Volume II

From Molecular Understanding to Innovative Applications of Humic Substances

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Section III

Physical, chemical and biological properties of natural organic matter and humic substances

The Specific UV-Absorbance of Dissolved Organic Matter (DOM) Explains the 5-fold Variation of the Copper Mobilisation by DOM in an Agricultural Soil Horizon

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Keywords: dissolved organic matter (DOM, DOC), specific UV-absorbance (SUVA), Cu mobility, WHAM, modelling, Cu affinity, complexation

1. INTRODUCTION

In soil column studies, increased Cu mobility is shown with increasing dissolved organic carbon (DOC) concentration (1). The variation of DOM composition likely influences the Cu affinity of DOM. A method was developed to measure the Cu-mobilizing-potential of isolated DOM (CuMP, mmol Cu/kg DOC) based on the Cu binding to DOM at defined environmentally relevant conditions, i.e. pH = 7, a solution Cu^{2+} activity = 5 10^{-12} M and soluble Ca= 1.5 mM (2). In the lab, we showed that the CuMP of DOM in soil solutions varied tenfold depending on incubation and extraction procedure. A positive significant correlation between the DOM specific UV-absorbance at 254 nm (SUVA) and CuMP was found, suggesting a relationship between aromaticity and Cu affinity of DOM in soil solutions. Here, we investigated if a similar variation and correlation is found in the field by analyzing leachates sampled in an agricultural field during five months. It was verified if the varying Cu mobilization by DOM could be predicted by the speciation program WHAM6, and if information on the DOM quality (SUVA) could improve this prediction.

2. MATERIALS AND METHODS

A field experiment was set up at an uncontaminated agricultural field (luvisol) in Belgium in 2006. On both sides of an 18 m long, 2 m wide and 2.5 m deep trench, 4 small experimental plots (4 m x 4 m) were located. Under each of the 8 plots, 2 passive capillary wick samplers (PCAPS) (3) with each 3 fiberglass wicks were horizontally installed at 45 cm depth. By creating suction using a hanging water column inside the fiberglass wick (100 cm), each wick sampled leachate water from the soil above their wick compartment (900 cm²). Prior to use, the wicks were combusted in a muffle oven (400 °C) for 4 hours to remove organic impurities (4). Preliminary experiments showed that Cu and DOM were not retarded on the wicks. The leachates of wick 1 and 2 of the 16 PCAPS were sampled approximately every two weeks between 22 November 2006 and 7 March 2007, resulting in 250 collected leachates with a volume larger than 10 mL. Cation, anion and DOC concentrations and SUVA (L/(g.cm)) were measured in the leachates.

The solution Cu concentrations in the 250 leachate samples were predicted with the assemblage model WHAM6 (6.0.13, Natural Environment Research Council) which includes WHAM model VI (5) for Cu binding to organic matter (particulate and dissolved), a surface complexation model for sorption on iron oxides and an ion exchange model for sorption on clay minerals. The input was the measured ion concentrations in the leachates (Na, Mg, Ca, K, Ni, Zn, Cl^-, NO_3^-, SO_4^{2-}), the free ion activity of Al and Fe calculated by the ion activity product of Al and Fe hydroxide and the following properties of the soil adjacent to the wick sampled (36-47 cm depth): pH, total Cu, % organic matter, % clay, % Fe, Mn and Al oxide. The total Cu concentration was multiplied by 0.30, this is the labile Cu fraction measured in the top soil. Fulvic acid (FA) was entered as the measured DOC concentration, multiplied by a factor 2, based on the assumption that 50% of the DOM is carbon, and multiplied by a factor 0.65, i.e. assuming that the fraction of DOM active as fulvic acids (%AFA) is 65% (6). The particulate humic acid concentration (HA) was entered as the difference between the total carbon concentration and the $CaCO_3$ concentration, multiplied by a factor 2 based on the assumption that 50% of humic acid is carbon.

3. RESULTS AND DISCUSSION

During the 5 month period, approximately 1.1 pore volumes percolated through the soil above the PCAPS. Copper concentrations in the leachates varied 7-fold with a median of 0.076 µM (range 0.030-0.195 µM). Concentrations of DOC ranged from 3.1 to 22.9 mg/L. A positive significant correlation ($p<0.0001$) was found between the Cu and DOC concentrations in the leachates, however the pearson correlation coefficient was rather low ($r=0.56$; Figure 1a). The [Cu]:[DOC] ratio varied about fivefold and increased with decreasing DOC concentration. The [Cu]:[DOC] ratio exhibited a positive significant correlation with the SUVA of DOM ($r=0.77$, $p<0.0001$; Figure 1b). This suggests an important role of the DOM quality, i.e. the aromaticity, on the Cu mobility of the DOM in the leachates of the agricultural soil.

Assuming %AFA to be 65%, the predicted free Cu^{2+} activity ranges $7.2\ 10^{-12}$-$1.4\ 10^{-11}$ M in the 250 leachates. Copper concentrations in the leachates are predicted to vary from 0.027 to 0.205 µM, which is in agreement with the measured values. However the predicted [Cu]:[DOC] ratio is almost constant (8.1 to 9.3 mmol Cu/kg DOC) and contrasts the 5-fold range of observed values (Figure 2a).

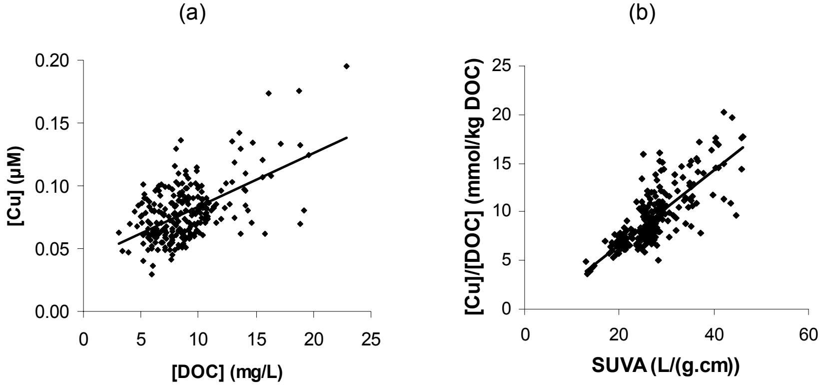

Figure 1 (a) The Cu concentrations in leachates of an agricultural soils are explained by DOC concentrations, however the correlation is weak; (b) the SUVA of the DOM explains the Cu:DOC concentration ratio in the leachates.

The importance of the quality of DOM for the prediction of the Cu mobilization by DOM can be introduced by relating the fraction of DOM active as fulvic acids (%AFA) to the SUVA of DOM, instead of assuming a constant value of %AFA=65%:

$$\%AFA = \frac{SUVA_{DOM}}{SUVA_{FA}} \cdot 100\% \qquad (1)$$

with $SUVA_{DOM}$ the SUVA (L/(g.cm)) of DOM measured in the leachates. The $SUVA_{FA}$ is the SUVA of FA with default properties as in Model VI, and is fitted by minimizing the sum of the squares of the difference between the predicted and measured [Cu]:[DOC] ratios in the 250 leachates. The best fit is found for $SUVA_{FA}$ = 38 L/(g.cm). This is a reasonable value as 37 L/(g.cm) is the measured SUVA of Suwannee River Fulvic Acid, a fulvic acid isolated by the International Humic Substances Society (IHSS) and used among others to determine the default parameters of FA in Model VI (5). The average predicted free Cu^{2+} activity is 9.2 10^{-12} M, the predicted Cu concentrations range from 0.036 to 0.216 µM. The variation in the predicted [Cu]:[DOC] ratio (from 4.6 to 16.3 mmol Cu/kg DOC) is much larger compared to the approach where SUVA was not taken into account (Figure 2b).

4. CONCLUSIONS

Speciation models are a useful tool to predict Cu complexation and mobility in soils, but lack parameters to take the varying DOM quality in soils into account. At a constant conversion of DOC to active fulvic acid, the WHAM model predicted an almost constant [Cu]:[DOC] ratio in leachates monitored during 5 months in an agricultural field. This contrasts the 5-fold variation of the measured [Cu]:[DOC] ratio. That ratio exhibited a positive significant correlation with the SUVA of DOM. Introducing this measure for aromaticity in the model to estimate Cu mobility, increases the predictive capacity of the model.

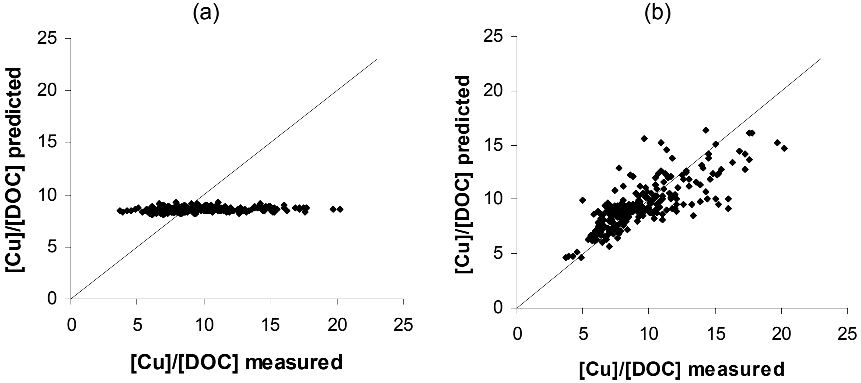

Figure 2. The [Cu]:[DOC] ratio (mmol Cu/kg DOC) predicted with WHAM6 versus the [Cu]:[DOC] ratio measured in the 250 leachates. (a) Under the assumption of constant DOM properties, with %AFA = 65%; (b) DOM quality based on SUVA as calculated by equation (1), with $SUVA_{FA}$ = 38 L/(g.cm).

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Strength of Methylmercury Binding by Fulvic Acid and Aquatic NOM

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Keywords: methylmercury, humic matter, NOM, stability constants, reduced sulfur

1. INTRODUCTION

Methylmercury (MeHg), a neurotoxin that bioaccumulates in organisms, is formed in natural waters by sulfate-reducing bacteria. Uptake by and bioavailability of MeHg to aquatic organisms are influenced by natural organic matter (NOM), a heterogeneous mixture of macromolecules. Abiotic transformations of MeHg, e.g., demethylation, also are influenced by NOM, and transport of MeHg through watersheds often is correlated with dissolved organic carbon concentrations.

MeHg is a soft Lewis acid, and according to Pearson's Soft-Hard Acid Base theory, it should complex strongly with reduced sulfur ligands. Xia et al. (*1*) used x-ray absorption near-edge structure spectroscopy (XANES) to show that up to 50% of the total S in aquatic and soil humic matter is in chemically reduced forms (S_{re}): thiols (RSH), thioethers (RSR) and disulfides (RSSR). Qian et al. *(2)* and Yoon et al. (*3*) used extended x-ray absorption fine structure spectroscopy (EXAFS) to show that reduced S in soil and aquatic organic matter forms complexes with MeHg. NOM forms strong complexes with MeHg, and researchers have reported high values of binding constants of MeHg with NOM. Here we used a competitive-ligand, equilibrium-dialysis technique with Br^- as the competitive ligand to measure binding strengths of methylmercuric ion ($MeHg^+$) with Suwannee River fulvic acid and extracts of dissolved NOM from a Minnesota bog and lake.

2. MATERIALS AND METHODS

IHSS Suwannee River fulvic acid, IR101-F (IHSS SRFA) was purchased from International Humic Substance Society (IHSS), St. Paul, MN, USA. Water samples were collected in acid-cleaned carboys from an acidic bog (wetland S2) and a small, moderately colored seepage lake (Spring Lake) in the Marcell Experimental Forest (Itasca County, Minnesota) and stored at 4°C. DEAE-cellulose, a hydrophilic weak anion exchanger (4), was used to extract aquatic humic matter. Filtered bog and lake water (0.2-0.6 μm Millipore AP15 glass fiber membranes) was passed through Whatman DEAE-cellulose columns; material retained on the column was eluted with 0.1 M NaOH, passed through an H+ Amberlite strong-acid exchange column, and freeze-dried.

