characteristics. The heterogeneity makes sound determination of the properties of HS a time consuming and tedious act.

Scientists have dealt with this by developing a number of simple proxies based on radiation adsorption, in the UV, Visible and IR range, and Fluorescence; e.g. UV Absorption at λ254, sUVa, SAR, RkSF, SFE, Humification Index, and Fluorescence peak ratios etc. These surrogates are readily used to provide information about the quality and amount of the DNOM that is only available through more sophisticated analysis. The spectroscopic characterisation of DNOM has been found to be useful, because it can characterise DNOM without major sample preparation, which can result in unnecessary artefacts (1). Also, it enables the measuring of a large number of samples, since it is simple, quick, and highly sensitive.

The problem is that our understanding of these proxies is not all that well funded and even some apparently antagonistic perceptions exist; The UV adsorption, conceptually linked to the amount of aromatic carboxyl and phenolic groups and to C=C systems (Williams and Fleming, 1995), is for instance commonly used as a proxy for the amount of DOC (3). At the same time it is generally perceived that specific UV radiation (sUVa = Abs.λ254/DOC) is an important proxy that varies considerably due to varying degree of aromaticity, total C-content and molecular weight (4) of the water-soluble HS. This proxy is frequently used by waterworks to reflect the reactivity of organics in water toward removal and toward organic halide (disinfection by-products; DBPs) formation potential (i.e. AOX form.pot.). A confounding issue is that especially iron (Fe) has also been suggested to contribute to UV attenuation, either directly or by interactions with DOC (5). The Specific Absorbance Ratio (SAR = Abs.λ254/ Abs.λ400) is expected to increase with decreasing degree of aromaticity (6) and molecular weight (7). The (E4/E6) ratio, conceptually associated with the length of the conjugated bonds (i.e. red- and blueshift; 2), is found to be inversely proportional to the degree of humification, condensation, aromaticity and molecular weight (4). It is also related to the adsorption ratio of organic micro-pollutants and thereby to their toxicity (8). The HIX (Fluorescence @ λ435–480 nm/λ 300–345 nm; 9) reflects the degree of condensation (10) and is associated with the amount of humic substances relative to non-humic material. This parameter has been found to have positive correlation with the C/H ratio of organic molecules (11). Fluorescence can be applied to differentiate and classify the various types of humic substances (12). There is usually a strong correlation with molecular weight and, in addition, the ratio of fluorescence-absorbance has been used as an indicator for molecular weight (13).

2. MATERIALS AND METHODS

This study uses correlation analysis and multivariate statistics to study the empirical relationships between optical properties and a large number of sophisticated chemical parameters determined on isolated DNOM material. Since 1986 several Humus-/NOM-isolates have been “produced” in the Nordic countries. The *NOMiNiC project* (Natural Organic Matter in the Nordic Countries; (14) and the *NOM-Typing project* (15) produced a set of 10+9 Reverse Osmosis (RO) and freeze dried DNOM isolates of runoff from coniferous-forested Nordic catchments, differing in climate, anthropogenic S-loading and relative coverage of mineral soil, bogs and lakes. 3 new samples, from more barren land and from higher altitude, have recently been added to this sample set. All materials are pre-treated and isolated in the same manner. These materials are distributed; free of charge, to colleagues that whish to use these materials in their line of research using their analytical techniques to characterize the material. Considerable numbers of characterization data have thereby been generated on these samples. This allows us to check if the interpretations of the proxies are valid and to disclose new relationships.

3. PRELIMINARY RESULTS AND DISCUSSIONS

A large number of strong correlations exist between the various parameters determined on the different sets of DNOM isolates. The empirical relationships between simple spectroscopic proxies and some central parameters are presented in Table 1. The challenge is to differentiate between strong correlations that are due to co-variations due to e.g. common catchment source (14) and real inherent relationships. Example of co-variations may be in that DNOM from peat’s are generally more coloured, more high molecular weight and more hydrophobic than DNOM from mineral soils. Another problem is to understand what is the governing parameter; A simple example is that colour (Abs.λ410) is found to be positively correlated to Fe (Table 1), though this is due to that the organic binding of the Fe increases its conc. and not that Fe increases the colour of the solution.