Inorganic content of water and NOM was determined by ion chromatography for Cl-, SO42-, NO3-, and ICP emission spectrometry for Na, K, Mg, Ca. Total carbon in dry samples was determined on a Carlo Erba CNS combustion analyzer. Lake NOM samples were analyzed for total S Huffman laboratories (Golden, CO). S2 NOM had 21.3% ash, 30.9% C and 0.37% S. Spring Lake NOM had 5.8% ash, 45.2% C and 1.84% S. IHSS SRFA has 1.0% ash, 53.0% C and 0.47% S. Dissolved organic carbon (DOC) was determined using U.S. EPA method 415.1 with UV-persulfate oxidation on a Tekmar Dorhman Phoenix 8000 TOC analyzer. A pH meter calibrated with standard buffers (pH 4.0, 7.0, 10.0) was used to measure pH. MeHg was determined using a Tekran model 2500 cold vapor atomic fluorescence spectrometer (CVAFS) for total Hg after distillation to separate MeHg (5). Visual MINTEQ (6) was used to calculate free MeHg+ using input data of MeHg bound to Br-, free Br-, K+ and Na+ concentrations and pH.

Experiments were conducted to determine effects of KBr concentration, Hg:Sre molar ratio and pH on MeHg binding constants with IHSS SRFA. A dialysis membrane method and competitive ligand exchange technique with Br- as the competitive ligand was used to determine binding constants. Spectra/Por cellulose-ester membranes (nominal pore size 100 mol. wt. cut-off, Spectrum Lab., Rancho Dominguez, CA) were used in dialysis separations. Prior to experiments, membranes were soaked in ultra-pure water for 72 h to remove NaN3 preservative. Experiments were conducted in the dark. MeHg recoveries were performed; only results with > 80% recovery, are reported. QC procedures included use of acid-cleaned glassware, clean-room techniques, and mass balance checks. A small amount of DOC leakage from the membranes was observed and corrected for in the binding constant calculations.

3. RESULTS AND DISCUSSIONS

Calculated values of distribution coefficients (K_{oc}) and stability constants (K) varied only slightly over a broad range of (i) Br^- concentrations and (ii) ratios of MeHg to reduced sulfur, S_{re}, (the putative binding site for $MeHg^+$) in the NOM. For example, at pH 3.0, K_{oc} ranged from $10^{7.9}$ to $10^{8.1}$ and K from $10^{15.8}$ to $10^{16.0}$ over MeHg:S_{re} ratios of 1:1220 to 1:12200 (all below the saturation limit of S_{re}). The importance of pH on binding constants depended on the model used to calculate binding constants. Over the pH range 2.98-7.62, distribution coefficients showed little pH dependence (slope = 0.2; r^2 = 0.4; range of K_{oc} = $10^{7.7}$-$10^{9.1}$), but stability constants calculated using thiol (RSH) groups with pK_a = 9.96 as ligands had a strong inverse relationship with pH (slope = -0.8; r^2 = 0.9; range of K = $10^{15.6}$-$10^{12.3}$). in contrast, a "pH-independent" model with thioethers or disulfides as binding sites yielded a range of K of $10^{8.6}$-$10^{10.2}$ with a small slope of 0.2 (r^2 = 0.4) over the same pH range.

The above values are similar to values for $MeHg^+$ binding by NOM reported by others. Hintelmann et al. (*7*) reported constants in the range $10^{13.02}$-$10^{14.56}$ for a "strong site" and $10^{12.15}$-$10^{13.07}$ for a "weak site" at pH 6.5 using equilibrium dialysis and Scatchard analysis. Amirbahman et al. (*8*) used a discrete log-K spectrum approach to model data from experiments with 500-MWCO equilibrium-dialysis membranes and obtained constants in the range $10^{10.39}$-$10^{10.54}$ for the weakest site, $10^{12.39}$-$10^{14.77}$ for a mid-strength site and $10^{14.47}$-$10^{14.84}$ for the strongest site for IHSS SRHA, IHSS Peat HA and a stream HA from Maine. Karlsson and Skyllberg (*9*) used a competitive ligand-exchange technique with halides (Cl^-, Br^- I^-) to determine binding constants of MeHg with soils and reported binding constants decreasing from K = $10^{17.2}$ at pH 2.0 to K = $10^{15.6}$ at pH 5.0.

A review of stability constants reported in the literature for MeHg with various ligands showed that inorganic ligands like Cl^-, OH^-, and Br^- do not bind as strongly with MeHg as inorganic and organic sulfur-containing ligands, especially at the low concentrations of these ligands typically found in natural systems. The stability constants for MeHg-NOM that we found are somewhat lower than stability constants of thiol-containing compounds like cysteine ($10^{16.5}$) and mercaptoacetic acid ($10^{16.9}$).

It is apparent from the comparison of previous studies on binding constants of MeHg with NOM that the values depend not only on the type of NOM and MeHg:S_{re} ratios, but also on the calculation model used to estimate them. Most recent studies have used thiol-based models, but both two- or three-site models have been used with pK_a values ranging from 4 to 10. These approaches have produced constants in the range $10^{10.39}$ for weak sites to $10^{17.2}$ for strong sites. There is a growing consensus (based on spectroscopic evidence from Hg-EXAFS) that reduced S sites are the predominant MeHg binding sites in NOM under ambient conditions (low MeHg:S_{re} ratios). However, the choice of pK_a values of the RSH groups and the number of weak and strong binding sites is based on model fitting. Despite the lack of molecular-level understanding of the exact binding site (thiol, disulfane, thioether or disulfide linkages), which can introduce large challenges in trying to account for the different binding constants reported in the literature, there is substantial consistency in stability constants values reported in literature and those calculated by us.

Slight differences in the magnitudes exist because of different methodologies, different pH and different types of NOM.

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Organo-Mineral Complexes and Their Effects on the Physico-Chemical Properties of Soils

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Keywords: soil structure, humic substances, clay minerals, humic molecule, aggregates, SOM

1. INTRODUCTION

Most arable soils contain 0.1 to 5% organic matter (OM) by weight. The lower end of this range represent sandy soils of arid zones, whereas the higher values are typical of clayey soils in temperate zones. All soils are physically and chemically influenced by the OM which they contain. In addition to the nutritional value of OM, it plays a critical role in the formation and stabilization of soil structure, which in turn produces desired tilth and drainage rates as well as resistance to erosion.

Soil mineral particles usually aggregate into granular structures. The stability of soil aggregates (or micro-aggregates – the small particle size fraction of the aggregates – see below) is an important indicator of soil quality due to their significance in the determination of soil porosity and hydraulic conductivity. The degree to which soil is organized into water-stable aggregates influences many of the agricultural and ecosystem properties of soils (4). Cropping systems or soil management that promote SOM accumulation (e.g. no-till) also promote soil aggregation. A number of soil properties contribute to the formation and stabilization of aggregates including: SOM, clay mineralogy, soil texture, exchangeable cations and salt content, $CaCO_3$ content, and Fe and Al oxides.

The scope of this presentation is limited to a thorough examination of clay-organic complexes and their function in soil structure formation.

2. MATERIAL AND METHODS

Due to the important role of humic substance (HS) in aggregate formation and dispersion in soils, an effort was made in the first stage of this study to suggest a model formula for a humic acid (HA) based on average values of the following: (i) elemental analysis including C/N ratios; (ii) functional groups concentrations; (iii) ^{13}C-NMR data on their contents of: aliphaticity, aromaticity, carbohydrate, carboxy, methoxy, keton, phenolic and other groups. With the input of the data listed above and the use of the

ACD/Chemsketch program, a model formula is proposed and its potential interaction with clay particles is shown (Fig. 1). Flocculation and dispersion characteristics of homoionic montmorillionite were studied as a function of the exchangeable cation (Na^+ and Ca^{2+}), humic acid (HA) and fulvic acid (FA) concentration (0-40 mg L^{-1}) and pH (4, 6, 8 and 10). The influence of polysaccharide concentration, molecule characteristics, and pH, on flocculation and rheological properties of Na-montmorillonite were studied. Aggregate-size fractions of two cultivated calcareous Mediterranean soils were characterized with regard to OM and other soil components involved in soil structure stabilizing reactions. The data determined in this study along with those collected from a large number of published articles by a number of authors were integrated and analyzed such that a comprehensive understanding of the interaction of SOM and HS with clays and their influence on soil structure could be obtained.

3. RESULTS AND DISCUSSIONS

Structural model for aggregate formation

Structural models for aggregate formation have been proposed by Tisdall and Oades (4), and the particle-size fractions that they suggested will be followed in this abstract and presentation.

Effects of polysaccharides (ps) and humic substances on clay suspensions and aggregate stability

The discussion and evidence presented earlier lead to a very general conclusion, namely, that OM in soils leads to a well aggregated structured soil. The question whether this is always the case, or whether adverse effects due to the presence of OM or some specific components of it, can also dominate soil properties, requires research. In an effort to corroborate the mechanism of macro-molecular interactions of clays, HS and PS under varying pH values and relative concentration and charges of the organic components were studied by our group (2, 3) using flocculation measurements and later on further confirmed by us using rheological measurements. Studies on flocculation and dispersion characteristics of homoionic montmorillonite were performed as a function of exchangeable cations, HA, FA and polygalacturonic acid (PGA) concentration and pH. A matrix of experimental conditions was employed and the corresponding flocculation values (FV) were measured. Edge-face (E-F) and edge-edge (E-E) interactions at various pH levels, as well as heteroflocculation and its response to pH and concentration of OM will be discussed. In general, PGA greatly increases the stability in suspension of the clay particles. This effect is pH dependent due to changes in the edge charge of the clay and the increase in dissociation of functional groups of the PGA with pH.

Aggregate-size fractions were separated and characterized for: particle size distribution, $CaCO_3$, organic C and N, fulvic acid fraction (FF), HA, total HS, ^{13}C-NMR spectra and extractable Al and Fe. Except for the organic C and HS in the Nevo Horon (MH) soil, all of the calculated ratios between the contents of each of the components in any size fraction and that calculated for the same component in the <2-μm fraction, exhibited a parabolic type curve, reaching a maximum for the 20- to 50-μm aggregates. In practically all combinations of aggregate-size fractions and all soil components (in both soils), the individual stabilizing component is present at higher relative concentration than in the <2-μm fraction. These components, therefore, act first as surface coating materials, with the excess material forming interparticle associations. Higher polysaccharide content was observed by ^{13}C-NMR measurements in the larger particle size fraction, reflecting the influence of recent plant material.

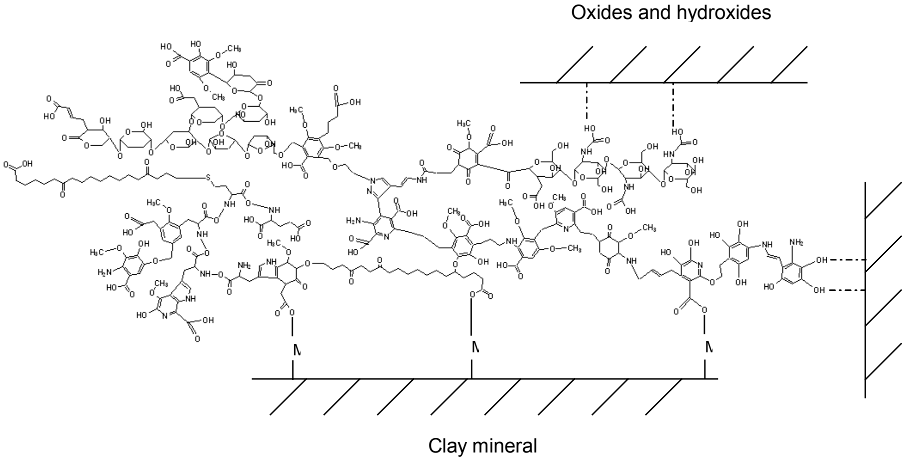

Figure 1. Interaction of soil humic acid (HA) molecules with clay minerals, oxides and hydroxides. The proposed macromolecular structure of the HA based on the following common characteristics: MW- 6386 Da; Elemental analysis (%): C- 53.9; N- 5.0-; H- 5.8; O- 35.1; S- 0.5; C/N: 10.7; Functional groups (cmol/g): carboxyl- 376; phenol- 188; total acidity- 564; Distribution of % C based on NMR analyses: aliphatic – 18.1, aromatic- 20.9, carbohydrates 23.7, methoxy – 4.9, carboxylic – 8.4, keton-4.5, phenolic – 4.2, other groups- 15.3 (Stevenson, 1994). The structure was built using the ACD/ChemSketch program.

4. CONCLUSIONS

A wide array of organic compounds, including HS, PS, amino acids, peptides, proteins and aliphatics are adsorbed to the mineral components of soils, clays and hydroxides in particular, to varying degrees, thereby playing an important role in the determination of aggregate stability. Adsorption is determined to a large extent by the properties and charge of the organic and mineral components, pH, ionic strength and the nature of the exchangeable cation on the clay surface.