Table 1. Selected correlations with r > 0,85 in the NOM-Typing data set. sUVa denotes Abs.λ254/DOC. SAR denotes Abs.λ254/ Abs.λ400. *Fe* and *Al* denote total elemental cons. in solution. *Org. Al* denote Al bound to organic matter. *Carbohydrates* is determined as the relative integral of the 13C NMR spectre between 90 – 112 nm. *AOX from pot.* denote the potential to form absorbable organic halogen compounds

							Fluorescence ratio		
	DOC	Colour	UV abs	sUVa	SAR	IR_{1400}/IR_{2850}	A/B	A/C	B/C
pH							0,85		
Fe	0,99	0,98	0,98						
Al	0,88	0,93	0,91						
Org. Al		0,94	0,90						
SiO_2	0,99	0,95	0,98			0,90			
% Ash	-0,88	-0,92	-0,90						
% N					0,87	0,95			0,92
NO_3				-0,86	0,86			0,87	0,94
Amino acid					-0,86				
Amino sugars								0,93	0,94
Carbohydrates							-0,85		
AOX form. pot.				0,90	-0,88	-0,87		-0,88	-0,90

4. CONCLUSIONS

Considerable new knowledge can be generated by studying the relationship between parameters characterizing a set of DNOM samples pre-treated and isolated in the same way.

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Difference between Humic Acids from Soils Derived under Different Tree Species in a Temperate Rain Forest, as Measured with ^{13}C CPMAS-NMR

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Keywords: ^{13}C CPMAS-NMR, decomposition, humic acids, tree species

1. INTRODUCTION

In recent interest has refocused on soil organic matter (SOM). The formation of humic acids (HAs) under different tree species was rarely reported. The objective of this study was to investigate the characteristics of HAs derived under different tree species.

2. MATERIALS AND METHODS

Three soils under different tree species were conducted at the Ta-Ta-Chia LTER site in central Taiwan, located at elevation about 2,600 m; average annual precipitation is 3,000 mm, and the average annual temperature is 10.3°C. The soils are classified as loamy silty, mixed, mesic, Dystric Cryochrepts (2). The three tree species was dominated by (1) Taiwan red pine (*Pinus taiwanensis*); (2) Taiwan hemlock (*Tsuga chinensis var. formosana*) and (3) dwarf bamboo (*Yushania niitakayamensis*). Plant tissues (fresh leaves), litters, and soils were collected in triplicates that were all examined by solid-state ^{13}C CPMAS-NMR. Soil humic acids were isolated, fractionated, and purified using the IHSS method. Humic acids also examined by ^{13}C-NMR, and the ^{13}C-NMR spectra were divided into different chemical shift regions described by Oades (1). Areas of the chemical shift were measured by computer program and expressed as percentage of total area (i.e., relative intensity).

3. RESULTS AND DISCUSSION

Table 1 gives selected soil physic-chemical properties. Table 2 displays the percentage distribution of all ^{13}C-NMR spectra of the fresh leaves, litters and soils. The relative lignin content of fresh tissues in bamboo (8.7%) of particular interest is lower than that in pine (16.7%) and hemlock (16.8%). After decomposition, the components in litters of cellulose (O-alkyl-C) and hemi-cellulose (N-alkyl/methoxyl-C plus acetal-C) tend to decline, and microbial re-synthesized carbohydrates (alkyl-C plus carboxyl-C) increase in litters. The microbial re-synthesized carbohydrates contents in bamboo and hemlock (37.1%) are greater than that in pine (21.4%). The microbial re-synthesized carbohydrates contents in bamboo (37.1%) are greater than that in hemlock (30.9%) (O/A and A horizons). Furthermore, hemlock and bamboo soils contain less amounts of cellulose in the A horizon but higher amounts of aromatic-C and phenolic-C, while pine soil shows the reverse trend. This may be because dwarf bamboo plant materials decompose easily, and pine plant tissues decompose slower than those of hemlock and bamboo.

Table 3 displays functional groups percentages of HAs under different tree species. The HAs of pine comprise consistent percentages of functional groups in the O/A and A horizons. On the other hand, the aromatic-C component in HAs of bamboo is greater changed. Concentrations of organic functional groups of HAs in pine and hemlock follow the general trend: alkyl-C > O-alkyl-C ≈ N-alkyl/methoxyl-C > aromatic-C > carboxyl-C > acetal-C > phenolic-C; while the trend in bamboo was: alkyl-C > aromatic-C > O-alkyl-C ≈ N-alkyl/methoxyl-C > carboxyl-C > acetal-C > phenolic-C. Thus, we suggest that the trend of debris relative decomposition rates in the three tree species are bamboo > hemlock > pine.