ACKNOWLEDGEMENTS

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Reactive and Refractory Components of Dissolved Organic Matter (DOM) in Peatland Soil Porewaters and their Relation to Climate Change

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Keywords: peatlands, porewater DOM, climate change

1. INTRODUCTION

The growth of northern peatlands during the Holocene created a globally important source and sink for greenhouse gases. However, the response of these large carbon reservoirs to global warming remains uncertain. We have therefore begun a multi-disciplinary study of porewater DOM in the Glacial Lake Agassiz Peatlands (GLAP) in northern Minnesota in which we will develop a multiphase groundwater flow model that incorporates solute transport, organic matter reactivity and peat accumulation.

In this presentation we will describe the results of experiments designed to identify the differences in reactive and refractory DOM pools from two distinctly different peatland geoforms; bogs and fens. Raised bogs develop over sites that support local recharge mounds that drive surface waters downward into the deeper peat. Bogs thus have acidic surface waters with low concentrations of inorganic solutes. In contrast, fens develop where mineral solutes are transported upward to the peat surface. Bogs tend to develop over sand and gravel deposits, whereas fens are generally underlain by silty or clayey sediments.

2. MATERIALS AND METHODS

Soil porewaters were sampled from numerous sites in bog and fen peat formations at 2-cm intervals to depths of 1-2 m using piezometer nests. DOM in the porewaters was concentrated by freeze-drying followed by simple ion-exchange filtration to remove residual salts that would interfere with subsequent analyses. 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.

3. RESULTS AND DISCUSSION

Ultrahigh resolution FT-ICR mass spectrometry allows DOM mixtures to be compared based on molecular features, including formulas of individual compounds. Kendrick plots (Nominal Mass vs. Kendrick Mass Defect) from formulas comparing HMW DOM in surface porewaters from a fen and bog are quite similar (Figure 1.a). However, dramatic differences are obvious when such plots for porewaters at depth are compared (Figure 1.b). These initial data suggest that organic matter evolution in peatlands is highly variable and a function of numerous locally-important variables such hydrology and nutrient availability.

4. CONCLUSIONS

Comparisons of molecular formulas obtained from FT-ICR mass spectra suggest that high molecular weight DOM is initially quite similar in fens and bogs. However, complex localized conditions drive the evolution of DOM in very different directions. Initial results confirm our hypothesis that peatlands are spatially highly variable ecosystems.

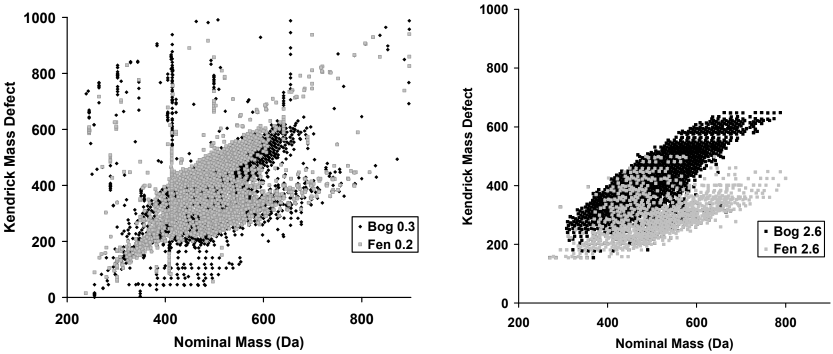

Figure 1. Kendrick plots of DOM from surface porewaters in a bog and fen (left), and DOM from the same formations at depth (right).

ACKNOWLEDGEMENTS

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Interactions between Dissolved Organic Matter and Organic Contaminants in Aquatic Environment

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Keywords: DOM, interactions, PAH, pharmaceuticals, SPME

1. INTRODUCTION

Dissolved organic matter (DOM) in aquatic environments is a complex mixture which is well known to play an important role in the fate of inorganic and organic pollutants. Indeed, DOM could bind these compounds modifying their distribution in aquatic environment, but also their bioavailability, biodegradation and subsequently their toxicity towards aquatic organisms (1).

In order to calculate the partitioning coefficient of each pollutant to DOM (K_{DOC}), it is necessary to quantify the free pollutant fraction separately from the DOM bound one. Few techniques allow the measurement of only free compound concentration without modifying the equilibrium during the analysis (e.g. fluorescence quenching, solid-phase micro-extraction, equilibrium dialysis).

The goal of this study was therefore to develop a reliable technique that permits to quantify rapidly total and free organic pollutant concentrations: solid-phase micro-extraction coupled to gas chromatography-mass spectrometry (SPME-GC-MS). Indeed, SPME fiber is introduced into the sample and only free pollutants are extracted according to their affinity with the fiber coating.

A series of tests was performed to optimize SPME parameters for the analysis of organic compounds (fiber coating, extraction time, injection system, desorption time…). After that, DOM was added to calculate K_{DOC} values.

2. MATERIALS AND METHODS

Organic pollutants: Four PAHs were used as hydrophobic model compounds: phenanthrene, fluoranthene, chrysene and benzo[a]pyrene. Secondly, neutral pharmaceuticals were tested: carbamazepine and diazepam, which are anxiolytic agents.

DOM preparation: Aldrich humic acid was added to pure water and then filtered on a 0.7 µm GF/F filter. Concentration of Dissolved Organic Carbon (DOC) was measured using a Shimadzu TOC-V CSN and was about 10 mg/L. For pharmaceuticals, natural samples of DOM from the Gironde estuary (La Réole) and the Mediterranean Sea (Toulon) were tested. These samples were concentrated by reverse osmosis previously being used with drugs at the required concentration in DOC (about 10 mg/L). The size distribution of macromolecules was determined by ultrafiltration in natural samples (figure 1).

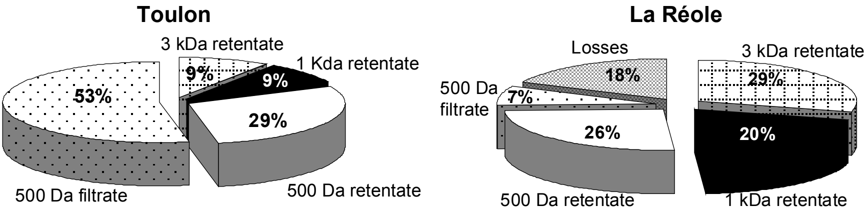

Figure 1. DOC distribution of ultrafiltration fractions (2).

SPME: For PAH, SPME analysis of the 10 mL samples were performed with commercially available PDMS (polydimethylsiloxane) and PDMS-DVB (divinylbenzene) coated fibers from Supelco. Different sizes of PDMS coating were compared (7 µm and 100 µm). A carbowax-DVB fiber was used for pharmaceuticals as recommended for N-heterocyclic molecules (3). After the immersion of the fiber in the sample, it was immediately desorbed into the GC/MS injection port. All samples and analyses were performed in triplicate.

GC-MS: The GC was an Agilent HP 6890 model equipped with a mass selective detector, model 5972 in the Selected Ion Monitoring (SIM) acquisition mode. During the GC/MS analysis (30 min) the SPME fiber is maintained in the injector port to condition it before each extraction at the appropriated temperature determined by the fiber coating.

Quantification: Corresponding deuterated PAHs and pharmaceuticals were used as internal standards to calculate the total concentration (free and bound fractions). Free compounds were quantified by external calibration or by comparison with a standard which does not interact with DOM (4). According to our tests, deuterated naphthalene (Nd8) was then chosen as this standard.

K_{DOC} calculation:

$$K_{DOC} = \frac{[PAH]_{DOC}}{[DOC][PAH]_{free}}$$

with $[PAH]_{DOC}$, the concentration of PAH bound to DOM, [DOC] the Dissolved Organic Carbon content, $[PAH]_{free}$ the concentration of PAH free in solution. If PAH-DOM interactions are linear on the range of the [DOC], they follow the Stern-Volmer equation:

$$\frac{[PAH]_{total}}{[PAH]_{free}} = K_{DOC}[DOC] + 1$$

3. RESULTS AND DISCUSSION

Preliminary results show that polydimethylsiloxane (PDMS) fiber coating with a 100 µm thickness seems to provide the best efficiency for PAHs with a time of analysis of only one hour, low limits of detection and quite good reproducibilities (Table 1).

Table 1. Detection limits and recoveries (PDMS 100 µm, 30 min extraction)

Compounds	Detection limit (ng/L of water)	Internal calibration recovery (%)	External calibration recovery (%)	Nd8 calibration recovery (%)
Phenanthrene	0.6 ± 0.2	102 ± 9	97 ± 7	103 ± 8
Fluoranthene	0.7 ± 0.2	104 ± 5	105 ± 9	110 ± 9
Chrysene	1.2 ± 0.1	92 ± 3	104 ± 22	111 ± 24
Benzo[a]pyrene	6.8 ± 1.4	94 ± 2	95 ± 25	98 ± 26

Interactions between PAHs and DOM have been studied by changing the concentration of DOC (0 to 5.5 mg/L) and keeping the total concentration of PAH constant (about 1 µg/L). K_{DOC} have been calculated by SPME using Stern-Volmer equation (figure 2) and are in the range of the ones found in the literature with Aldrich humic acid (table 2).

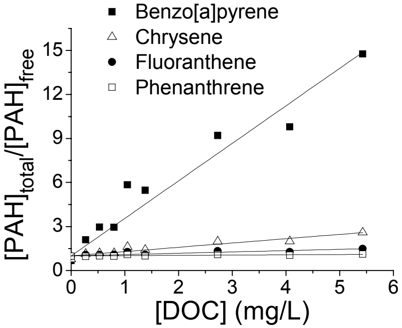

Figure 2. Stern-Volmer representation ($[PAH]_{total}$ = 1 µg/L).

Table 2. Log K_{DOC} measured by SPME compared to literature

	Log K_{DOC} (this study)	Log K_{DOC} (literature)	Method (literature)
Phenanthrene	4.3 ± 0.1	4.61 ± 0.04	Fluorescence quenching (5)
Fluoranthene	4.95 ± 0.05	5.06 ± 0.01	Fluorescence quenching (5)
Chrysene	5.47 ± 0.03		
Benzo[a]pyrene	6.41 ± 0.03	6.3 ± 0.1	Dialysis (6)

Regarding pharmaceuticals, SPME-GC-MS does not show interaction with Aldrich humic acid but La Réole and Toulon DOM samples seem to weakly associate drugs. It may be due to the composition of DOM, since natural samples are composed of much more small molecules than Aldrich humic acid and have probably a different structure. However, more analyses have to be made to determine whether or not interactions statistically occur.

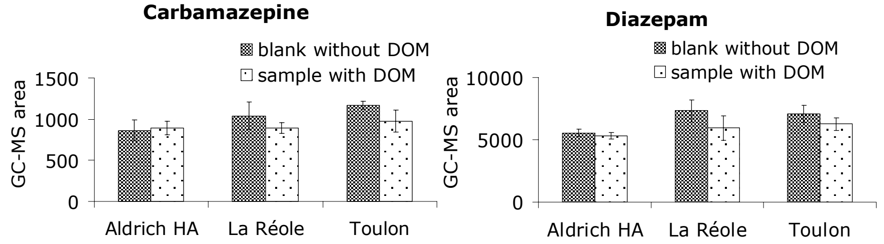

Figure 3. Free pharmaceuticals measured by SPME-GC-MS.

4. CONCLUSIONS

Thus, SPME seems to be a good tool to study the interactions between organic pollutants and DOM in complex aquatic environments. Log K_{DOC} were calculated between PAHs and Aldrich humic acid. Results are in accordance with literature so this technique can be applied to natural samples. Preliminary tests on pharmaceuticals show no or weak interactions with DOM but more analysis are needed to conclude.

ACKNOWLEDGEMENTS

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Greenhouse Effect and Mitigation by Soil Carbon Sequestration in Tropical Areas: Importance and Characteristics of Humic Substances

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Keywords: carbon sequestration, humic acids, tropical soils

1. INTRODUCTION

The energy matrix of Brazil is composed by 44% of renewable energy, mainly from hydroelectricity and bio-ethanol uses, compared with the world mean of only 14% of renewable energy. However greenhouse gases (GHG) emission is relatively high, mainly due to land use changes, including deforestation that can be reduced and/or mitigated.

Also Brazil has increased dramatically adoption of soil conservative systems, including use of no-till or direct drilling with around 22 M ha under no-till. Besides contributions to soil fertility, local and regional environmental aspects, no-till has been proven to be successful in promoting soil carbon sequestration in Brazil (Bayer et al., 2006). In the present work are described field studies on others areas with potential to soil carbon sequestration, as pastureland grass (*Brachiaria decumbens*). To evaluate the dynamic and reactivity of humic acids (HA) and soil organic matter (SOM) in these areas spectroscopic tools, as Electron Spin Resonance (ESR) and UV-Vis fluorescence, were applied helping to explain reactions in the soil.

2. MATERIALS AND METHODS

This study refers to a long-term experiment located in São Carlos city, state of São Paulo, Brazil, at experimental farm of Brazilian Corporation for Agricultural Research (Embrapa), at Southeast Cattle Research Center (21º58'S, 47º50'W), on a *Brachiaria decumbens* pastureland, established 27 years ago, following a corn field. Soil was a low fertility, acid Oxisol, with 25% clay content. Treatments were: T00 – 27 years under Brachiaria decumbens pasture, without limestone and fertilizer additions; t0- without surface liming, but with 400 kg y^{-1} N- ammonium sulfate and K_2O; t2m- 2 t ha^{-1} surface limestone with same t0 NK fertilizer use and 1 t ha^{-1} annual reinforcement of limestone; t4sa- 4 t ha^{-1} of surface limestone without NK fertilizer use; CV- dense cerrado vegetation in transition to a native mesophyle semideciduous tropical forest and tilled soil- pasture area changed to crop, with 0-20 cm depth till.

Total carbon analysis were performed in soil and HA samples using a total carbon analyzer (LECO model CR 412) and CHN (Perkin Elmer model), respectively. Soil bulk density was assessed and used to calculate the soil C stocks.

HA samples were extracted from soil samples and purified according to the IHSS recommended method from two depths: 0-10 cm and 10-20 cm, in all treatments reported before.

The ESR measurements of HA were performed in a Bruker EMX spectrometer operating at 9 GHz (X- band) at room temperature. The relative concentrations for semiquinone-type free radicals were obtained using Singer's method using as a secondary standard a ruby crystal calibrated with strong pitch reference of known free radical content.

Ultraviolet and Visible Fluorescence spectra in the emission and synchronous-scan excitation modes were acquired in a Perkin Elmer LS-50B luminescence spectrometer. Each HA sample was dissolved in 0.05 mol L^{-1} $NaHCO_3$ with 20 mg L^{-1} concentration, and pH 8.

3. RESULTS AND DISCUSSION

In the pastureland areas were measured the carbon storage potential of a 25% clay content Oxisol under well managed tropical grass pasture, *Brachiaria decumbens*, established 27 years ago after corn crop, in comparison to native dense Savannah (Cerrado) vegetation, in São Carlos, SP, Brazil. Greatest SOM content occurred under pastureland in the 10 cm surface soil layer (Figure 1). Compared to native Cerrado, the C stocks in pasture increased from 1.7 to 3.5 Mg C ha^{-1} y^{-1} for the 0-100 cm layer, depending on nitrogen availability. So changing the soil management from Cerrado vegetation to good managed grass pasture will result in an annual sequestration rate ranging from 6.1 to 12.8 Mg CO_2 ha^{-1} y^{-1}. Nowadays Brazil has around 200 Mi ha under pastureland, being 80 Mi ha with *Brachiaria decumbens.* So considering only the potential of land associated with *Brachiaria decumbens* it is possible to estimate, using the mean value of 9.45 $MgCO_2$ ha^{-1} y^{-1} as soil carbon sequestration rate, obtained from our data, a total of carbon sequestration of 756 Tg CO_2 per year by pastureland under *Brachiaria decumbens* that corresponds to practically half of total Brazil's GHG emission (1,500 Tg CO_2 - equivalent per year) or around 44% of total Planet emission due to deforestation and land use change (1.700 Tg CO_2) (Lal, 2004), suggesting a huge mitigation potential. However it is important to consider that this result was obtained in a well managed (including soil liming and fertilization) *Brachiaria decumbes* pastureland area in a experimental site of Brazilian Agricultural Research Corporation (Embrapa) and without consider GHG emission due to nitrogen fertilizer production. So our next effort will be to evaluate different areas, including private farm sites, in all country, and improve the GHG emission/sequestration balance. Naturally other efforts in the bovine production, as reduction of methane and nitrous oxide emissions, are also in progress worldwide as well as in Brazil.

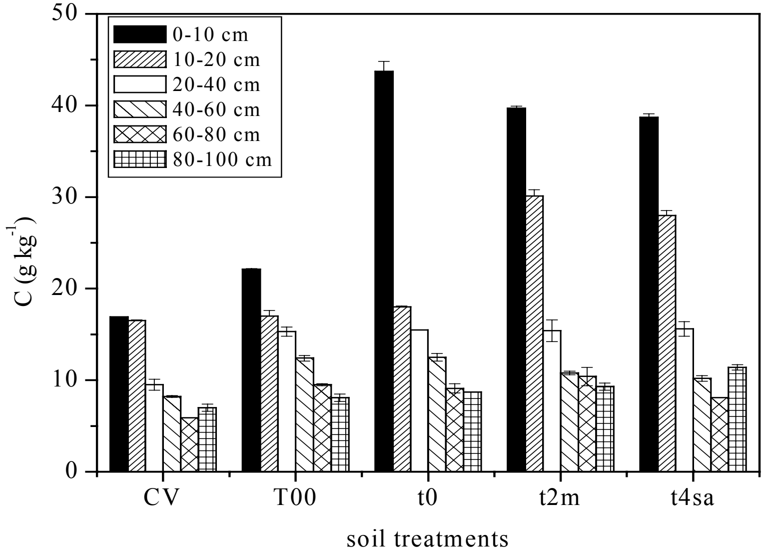

Figure 1. Carbon (g kg^{-1}) content in soil samples under different treatments (as described in Material and Methods section) until 100 cm depth.

ESR studies (Figure 2) demonstrated that HA from pasture soil had higher semiquinone-type free radicals than those from Savannah (Cerrado) soil under native vegetation and tilled soil. Higher semiquinone-type free radical content occurred in t2m and t4sa treatments, more probably due to indirect effect of limestone addition. Limestone use is known to increase soil pH, stimulating soil microbial activity and generating more humified humic acids. In these conditions the increase in the humification degree of HA indicate higher chemical stability of organic matter and consequently higher lifetime of carbon compounds in soil delaying or avoiding CO_2 emission to atmosphere. These ESR data agreed with Fluorescence Spectroscopy of dissolved HA (data not shown).

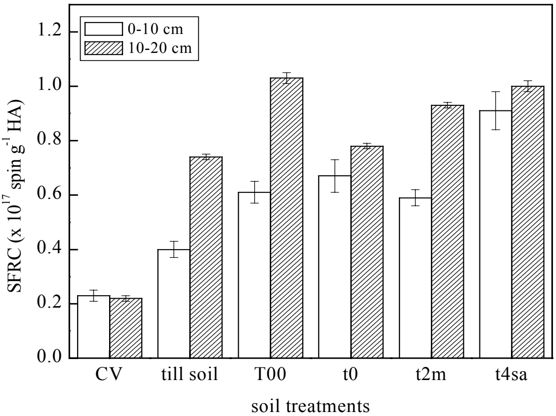

Figure 2. Content of semiquinone-type free radical (SFRC) given in spins g^{-1} (x10^{17}) of soil HA determined by ESR under different soil treatments in the pastureland Brachiaria Decumbens areas. Symbols are same of Figure 1 (CV, T00, to, t2m and t4sa) and till soil is another reference area with grains under conventional tilled soil.

4. CONCLUSIONS

Considering the large Savannah (Cerrado) land area (about 200 million hectares), which is, and could be, used for cropland and pasture the adoption of soil conservative systems (as no-tillage) and well managed pastureland has the potential to turn Cerrado soils into a significant atmospheric C sink and contribute to mitigating global carbon dioxide emissions in addition to increase food and biofuel production.

The spectroscopic analysis permitted to identify increase of humification degree of HA, as detected by ESR and UV-Vis fluorescence, in areas of pastureland (*Brachiaria decumbens*) under soil liming and fertilization compared with native Cerrado.

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What Is Making up Dissolved Organic Matter in Anthropized Aquatic System?

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Keywords: isolation, hydrophilic/hydrophobic organic matter, characterization, elemental analysis, FTIR, SUVA, fluorescence, size exclusion chromatography, pyrolysis-GC/MS

1. INTRODUCTION

In anthropized aquatic system, the sources of Dissolved Organic Carbon (DOC) differ from natural aquatic system because of various water discharges (urban runoff, landfill leachate and urban sewage domestic and industrial treated or not) and the primary productivity induced by these discharges. For example, in dry weather, the French major wastewater contributes to 15% of the Seine River flow at Paris. During the past few decades many studies have been published regarding the isolation and the characterization of Dissolved Organic Matter (DOM) but often focused on the so called “humic substances” (HS). However in anthropized continental waters, the humic character of DOM is weaker because of various urban discharges (Imai et al., 2002). Very few information is available concerning the Non Humic Substances (NHS), e. g. the hydrophilic fraction of DOM, because of its very difficult isolation and purification.

The present study aims (1) to isolate DOM from Seine-Basin: urbanized and low urbanized sites and (2) to characterize these isolated DOM fractions by various analytical techniques such as elemental, spectroscopic (FTIR, UV, fluorescence), size exclusion chromatography and pyrolysis-gaz chromatography coupled to mass spectrometry (Py-GC/MS) in order to acquire better understanding of DOM in anthropized water.

2. MATERIALS AND METHODS

DOM fraction was isolated from Seine-Basin following the reverse osmosis concentration and DAX-8/XAD-4 resins adsorption techniques (Figure 1) recently implemented (Leenheer, 1981; Croué, 2004). The first site is the Marne River at Méry/Marne upstream from Paris which is considered as a slightly urbanized site. The treated effluent of Seine-Aval WasteWater Treatment Plant (WWTP) was sampled because this WWTP collects more than 70% of dry weather flows (combined sewer) of Paris and its suburbs (≈ 8 million equivalent inhabitants) and contributes for 15% of the

Seine River flow in dry weather. These effluents are treated in this WWTP by primary settling and aerobic activated sludge. The Seine River downstream from Paris at Andrésy and Méricourt was also sampled. One IHSS standard fulvic acid (Suwannee River Fulvic Acid SRFA 1S101F) was used like “natural organic matter reference” for the following investigations.

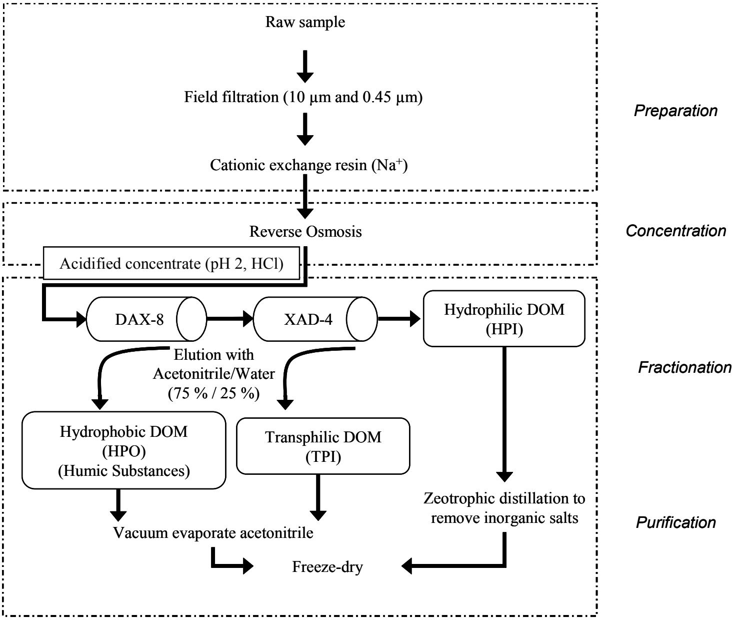

Figure 1. Comprehensive isolation protocol of DOM: The RO/DAX protocol.