Table 1. Selected physical and chemical properties of soils tested

Tree species	Horizon	Depth	pH	C	N	C/N	CEC	Sum of Cations
		(cm)	(1:1)	— (g kg^{-1}) —			(c mol (+) kg^{-1})	
Pine	O/A	0-15	3.1	128.3	7.4	20.2	12.0	0.7
	A	15-25	3.6	89.3	6.5	16.2	8.0	0.3
Hemlock	O/A	0-11	3.3	331.4	15.2	25.4	16.0	1.4
	A	11-20	3.9	17.0	0.9	23.2	13.0	0.7
Bamboo	O/A	0-19	3.8	69.1	4.4	18.3	14.0	0.3
	A	19-25	4.3	4.7	1.2	4.6	12.0	0.1

Table 2. Percentages of functional groups of fresh tissues and soils by ^{13}C-NMR

Tree species		Alkyl	N-Alkyl	O- Alkyl	Acetal	Aromatic	Phenolic	Carboxyl
		-C[‡]	-C	-C	-C	-C	-C	-C
Pine	FT	17.6	11.2	34.9	14.6	10.2	6.5	5.0
	L	16.8	11.1	38.2	12.2	11.0	6.1	4.6
	O/A	27.8	13.0	21.9	9.2	12.1	6.3	9.7
	A	26.9	13.5	20.5	9.8	12.5	7.0	9.8
Hemlock	FT	17.6	11.2	34.9	14.6	10.2	6.5	5.0
	L	19.4	12.3	35.7	12.9	9.4	6.8	4.5
	O/A	23.5	13.3	29.4	10.4	10.9	5.2	7.4
	A	35.9	4.2	13.6	11.5	19.0	7.4	8.3
Bamboo	FT	18.2	14.3	41.8	12.1	6.5	2.2	5.0
	L	19.4	12.3	35.7	12.0	9.4	6.8	4.5
	O/A	26.4	13.6	23.7	8.3	10.7	6.5	10.7
	A	20.8	9.9	13.2	10.7	19.3	11.9	14.2

[‡]: Functional groups (chemical shift/ppm): Alkyl-C (0-45), N-Alkyl-C (45-65), O- Alkyl -C (65-90), Acetal-C (90-110), Aromatic-C (110-140), Phenolic-C (140-160), and Carboxyl-C (160-200).

Table 3. Percentages of functional groups of HAs by ^{13}C-NMR

Tree species	Horizon	Alkyl	N-Alkyl	O- Alkyl	Acetal	Aromatic	Phenolic	Carboxyl
		-C	-C	-C	-C	-C	-C	-C
Pine	O/A	35.6	15.0	15.2	5.7	13.7	3.8	10.9
	A	36.6	14.6	14.8	5.5	14.5	3.1	11.0
Hemlock	O/A	30.4	17.1	18.9	7.3	12.9	4.5	8.9
	A	45.2	14.2	13.9	5.0	9.4	2.9	9.4
Bamboo	O/A	34.3	14.9	16.5	5.2	15.0	2.5	11.6
	A	29.8	14.0	14.2	5.4	20.8	3.0	12.7

4. CONCLUSIONS

The debris of pine forest does not decompose easily in forest soil environments at high elevation and mountains. The relative decomposition rates of plant tissues of the three species show the following trend: bamboo > hemlock > pine forest.

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Humus Level in Soils of South Kazakhstan Irrigated Massifs

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1. INTRODUCTION

Soil cover of Republic Kazakhstan occupies the 9th place in the world by its area, and the 1st place by the area per capita. The soil cover differs from the soils of other countries by low resistance to anthropogenic influence, hence it is subjected to the processes of degradation and desertification (1).

The problem of degradation of the irrigated soils used in the specific conditions of land tenure system is of concern in environmental investigations. The process of soil dehumification is one of the major factors of soil degradation (2). The development of dehumification process in irrigated soils, its mechanism, and principal causes leading to dehumification were considered in our previous works (3, 4). Our researches proved that the level of total humus in the soils of Akdala massif newly irrigated for rice cultivation decreased by 19-25% in comparison with the initial status. The loss of the most mobile water soluble form of humus achieved 12-36% for one season. The loss of humus achieved already 30-40% in the soils of old irrigated massifs in Kyzylorda oblast and its level made less than 1% on about 60% of the arable lands in the oblast (5). The loss of humus was promoted by mobilization and migration processes, which are rather intensive in the conditions of permanent flooding. Such loss, undoubtedly, causes the worsening of soil fertility, decreases its buffer capacity and resistance to unfavorable anthropogenic factors. In this connection the research and assessment of modern humus level in the irrigated soils is one of the most urgent tasks in modern environmental and soil sciences.