Elemental analyses (C, H, O, N, S) were carried out by the laboratory Service Central des Analyses of the Centre National de la Recherche Scientifique (CNRS, Solaize). Specific UV absorbance (SUVA) at 254 and fluorescence 3D were obtained using respectively a spectrophotometer Lambda Perkin Elmer and a spectrofluorophotometer Jasco FP-750 with 1 cm long quartz cells. Infrared spectra of 2-5 mg of DOM fraction isolates in potassium bromide pellets were determined on a Fourrier Transform InfraRed spectrometer (Perkin Elmer Spectrum BX). All spectra were normalized after acquisition with a maximum absorbance of 1.0 for comparison.

3. RESULTS AND DISCUSSION

The distribution of DOC (Figure 2) shows the importance of NHS (transphilic and hydrophilic) in WWTP discharge and DOM from Seine River downstream from Paris contrary to DOM upstream form Paris from slightly urbanized site where hydrophobic fraction prevails.

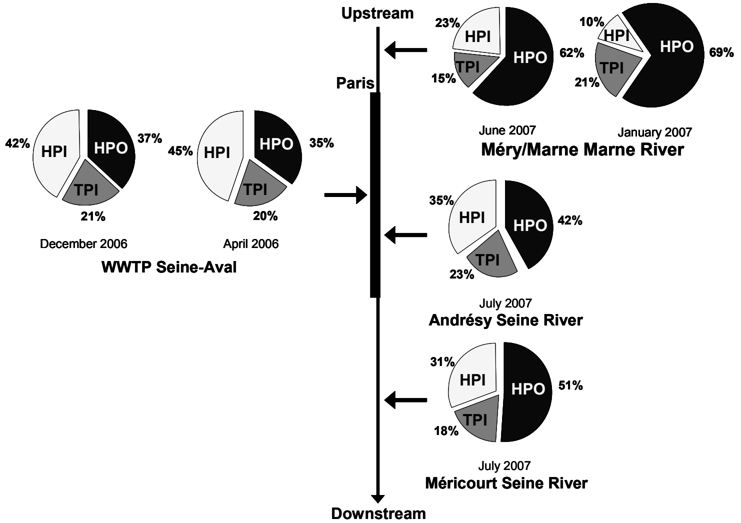

Figure 2. Distribution of DOC of sample from Seine-Basin.

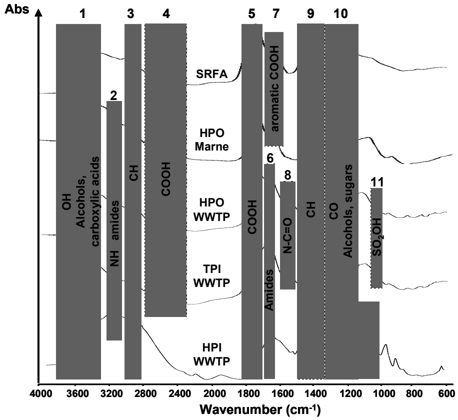

Figure 3. FTIR spectra of isolated DOM.

The results of elemental analysis show the efficiency of isolation protocol to remove inorganic constituents from the sample because the sums of the elemental masses are higher than 90 % in each of the fractions. Nitrogen and sulphur are particularly higher in WWTP fractions (respectively between 5 to 12 % for N and less than 1 % for S) than in SRFA or DOM from Méry/Marne, a slightly urbanized site (between 0.7 to 3 % for N and 2% for S). Moreover H/C ratio and SUVA highlight a weak aromaticity (0.9-2 m-1.l.mgC-1) for treated wastewater DOM compared to DOM from low urbanized site and SRFA (2.7-4.3 m-1.l.mgC-1) probably due to less and condensation processes.

On the FTIR spectra (Figure 3), eleven bands can be allotted to various chemical bonds vibrations. Treated wastewater DOM differs from SRFA particularly in amides group presence which is interpreted as an indicator of intense microbial activity.

Concerning the size exclusion chromatography results revealed significant differences about molecular weight according to the DOM fraction nature and the sample origin.

Py-GC/MS revealed contrasts between the chemical compositions of the samples examined, and revealed certain relationships between this composition and the initial origin of some of the compounds.

4. CONCLUSIONS

In conclusions, hydrophilic DOM appeared as the most important fraction in WWTP discharge contrary to natural aquatic ecosystem where hydrophobic DOM prevails. Various analytical techniques (elemental, spectroscopic: UV, FTIR and fluorescence, size exclusion and Py-GC/MS) allow to characterize DOM from Seine-Basin. Results revealed a weak aromaticity and a high rate of proteinaceous structures of treated wastewater DOM compared to DOM form low urbanized site probably due to less condensation processes and intense microbial activity.

ACKNOWLEDGEMENTS

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Humic Substances in Soils and in Their Drainage Waters

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Keywords: soil organic mater (SOM), dissolved organic matter (DOM), humic acid, fulvic acid, amino acids, neutral sugars, extraction, isolation

1. INTRODUCTION

In this study we seek to examine the links between the compositions of soil organic matte (SOM) and of that in drainage waters leaving the soil. We compare aspects of the compositions of the OM in surface run-off and deep drainage waters with those in the surface horizons of two broadly similar soils; one soil is in long-term grassland, and the second is in long-term cultivation.

2. MATERIALS AND METHODS

A pelo-stagnogley soil (in the Hallsworth series) in long-term grassland, at the IGER Research Farm in North Wyke, Devon, England has, in the top 20 cm, 37% clay, 48% silt, and 4.6% organic C. A second soil (pelo-stagnogley, in the Denchworth series) at the former BBSRC Brimstone Research Farm, Coleshill, Oxfordshire, has been cropped long term to wheat and had in the surface 20 cm, 3.3% organic C, 54% clay, and 39% silt. The Hallsworth soil (Hs) has tile drains at 0.85 m and 40 m apart, and with transverse mole drains 2 m apart, and an undrained site has V notch interceptor drains to collect the run-off waters. Drains in the Denchworth soil (Ds) are at 0.9 m. Organic components, isolated in sodium pyrophosphate solutions (Pyro) at pH 7, pH 10.6, and at pH12.6 (with Pyro + 0.1 M NaOH), were diluted to < 50 ppm, the pH was adjusted to 2 and the solutions passed on to XAD-8 [(poly)methylmethacrylate] and XAD-4 (styrenedivinylbenzene) resins in tandem. Humic acids (HAs) and fulvic acids (FAs) were recovered from the XAD-8 resin, and XAD-4 acids were isolated from the XAD-4 resin. The same resin technique was used for the water samples. The resin procedure, and details for the CPMAS ^{13}C NMR spectroscopy, and for the neutral sugars and amino acids analyses are described by Hayes et al (1) The δ^{13}C and δ^{15}N analyses were carried out by Dr. C.E. Clapp, Department of Soil, Water and Climate, University of Minnesota.

3. RESULTS AND DISCUSSION

There were significant differences in the compositions of the fractions isolated at pH 7, 10.6 and 12.6. Since the compositions of the fractions isolated at pH 7 resembled most closely those isolated from the drainage waters, only results from that fraction are presented here. The isolates at pH 7 from both soils represent the most highly biologically transformed and oxidized components of the SOM. However, only 33% of the total extracted was in the HAs, FAs, and XAD-4 fractions in the case of the Ds, whereas 57% was in these fractions from the Hs. The OM contents of the drainage waters from the Hs and Ds were 2.63 and 5.11 mg l^{-1}, respectively; that of the run-off waters was 11.6 mg l^{-1}.

Table 1. C (%) in seven regions (ppm) of the CPMAS ^{13}C NMR spectra of the humic acids (HA), fulvic acids (FA) and XAD-4 acids (XAD) in the Hallsworth (Hs) and Denchworth (Ds) soils and drainage waters (Hw and Dw), and in the Hallsworth run-off (Hro) waters

Sample	220-190	190-160	160-140	140-110	110-65	65-45	45-10 ppm
HsHA	4	16	7`	22	18	12	21
HwHA	1	13	5	23	15	12	31
HroHA	3	15	9	27	13	13	21
HsFA	5	21	4	21	17	11	22
HwFA	4	17	1	16	13	–	49
HroFA	4	20	5	21	13	7	30
HsXAD	1	19	1	6	53	–	21
HwXAD	2	18	1	6	28	14	32
HroXAD	3	19	2	9	36	11	21
DsHA	3	14	6	28	15	11	22
DwHA	2	13	6	24	12	8	35
DsFA	6	20	5	23	18	9	19
DwFA	4	20	2	16	12	–	46
DsXAD	0	16	1	4	58	–	21
DwXAD	2	21	1	7	29	15	25

The higher aliphatic (45-10 ppm) content in the case of the HwHA is the major difference between the functionalities abundances of the HsHA and HwHA samples. The

HsFA has significant carboxyl enrichment (190-160 ppm), but is otherwise similar to HsHA in terms of the distributions of the functionalities. The data for the HwFA show definite differences in their aromaticity (140-110 ppm) and aliphatic functionalities. It is clear that the HroHAs and FAs are different from those from the SOM. The run-off samples were formed from decaying vegetation and the manures of grazing animals, and the enrichments in O-aromatic (160-140 ppm) and methoxyl (65-45 ppm) resonances indicate lignin residues and incomplete humification. The HsXAD, HwXAD, and HroXADs have features in common, but these components are different from the corresponding HAs and FAs, and are not humic substances on the basis of the classical definitions

In general the trends observed for the Hallsworth samples applied for the isolates from the Denchworth soils and waters, but a detailed examination of the data in Table 1 will show definite differences between the isolates from the grassland and arable soils.

Table 2. Total neutral sugars (NS), (Mannose + Galactose)/(Arabinose + Xylose) (M+G)/A+X) ratios, amino acids (AA) contents, and $\delta^{13}C$ and $\delta^{15}N$ values for the humic acids (HA), fulvic acids (FA), and XAD-4 (XAD) acids isolated at pH 7 from the Hallsworth (Hs) and Denchworth soils (Ds), from the drainage (Hw, and Dw) waters from the soils, and for the run-off waters (Hro) from the Hallsworth soil

Sample	NS (mg/g)	(M+G/)(A+X)	AA (nmol/mg)	$\delta^{13}C$	$\delta^{15}N$
HsHA	50	0.7	750	-29.3	2.5
HwHA	28	0.6	287	-31.0	3.1
HroHA	28	0.3	297	-31.8	3.1
HsFA	45	0.5	457	-28.1	1.6
HwFA	4	0.4	94	-30.2	1.3
HroFA	12	0.3	99	-30.6	0.8
HsXAD	152	1.4	390	-28.4	3.2
HwXAD	9	0.9	141	-28.9	3.2
HroXAD	53	1.4	258	-29.0	3.6
DsHA	35	0.6	559	-29.4	3.1
DwHA	7	0.7	154	-29.1	1.0
DsFA	54	0.5	315	-29.6	2.9
DwFA	3	0.7	60	-29.1	1.5
DsXAD	101	0.7	382	-27.8	5.4
DwXAD	3	1.0	87	-28.1	3.0

These differences are especially evident in the NMR spectra (not shown).

The data in Table 2 confirm similar sugar and amino acids trends for the HsHA and HsFA samples. There are significant differences, however, between the HwHA and HwFA samples, although there are some similarities between the data for HwHA and HroHA samples. Large differences are evident between XAD samples and the HAs and FAs.

Again, compositional trends for sugars and amino acids are broadly similar for the DsHAs and DsFAs, and there are some similarities between the DwHAs and DwFAs. There is little relationship between the DsXADs and DwXADs. The (M+G)/(A+X) ratios strongly suggest that the sugars in the HAs and FAs of the run-off samples had origins in plants (we regard a ratio of 7 or less as indicative of plant origins, predominantly) while the origins of all of the XAD samples were more likely to have resulted from microbial involvements in the transformations of the organic residues. The $\delta^{13}C$ data indicate that all of the fractions were from C3 plants, although small ^{13}C enrichments are evident in the cases of some samples (HsFA, Hs and HwXADs, and Ds and DwXADs). These enrichments are likely to have arisen from microbial metabolites. The $\delta^{15}N$ data would suggest inputs from the fertilizer N applied.

4. CONCLUSIONS

The greater contents of DOM in the drainage waters from the Denchworth soil in long-term cultivation suggests that biological oxidation is still occurring faster in that soil (than in the grassland soil) as the result of the cultivation. The run-off water samples were taken in a rainfall event during the autumnal flush of transformations of senescent vegetation, and the classical humic fractions in these were less well humified than those that had formed in the soils. The pyrophosphate complexed divalent and polyvalent cations that immobilize humic substances in soil, thereby releasing humic materials soluble in aqueous media. This study shows that the materials released in that way have definite similarities with those that are slowly released into, and lost in the drainage waters.

ACKNOWLEDGEMENTS

We acknowledge the contributions of Drs T.M. Hayes and J. O. Skjemstad.

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Size Exclusion Chromatography Investigation of the Photocatalytic Degradation of Natural Organic Matter

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Keywords: photocatalysis, size exclusion chromatography, small organic acids, bioavailability

1. INTRODUCTION

Natural organic matter (NOM) is ubiquitous in natural waters. Although NOM is in itself harmless, it is a source of unwanted disinfection byproducts (DBPs) in drinking water treatment, including trihalomethanes. As a source of DBPs, it is critical to understand the behavior of NOM in established and emerging water treatment steps.

Among the so-called advanced oxidation processes (AOPs), heterogeneous photocatalysis with titanium dioxide (TiO_2, Figure 1) as the photocatalyst has been shown to be an effective oxidative treatment for a wide variety of organic micropollutants (1). It continues to attract the attention of researchers around the globe, especially from countries with an ample supply of sunshine, which can be used to provide the otherwise expensive photons required for heterogeneous photocatalysis.

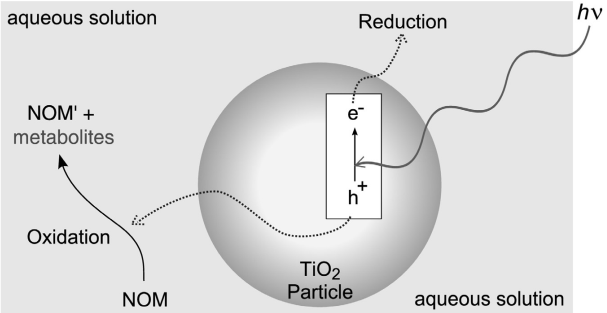

Figure 1: Simplified scheme of heterogeneous photocatalysis with TiO_2 suspensions.

With the work presented here, we aimed to:

1. contribute to the understanding of the photocatalytic degradation of NOM by quantifying the changes in molecular size occurring during the course of irradiation,

2. examine the effect of the presence of Fe^{3+}, Mn^{2+}, Cu^{2+} and Zn^{2+} in the range of 0 to 10 µmol/L on the photocatalytic process,

3. examine the production of small organic acids upon irradiation, and
4. characterize the bioavailability of the organic material after irradiation.

2. MATERIALS AND METHODS

Experimental design:

We used a simple 2^4 full factorial design to systematically study the effect of adding the metals separately and in every possible combination (2). The distribution of points in full factorial designs allows the recognition of single factor effects as well as those effects caused by a combination of factors.

Size exclusion chromatography with organic carbon and ultraviolet detection (SEC-DOC, SEC-UV):

The size exclusion chromatography setup has been previously described (3).

Low molecular weight organic acids (LMWOA):

The detection of LMWOA by means of ion chromatography has been reported in detail by Brinkmann et al. (4).

Sample preparation:

20 mg of P25 (TiO_2 powder, BET surface area = 50 ± 15 $m^2\ g^{-1}$, mean particle diameter = 20-30 nm, approx. 75% anatase and 25% rutile, Degussa, Germany) were added to 40 mL of filtered (0.45 µm) lake Hohloh water (this brown water is described in 5). Samples were then sonicated for 10 min and used immediately for the irradiation procedure. For the experiments with added metals, appropriate amounts of $CuSO_4$, $FeCl_3$, $ZnCl_2$, $MnCl_2$ were dissolved in lake Hohloh water and let stand for a minimum of 3 days in the dark. This spiked water was used to prepare the TiO_2 suspensions.

Irradiation procedure:

The samples were irradiated using a solar UV simulator (Oriel Corp., USA) with additional WG 295 filters (6 mm, Schott, Germany) and an atmospheric attenuation filter (Oriel Corp., USA) installed in the irradiation beam to match the specturm of the Xe lamp to the typical solar UV-spectrum. The samples were stirred, open to the atmosphere and irradiated from above by a homogeneous light field.

Bioavailability:

Irradiated samples (50 mL) were incubated under sterile conditions with mixed bacterial sludge from two wastewater treatment plants and stirred and incubated at 36 °C for two days.

3. RESULTS AND DISCUSSION

First, we performed irradiation experiments in the absence of added metals. The SEC-DOC (Figure 1a) and SEC-UV (not shown) chromatograms of the original and irradiated samples were divided into three fractions (F1-F3). Upon irradiation, we observed a rapid decrease in F1, accompanied by an increase in both F2 and F3 (Figure 1a & b). This shows a redistribution of molecular sizes in the NOM during irradiation, where the larger molecules are degraded to smaller ones without noticeable decrease in overall DOC content (Figure 1b). With continuing irradiation, the rate of formation for F2 (presumably from F1) decreases because of a smaller F1, the DOC content of F2 passes through a maximum and starts to decrease. At the same time, the rate of increase of F3 decreases, leading to an overall decrease in DOC content.

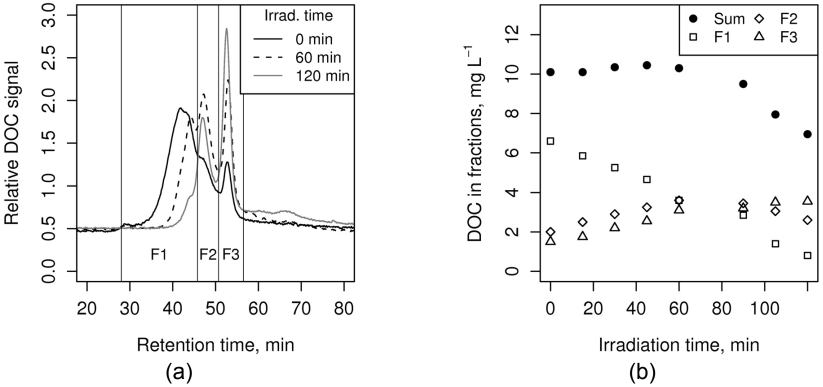

Figure 2: (a) SEC-DOC chromatograms showing the defined fractions (F1-F3) and their time evolution under UV irradiation in the presence of 0.5 g L^{-1} P25; (b) DOC content of each fraction as a function of irradiation time.

The effect of adding Fe^{3+}, Mn^{2+}, Cu^{2+} and Zn^{2+} in the range of 0 to 10 µmol/L was analyzed for the DOC content of the fractions after 30 and 60 minutes of irradiation. Differences in the size of the fractions for samples containing different metals were more pronounced after 60 min than after 30 min. We found that the addition of Cu^{2+} had a delaying effect on the degradation of F1, leading to smaller F2 and F3, respectively. Addition of Fe^{3+}, Zn^{2+} and Mn^{2+} had no *independent* effect on the DOC content of either of the fractions. However, they were involved in interaction effects, as revealed by fitting a linear model including interaction terms to the data. That is, the effect of these metals was *dependent* on the levels of the other metals.

After incubation of the irradiated samples with a bacterial culture, F1 was larger and both F2 and F3 smaller than before incubation. This indicates that F2 and F3 are both bioavailable. We attribute the increase in F1 to the synthesis of high molecular weight molecules by the bacteria. Of the organic acids produced during irradiation (formic > oxalic > succinic ≈ glutaric), all except oxalic acid were completely biodegraded.

4. CONCLUSIONS

The photocatalytic degradation of NOM is influenced by the presence of added metals, especially Cu^{2+}. Other metals tested had no influence on their own but were found to interact with Cu^{2+} and each other to favor or retard the degradation of the NOM fractions in a non-trivial way. The systematic assessment of these effects gives insight into the various interactions which govern this complex system.

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Interaction between Pentachlorophenol and Soil Organic Matter: Sorption-Desorption Reaction and Hysteresis

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Keywords: pentachlorophenol (PCP); sorption-desorption; humic acids (HA); hysteresis

1. INTRODUCTION

Pentachlorophenol (PCP) is one of the ionizable hydrophobic organic contaminants (HOCs) and has been designated as a priority pollutant. Understanding the behavior of PCP requires an assessment of the processes influencing its fate, transport, bioactivity and persistence in soils. The objectives of this study were to investigate the sorption/desorption of PCP by humic acids and soil organic matter in order to improve our understanding of soil parameters that control the transformation and transport of ionizable HOCs.

2. MATERIALS AND METHODS

Two kinds of humic acids (HA) were used. One was a commercially available HA, designed as HA_1 (with a 36.8% C content). The other designed as HA_2 (with a 57.4% C content) was extracted and purified from a paddy soil. Six soils were used for further investigation of the potential contribution of SOM to sorption by comparing the different retention behaviors before and after the removal of organic carbon with H_2O_2 treatment. PCP was Aldrich standard with purity more than 98%.

Sorption was conducted in glass screw- PTFE-cap centrifuge vials. The solid to liquid ratio was adjusted to achieve 30-70% of total solute sorbed. Each series comprised 15 samples. The vials were shaken in the dark until the sorption equilibration was achieved and the suspension was then centrifuged at 4000 rpm for 20 min. The supernatant was analyzed by HPLC. A single-dilution desorption step was further conducted for soil sorbents to investigate the sorption-desorption hysteresis.

3. RESULTS AND DISCUSSION

Typical sorption isotherms of PCP on HAs were fitted to the logarithmic form of the Freundlich equation. The Freundlich model fitted very well the individual isotherms (r^2 = 0.990 and 0.986 for HA_1 and HA_2, rspectively; $p < 0.01$, $n = 15$). *N* values (0.770 for HA_1 and 0.740 for HA_2) were less than 1, resembling L-type isotherms. A L-type curve indicated a relatively high PCP-sorbent affinity at low concentration; the extent of sorption decreased as the PCP concentration increased, because it became increasingly more difficult for PCP to find vacant adsorption sites. The system pH might emerge as a primary factor that controlled the sorption behavior of PCP by affecting the surface characteristics of the HAs and the distribution of PCP species in solution. The equilibrium pH values for the HA_1 and HA_2 after PCP sorption were 5.26 and 4.94, respectively, approaching nearly the pK_a value of OM (~5) and PCP (4.75) (1). Therefore, both the OM and the PCP molecules would be partly dissociated and PCP sorption increases with the increase in the fraction of neutral PCP. With a functional group of OH, PCP would dissociate over the range of equilibrium pH and be present as two chemical species with very different physico-chemical properties. Results presented by Lee et al. (2) suggested that for pH less than 7, PCP sorption increased with an increase in the fractions of the neutral PCP species. Simultaneously, the component of aromatics in HAs allowed the neutral PCP to sorb through hydrophobic bond formation. And the conjugation between neutral PCP and carbohydrates, and the formation of intermolecular hydrogen bonds between PCP and COOH groups were also likely involved (3). Therefore, the pH-dependent chemical characteristics of PCP and the surface properties of HAs co-contributed to the complexity of PCP behavior in the HAs-liquid interface, and thus could result in the high sorption capacity observed.

Table 1. Parameters influencing changes of PCP sorption on soils tested before and after H_2O_2-treatment

Soil No.	TOC (g kg^{-1})		pH		K_d (L kg^{-1})[b]	
	H_2O_2-BF[a]	H_2O_2-AF	H_2O_2-BF	H_2O_2-AF	H_2O_2-BF	H_2O_2-AF
1	18.3	3.2	6.2	4.8	23.6(1.0)	11.4(0.9)
2	24.3	2.9	6.5	4.8	15.9(0.2)	6.6(0.9)
3	17.9	1.5	5.8	4.9	35.6(0.5)	7.7(0.4)
4	6.6	0.9	5.6	5.2	6.2(0.3)	4.9(0.8)
5	11.0	1.8	5.3	4.7	52.5(2.3)	33.5(2.2)
6	5.5	3.4	9.0	7.5	2.7(0.2)	0.6(0.1)

[a] H_2O_2-BF: before H_2O_2-treatment; H_2O_2-AF: after H_2O_2-treatment;
[b] Values are means with standard errors in the parentheses.

The H_2O_2 treatment may be considered as a drastic treatment of the soil, but no significant difference in specific surface area was observed (data not shown). The only changes detected were the total soil organic carbon (TOC) and pH (Table 1). The two parameters were approximately parallel before and after the H_2O_2-treatment. The H_2O_2 treatment decreased 7.14%-26.15% of pH and removed, on average, about 80% of TOC (Table 1), resulting in the drastic decrease in the sorption of PCP as shown in the K_d values. This indicates that the sorption capacity in the investigated soils was related to the organic carbon content.