The aim of the present study was to investigate and assess the current humus condition of the South Kazakhstan irrigated soils. Such investigations are necessary to determine the soil degradation reasons and to develop the practical measures for the stabilization and improvement of soil humus condition. It is known that the soils with a stable and high humus level are necessary to receive stable, high and ecologically pure crops. Such investigations can be also an introduction for developments of the methods of regulation and optimization of humus condition in the irrigated soils.

2. MATERIALS AND METHODS

Soil survey works on studying of soil humus condition have been carried out within the framework of the international project “Co-ordination of scientific activities towards elaboration of common strategy for environmental protection and sustainable management in Syr-Darya River Basin, in Uzbekistan and Kazakhstan”. Humus condition was estimated by the total humus level in soils, contents of humus water soluble part, degree of its solubility and saturation with nitrogen. All humus analyses were carried out using standard methods.

The investigations have been carried out in Shieli and Akdala massifs. Shieli massif is located in the South Kazakhstan, in lower flow (predelta) of the Syr Darya River, Akdala massif is located in South-East Kazakhstan, in the ancient delta of the River Ili. The major part of the massifs has a favorable relief fro irrigation. Rice is the major plant cultivated at both massifs.

3. RESULTS AND DISCUSSION

Alluvial-meadow, meadow-boggy, boggy soils were mainly used for rice cultivation at Shieli massif and takyr-like soils – at Akdala massif. As a result of the long-term use of the soils for rice cultivation, the soils of both massifs developed according to the classification of Kazakhstan soil scientists (6, 7, 8) and have turned to the irrigated (paddy) boggy soils. The changes of soils under this culture are provided with the specific conditions of its cultivation – constant flooding.

Content of total and water soluble forms of humus in soils covering investigated massifs were established. The obtained results were elaborated by statistical methods. According to the classification the investigated soils can be referred to group of low humus level. A small variation concerning humus level is observed between two investigated massifs. The soils of Akdala massif are characterized by a lower level of humus (1.28 ± 0.136%), the soils of Shieli massif – by a higher level (1.45 ± 0.191%). The different design is observed at the content of water soluble form of humus. The soils of Akdala massif have higher level of this form (0.006 ± 0.0007%), the soils of Shieli massif, the lower one (0.003 ±0,0002%).

The content of nitrogen in humus from investigated soils was also determined. The presence of nitrogen, as one of the main nutritive element for plants, is one of the main parameters of a qualitative humus composition. The soils of newly irrigated Akdala massif were characterized by lower saturation with nitrogen (5,6 ±0,24%), whereas the saturation with nitrogen in the soils of Shieli massif achieved higher level (6,8 ±0,40%).

All results were elaborated statistically (9). Analytical data received as a results of the researches, are characterized by a statistical stability and can be used for the assessment of the current humus condition of the soils investigated at the massifs of rice cultivation.

Over the last 30 years irrigated areas have increased rapidly, helping to feed a growing population. Although the achievements of irrigation in ensuring food security have been impressive, the investigations indicate some environmental problems. In addition to large water use, water quality reduction and salinization, soil degradation is usually considered the most significant problem of the irrigation sector. The process of soil dehumification is one of the major factors of soil degradation leading to a number of economic and social consequences including the reduction of harvesting capacity, decrease of agricultural export potential and slow development of food industry.

4. CONCLUSIONS

1. The development of large-scale irrigation of agriculture in the arid region of southern and south-eastern Kazakhstan has resulted in the process of soil dehumification.
2. Process of soil dehumificaion is observed, practically, at all arable and forage lands, although bigger humus loss is observed in irrigated soils. The loss of humus is promoted by mobilization and migration processes, which are rather intensive in the conditions of permanent flooding.
3. Current humus condition of the irrigated soils in the southern Kazakhstan is characterized by a very low degree of protection of total humus.
4. The humus of the soils has different degree of solubility in dependence on the beginning of massif irrigation. The degree of solubility is higher in soils of newly irrigated Akdala massif, than that in the soil of the massif irrigated from a long time (Shieli).
5. The investigation and assessment of the current humus condition in southern Kazakhstan is really important and investigations are necessary for the determination of the soil degradation reasons and development of practical measures for the stabilization and improvement of soil humus condition.