The hysteresis index (*HI*) was studied for the convenience of comparing the irreversibility of sorption-desorption. The values of *HI* at 20 °C and five representative concentration levels before and after H_2O_2-treatment were investigated (Figure 1). As shown in Figure 1, the *HI* generally decreased after the treatment of H_2O_2, especially for the soils 1, 2, 4 and 5. With the 86% removal of organic carbon, the *HI* values were negative, indicating that PCP desorption was completely reversible in soil 4 after treated with H_2O_2 (A zero or negative *HI* value indicates that sorption-desorption hysteresis is statistically insignificant). It was noteworthy that although the H_2O_2 treatment greatly decreased both TOC and pH values, soil 1 showed a steady but low decline leading to the inverse 10-15% increase of *HI* at the highest concentrations. In soils 3 and 6, the *HI* values increased unexpectedly at the majority of the concentrations tested. This is attributable to exposed sorption sites of clay and oxide minerals and their possible minor surface changes, which might not be detected by the used characterization methods.

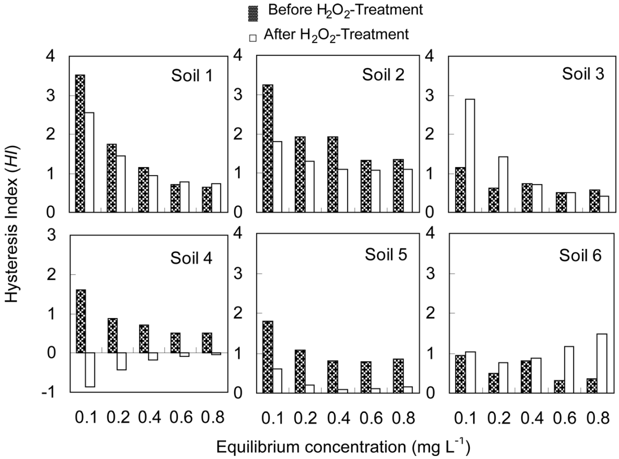

Figure 1. Hysteresis index (*HI*) for pentachlorophenol sorption on the six soils at five representative concentrations before and after removal of the organic carbon by H_2O_2-treatment.

4. CONCLUSIONS

The results obtained in this study provide a basis for understanding and quantifying the sorption-desorption behavior of PCP and its hysteresis in soil/sediment environments. This information is essential for the prediction of the fate of PCP in soils and sediments.

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Modeling Zn Adsorption and Desorption to Soils

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Keywords: adsorption, desorption, kinetics, soil organic matter, WHAM

1. INTRODUCTION

The rate of reaction of trace metals between soils and solutions is important in predicting trace metal behavior as equilibrium may not be attained. Although chemical equilibrium models are commonly used for soil systems, there has been less progress in developing predictive kinetic models.

Kinetics of Zn adsorption/desorption have been modeled based on the linear adsorption isotherm assumption (1, 2), where adsorption and desorption rate coefficients remain constant at constant pH and are constrained by the equilibrium partition coefficient. This approach is in agreement with Zn adsorption/desorption behavior only within a narrow range of Zn concentrations. However, a nonlinear adsorption isotherm is often observed over wide range of Zn concentrations, indicating that a general kinetics model needs to account for the nonlinearity of Zn binding to soils. In the nonlinear adsorption process, the adsorption rate could vary with the Zn loading in soils. Moreover, Zn adsorption and desorption reactions with soil particles are controlled by both soil composition, such as soil organic matter (SOM) content, and solution chemistry, e.g. pH and Zn concentration.

2. MATERIALS AND METHODS

Soil samples were obtained from the 0-20 cm layer, air-dried, and then sieved using a 2-mm screen. These soils have low background Zn concentrations ranging from 16 to 127 mg kg^{-1}. Soil organic carbon (SOC) concentrations were 0.76% to 12.90%.

The stirred-flow reaction chamber has a reaction volume of 6.4 cm^3 and contained 0.3 g soil for most experiments. Zn concentrations were 0.83 to 3.62 mg L^{-1} in 3 mM $Ca(NO_3)_2$ background electrolyte. The pH (5.5 – 6.5) was controlled using 3 mM MES, which does not complex Zn ions. After 4-h adsorption at a 0.9 mL min^{-1} flow, Zn-free electrolyte was pumped through the flow chamber. Samples were analyzed by ICP-MS.

The basic formulation for the change in the concentration of sorbed zinc is a two-site adsorption and desorption kinetics model has been described previously (1). The Zn adsorption/desorption reaction is controlled by two groups of sites, one fast and another slow. The kinetic equations for Zn adsorption /desorption on the two sites are $\frac{dC_{p1}}{dt} = -k_{d1}C_{p1} + k_{a1}C_{ion}$ and $\frac{dC_{p2}}{dt} = -k_{d2}C_{p2} + k_{a2}C_{ion}$ where $k_{a1,2}$ (L (g min)$^{-1}$), $k_{d1,2}$ (min^{-1}), and $C_{p1,2}$ (μg Zn g^{-1}) are the adsorption and the desorption rate coefficient and particulate Zn concentration for sites 1 and 2 respectively, and C_{ion} (μg Zn L^{-1}) is the solution concentration of ionic Zn. The equation for ionic Zn concentration follows from a mass balance for the reactor volume.

In our previous study (1), we used a linear adsorption isotherm, in which adsorption rate coefficients remained constant at constant pH, irrespective of Zn loadings in soils. Using a mechanistic model for Zn adsorption on soils would generalize the model. It would be possible to calculate the change of the adsorption rates during the nonlinear binding process under different reaction chemistry conditions.

The basic model assumption is that the Zn concentration in the particulate phase, which determines the free sites concentrations, determines the corresponding individual adsorption rate constants for Zn adsorption to these free sites as follows. At a specific particulate Zn concentration C_{p1} and solution conditions, it is possible to compute concentration of ionic Zn in solution, C_{ion}, that is in equilibrium with this particulate Zn concentration and the corresponding partition coefficient $K_{p1}(C_{p1})$ based on the adsorption isotherm. Thus, with desorption rate coefficient k_{d1}, the adsorption rate at equilibrium is found from eq 1 with $\frac{dC_{p1}}{dt} = 0$. Then, $k_{a1} = k_{d1}C_{p1} / C_w = k_{d1}K_{p1}(C_{p1})$ where the $K_{p1}(C_{p1})$ is the partition coefficient for the fast site at the specific particulate Zn concentration C_{p1}. This equation determines the adsorption rate coefficient at each time during the reaction. A similar equation applies for the slow site. SOM was considered as the sole adsorbent for Zn binding to the tested soils (1).

The mechanistic model WHAM was used to obtain K_{p1} and K_{p2} at various reaction conditions. In WHAM V calculations (3), Zn can bind to either monodentate or bidentate sites, and can also form outer-sphere complexes in the electrical double layer (DL) via electrostatic interactions. We associate the fast adsorption reactions to both Zn binding to monodentate sites and formation of outer-sphere complexes in the DL. The slow adsorption reactions are assumed to be Zn binding to bidentate sites in SOM. Thus the partition coefficients $K_{p1}(C_{p1})$ and $K_{p2}(C_{p2})$ can be calculated using WHAM at various reaction conditions.

3. RESULTS AND DISCUSSION

Three parameters must be fit for the model, desorption rate constants for the two sites and the fraction of the organic matter that is active in the partitioning. The partitioning coefficients are defined by WHAM. The data for all soils and all conditions tested were globally fit to obtain the best value for the desorption rate constants. These are 3.0×10^{-2} and 5.1×10^{-6} sec^{-1} for the fast and slow sites respectively. This result is very similar to that of Gee et al. (4) who found the desorption rate constant for zinc from particulate matter in South San Francisco Bay was 1.74×10^{-6} sec.

As shown in Figure 1, the model fits are consistent with the experimental data. The kinetics model incorporating WHAM can account for the variations in Zn loading, solution pH and concentrations of SOC.

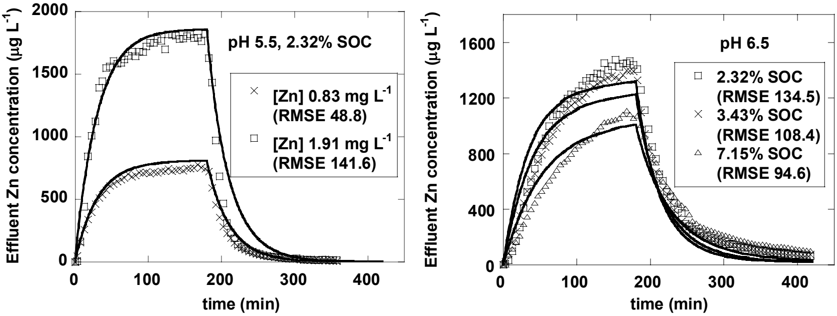

Figure 1. Kinetics of Zn adsorption and desorption on soils.

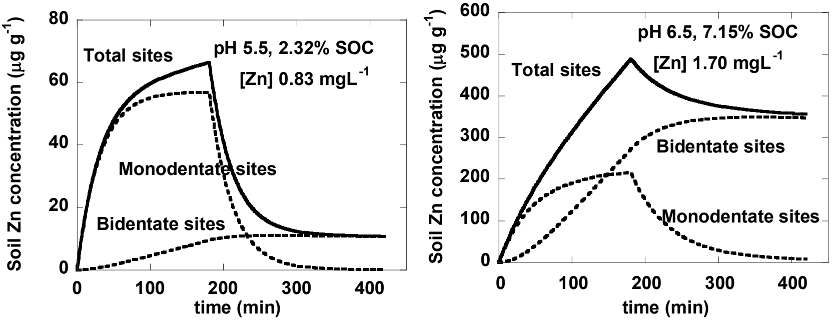

Figure 2. Kinetics of Zn adsorption/desorption on monodentate and bidentate sites.

The role of monodentate (fast) and bidentate (slow) sites on controlling Zn adsorption /desorption kinetics depends on reaction conditions and reaction time (Figure 2). At pH 5.5, binding of Zn to monodentate sites dominated the overall adsorption/desorption reactions. With an increase in pH to 6.5, more Zn was bound to bidentate sites than to monodentate sites after adsorption. During 4 h desorption experiments, the overall Zn release was mainly controlled by the monodentate sites. The amount of Zn adsorbed in bidentate sites continued to increase even during the desorption process, indicating a re-distribution of Zn from monodentate sites to bidentate sites.

4. CONCLUSIONS

Differences in the measured net desorption rate under various solution chemistries result from changes in the readsorption rate, not the desorption rate as has been claimed. Changes in solution speciation, for example by the addition of strong chelating agents, affect the rate of readsorption, not that of the desorption. The initial sorption to monodentate sites is followed by a slow transition to bidentate sites. Desorption of metal from the monodentate sites is rapid, but desorption from the bidentate sites is slow.

Desorption of Zn from soil is a slow process with many days required for a new equilibrium state to be attained. This is likely to have important effects on Zn bioavailability and Zn flux from soil in the field. Because laboratory studies of metal sorption desorption have usually been run for relatively short times relative to the reaction rates, the experimental soil – soil solution distribution will depend on the experimental conditions. Comparison of the results of different investigators and extrapolation to new conditions is difficult.

ACKNOWLEDGEMENTS

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Use of Diffusive Gradient in Thin-Films (DGT) for the *in vitro* Study of the Effects of Humic Fractions and Metals on the Growth of a Phytopathogenic Fungus

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Keywords: humic fractions, compost, peat, coconut fiber, phytopathogenic fungi, *Sclerotinia sclerotiorum*, DGT technique

1. INTRODUCTION

Recent studies have demonstrated that composts and their humic fractions (HAs) can exert a suppressive activity on different soil-borne plant pathogenic fungi, thus allowing a more limited use of chemical fungicides and favouring sustainable crop protection (1-4). The soil-borne fungus *Sclerotinia sclerotiorum* is non specific phytopathogen commonly present in the rhizosphere of a large number of vegetables and crops with a life cycle occurring mostly in the soil (5). When the mycelial growth encounters adverse nutritional or environmental conditions, it forms black seed-like resistance structures called sclerotia. Diffusive gradient in thin-films (DGT) is a new and interesting technique initially developed to measure trace metal concentrations in natural waters (6), and later used to measure solute fluxes and concentrations in sediments and soils (7). Solutes that have been measured by the DGT technique include Ni, Cu, Fe, Mn, Zn, Cd. Mg, Ca and others (8). This technique, which has been shown to measure bioavailable metals, uses a simple device that accumulates solutes on a binding agent after passage through a hydrogel, which acts as a well defined diffusion layer (6). The binding agent used is selective to the species being measured. It relies on the establishment of a steady state concentration gradient in the diffusion layer so that Fick's First Law can be used to calculate unknown concentrations.