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The Role of Lichens in Carbon Preservation in Soils of Tundra Zone

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1. INTRODUCTION

Humic substances (HS) comprise the major part of stable organic matter in soils. It is considered that the main structural precursor of HS in the boreal zone is lignin and its transformation products due to its high content in plant tissue and relative resistance to biodegradation. Vegetation of large territories in the tundra zone is dominated by lichens and mosses, almost lacking lignin. Little is known about the quantitative contribution of such lignin-free vegetation to the soil organic matter pool. Lichens – symbiotic associations of a fungus and a photobiont (green algae or cyanobacteria) – represent a promising object for investigating carbon immobilization in a lignin-free environment. As primary producers of biomass they are pioneers on the rocky surfaces and apparently do not depend on organotrophic nutrition. The aim of this work was to study the role of lichen-derived carbon in accumulation of stable organic matter in tundra soils. We compared δ^{13}C signatures of dominating plant species with that of underlying soils and investigated relative mineralization rates of selected plant species in a laboratory incubation experiment.

2. MATERIALS AND METHODS

The samples of dominating vegetation, litter and soil were taken from several representative field plots in Khibiny mountains (Murmansk region, Russia; tundra zone) and from reference plots in Moscow and Leningrad regions (boreal zone). Stable isotope measurements (δ^{13}C) were conducted with a FinniganMAT DeltaPlus IR-Mass-Spectrometer equipped with an elemental analyzer (CE2500) and a conflow interface. The isotopic fractionation during organic matter degradation was determined in respiration experiments by measuring the isotopic composition of produced CO_2 and by measuring depth profiles of δ^{13}C of organic matter. The relative mineralization rates of lichens, mosses and shrubs, most abundant in the studied sites, were assessed using milled plant biomass that was added to fresh tundra soil and incubated at 20°C in the dark for 17 days.

The CO_2 evolved was trapped by 0.5 M NaOH and precipitated by 0.5 M $BaCO_3$ for $\delta^{13}C$ measurements. The amount of CO_2 produced during soil respiration and mineralization of added carbon sources was quantified by titrating the excess of alkaly with 0.1 M HCl.

3. RESULTS AND DISCUSSION

In the measurements of the ^{13}C-composition of lichens of different tundra sites we determined $\delta^{13}C$ values between –21‰ and –22‰ for *Parmeliaceae* (genera *Cetraria, Flavocetraria, Alectoria*), from –23.5‰ to –25.5‰ for *Cladoniaceae* (genus *Cladonia)*; and from –24‰ to –34‰ for the members of the order *Peltigerales* (genera *Peltigera, Lobaria, Nephroma, Solorina*). The stable carbon isotopic composition of mosses was between -27‰ and –29.5‰, while that of higher plants was in the range of –28.2‰ to –30.3‰ (except for *Empetrum nigrum* with $\delta^{13}C$ value of –25.7‰). The $\delta^{13}C$ values of tundra soils, developed under shrub-moss-lichen cover were from –24.00 ‰ to –25.00 ‰, while ^{13}C-composition of reference soils developed in boreal zone under lignified vegetation was in the range of –27.00 ‰ to –29.00 ‰. The ^{13}C-composition of soils became „heavier" with depth. It was due to isotope fractionation during mineralization of organic matter as $\delta^{13}C$ values of CO_2 respired from the soils were always „lighter" than that of soil organic matter. The ^{13}C-composition of tundra soils was closest to that of different *Cladonia* species that suggests the dominant role of these lichens in carbon sequestration in the studied sites. This was proved by the results of laboratory incubation experiment when we compared the relative mineralization rates of representative lichen, moss and shrub species. The highest mineralization rates were found in peltigerous lichens, *Cetraria nivalis* and the shrub *Empetrum nigrum*. Mineralization rates of *Cladonia spp.* were one of the lowest suggesting long preservation of their biomass and its transformation products in soils.

4. CONCLUSIONS

The ^{13}C-composition of tundra soils developed under moss-shrub-lichen cover reflects that of *Cladonia spp*. They dominate in the vegetation of investigated tundra soils and are characterized by low mineralization rates in comparison to other lichens, mosses and shrubs studied. The results of the present work suggest importance of *Cladonia* lichens as carbon source for humification in the soils of tundra zone.

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