The objectives of this work were to study: (i) the effects of three different HAs, extracted from conventional and new plant growth substrates, and some metals on the growth of *S. Sclerotiorum*, and (ii) the capacity of these HAs to alter the DGT concentrations of these metals, i.e. to regulate metal availability, when present both in the growing medium of the fungus.

2. MATERIALS AND METHODS

The HAs were isolated from three different organic substrates: a compost (C-HA), a coconut fiber (CF-HA) and a peat (P-HA). The elemental and acidic functional group composition of the HA examined are shown in Table 1. Stock suspensions of each HA were prepared at concentrations of 1000 mg/L by using sterile double distilled water. ICP standard solutions at concentration of 1000 mg/L (Fluka) of the metals Cu, Zn, Mn, Fe and Al were used. Aqueous solutions of potato dextrose agar (PDA, 4%, w/v) were steam sterilized, cooled at about 55°C and then added with each metal solution (M) at concentrations of 0 (control, only PDA), 5, 10 and 25 mg/L. Each substrate was also added with appropriate aliquots, withdrawn under stirring, of any HA sample at concentration of 0 (only metals) and 100 mg/L. The media obtained were poured in Petri dishes, and left to cool and solidify at room temperature. Then, the fungus was inoculated in the middle of the plates (4) and the DGT unit was placed at the edge of these. The plates were kept in an incubator in the dark at a constant temperature of 20°C. After appropriate time periods, and until the fungus reached approximately the border of the plate (96 h), the apparent morphology and radial growth of the mycelium were evaluated. Starting from sclerotial initiation (144 h after inoculation) and until 264 h, the number and dimensions of sclerotia were measured. All the experiments were replicated six times, and data were statistically analyzed by one-way analysis of variance (ANOVA), and the means of treatments separated by the least significant differences (LSD) test.

At the end of the experimental time, the DGT discs were removed from the plates and disassembled. Metals present in the binding gel were extracted by using 0.5 mL of Aristar nitric acid for 24 h and analysed by inductively coupled plasma–optical emission spectroscopy (ICP-oes).

3. RESULTS AND DISCUSSION

With respect to the corresponding control, no morphological changes were observed for the fungal mycelium as a function of the presence of metals (M) at 5 and 10 mg/L and any HA examined. Conversely, the fungal growth was highly stimulated by the presence of M at the lower concentration (up to more than 6 times the control at 48 h after inoculation) with a decreasing trend during growth (Fig. 1). The presence of any HA in combination with M at 5 mg/L greatly reduced the M effect, especially at 48 and 72 h after inoculation (Fig. 1). This is probably due to some metal adsorption/complexation by HAs. In the treatments with the sole M at concentration of 10 mg/L, a significant mycelial stimulating effect was observed only at 48 h whereas a general almost total inhibition of the fungal growth was observed in the combinations of M at 10 mg/L with any HA. At the highest M dose (25 mg/L), either alone or in combination with each HA, the fungal growth was completely inhibited thus suggesting the occurrence of toxic metal concentrations.

As it concerns sclerotial initiation, the presence in the growing medium of M alone at both doses was able to promote sclerotia initiation and development with respect to the control, i.e. sclerotia appeared earlier and their number and dimension in the plates increased (Fig. 2). Also the combinations of M at 5 mg/L with any HA, especially C-HA, promoted high significantly the formation of sclerotia with respect to the control (Fig. 2). These results may be interpreted as the occurrence of adverse nutritional conditions.

The DGT concentrations measured in the M treatments at 5 and 10 mg/L are referred in Table 2. The combinations M at 5 and 10 mg/L + C-HA or P-HA produced DGT concentrations always lower than the sole M at both concentrations (Table 2) suggesting a relevant role of HAs in the sorption of metals, i.e., a reduced metal availability for the fungus, especially in the case of C-HA. The combinations M at 25 mg/L + any HA fractions caused a DGT concentration generally similar to the M treatment at 25 mg/L alone (data not shown) indicating that the sorption capacity of HAs is not able at a metal dose so high to influence the DGT concentration.

Table 1. Elemental and acidic functional group composition (on a moisture- and ash-free basis) of the HAs examined

SAMPLE	C	H	N	S	O	Total Acidity	COOH	Phenolic OH
	($g\ kg^{-1}$)					($meq\ g^{-1}$) C		
C-HA	583	56	69	6	286	5.6	3.6	2.0
CF-HA	581	51	19	1	348	6.1	4.6	1.5
P-HA	599	56	21	1	323	5.7	3.3	2.4

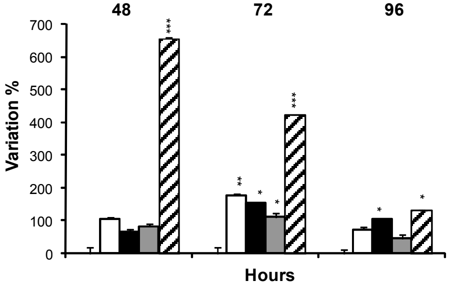

Figure 1. Effects of M alone (striped bars) at 5 mg/L and its combinations with C-HA (white bars), CF-HA (black bars), and P-HA (grey bars) at 100 mg/L on the variation (%) of radial mycelial growth of *S. sclerotiorum* with respect to control (only error bars).

Table 2. Effects of different substrates on the DGT concentration (μg/L) of the metals

SAMPLE	Fe	Mn	Cu	Zn	Al
Control	1,49	0,30	2,57	27,40	3,18
M 5 mg/L	70,22	104,33	82,64	180,87	116,03
M 5 mg/L + C-HA	3,68	1,24	7,84	70,15	5,66
M 5 mg/L + CF-HA	n.d.	n.d.	n.d.	n.d.	n.d.
M 5 mg/L + P-HA	23,80	23,21	56,84	53,87	20,07
M 10 mg/L	116,27	170,69	180,84	382,03	261,60
M 10 mg/L + C-HA	24,30	16,24	34,28	62,64	31,75
M 10 mg/L +CF-HA	20,81	13,82	136,54	48,03	59,04
M 10 mg/L + P-HA	57,58	31,32	172,26	77,19	95,07

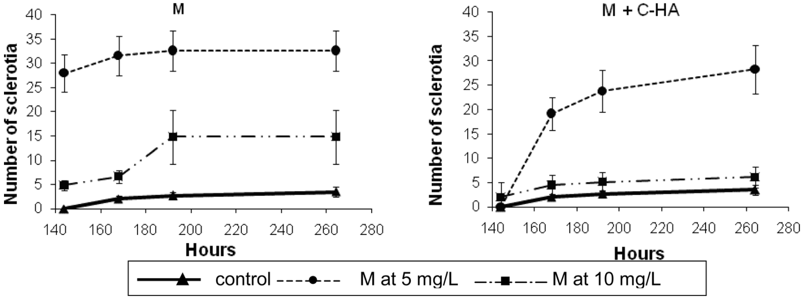

Figure 2. Effects of M alone at concentrations of 5 and 10 mg/L and its combinations with C-HA at 100 mg/L on the number of sclerotia formed by *S. Sclerotiorum* in comparison to the control, as a function of time.

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A Relationship of P Chemical Speciation and PZSE in Allophanic Synthetic Surfaces Enriched with Natural Humic Acids from Chilean Andisols

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Keywords: Chemical Speciation, Chilean Andisols, PZSE

1. INTRODUCTION

Humic acids (HA) and fulvic acids (FA) represent two important fractions of soil humic substances. These substances in Chilean Andisols represent around 70% of the total organic carbon (OC) content. A high P adsorption capacity of allophanic soils is closely related to their surface acidity dependent of pH, and the presence of Al(Fe)-humus complexes. The aim of this study was establish the relationship between P chemical speciation using GEOCHEM-PC (1) and surface charge determined by potentiometric titration (Point of zero salt effect, PZSE) modeling with the constant capacitance model (CCM, 2) on synthetic alluminosilicates with iron oxide and natural humic acid obtained from Chilean Andisols.

2. MATERIALS AND METHODS

Soil samples: from 0-15 cm depth of two Andisols (Osorno and Vilcun) collected from uncultivated areas were used. The OC fractionation was realized according Schnitzer method (1).

Synthetic aluminosilicates: (Al-Si allophane) were prepared from $AlCl_3$ and potassium K_4SiO_4 (1). Allophane were coated with two different percentage of iron oxide, 6% (Al-Si-Fe-I) and 12 % (Al-Si-Fe-II) and also with two levels of natural HA (2% and 6%)

Potentiometric titration: data were obtained from the suspended sample in 0.1 M KCl solution giving a final volume of 100 mL with and without $1x10^{-3}$ M KH_2PO_4 (3).

Chemical speciation: The P chemical speciation was performed using the computer program GEOCHEM-PC (1).

3. RESULTS AND DISCUSSION

Results of soil samples characterization showed the OC was similar for both soils, with lower HA and FA percentage in Osorno soil (4.0 HA y 3.0 FA) than Vilcun soil (6.0 HA y 5.0 FA). Higher OH-phenolic content for Vilcun soil (3,5%) is the main difference in the functional groups, suggesting a lesser condensation degree, with a lesser humidification than HA in Osorno soil (1.5%). The pK_{a1} and pK_{a2} obtained using GEOCHEM-PC and CCM do not present important differences (Table 1). However the program considers the speciation to constant ionic strength, while CCM is able to consider different ionic strength. Then, the acidity constants determined by CCM are more close to real values.

Table 1. Acidity constants from samples

Osorno	pK_{a1}	pK_{a2}	pK_{a1}	pK_{a2}
	(a)		(b)	
Al-Si-Fe-I - 2%	3.0	6.9	4.7	8.2
Al-Si-Fe-II- 2%	2.9	6.8	3.9	7.1
Al-Si-Fe-I - 6%	2.8	7.0	4.9	7.6
Al-Si-Fe-II- 6%	3.5	6.8	3.1	6.5
Vilcún	pK_{a1}	pK_{a2}	pK_{a1}	pK_{a2}
	(a)		(b)	
Al-Si-Fe-I - 2%	3.5	6.7	3.5	6.9
Al-Si-Fe-II- 2%	3.6	6.8	3.7	6.8
Al-Si-Fe-I - 6%	3.4	6.7	3.2	6.0
Al-Si-Fe-II- 6%	4.0	6.8	3.1	6.7

(a) GEOCHEM-PC
(b) CCM

4. CONCLUSIONS

If both the CCM and GEOCHEM-PC were able to describe the surface behavior of both synthetic aluminosilicates cover with natural humic acids isolated from Andisol.

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Correspondence Analysis of Microbe Species in the Formation of Humic Acid

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Keywords: microbe, correspondence analysis, humic acid

1. INTRODUCTION

Microorganisms are the driving force behind the formation, transformation, degradation and mineralization of humic substances (1). A recent study (2) linked to global warming revealed that over a 6 y period under elevated CO2, soil carbon was reduced by half, with this decline driven by the activity of the soil microbial community. However, their relative abundance and role during turnover of humic substances are still unclear (3, 4).

Correspondence analysis (CA) is a technique for studying the associations among a set of variables by graphically displaying the patterns and structures existing in the data which serves as a window onto the data. The method has been widely applied to multivariate data analysis in, for example, sociology, environmental science, and marketing research. In this paper, we explore the feasibility of applying the method to deal with high dimensional microarray data produced in experiments in the relationship between microbe species and humic acid formation studies. Combined with a bootstrap procedure using sample replicates, we show how the nice feature of the relationship between microbe species and humic acid formation. The significance of our approach in microarray data analysis in the studies is discussed at the end of the presentation.

2. MATERIALS AND METHODS

Materials: Sampling site locates in Liujia village, Nong An county, Jilin province, China. (N: 44°11'39.7'', E: 125°07'58.5''). Samples for this study were collected from 0-40 cm depth, uncultivated soil. Microbial species for test: B1: *Arthrobacter citreus*; B2: *A. globiformis*; B3: *Agrobacterium radiobacter var. I*; B4: *Pseudomonas pseudoalaligenes*; B5: *P. fluorescens var. I*; B6: *Bacillus subtilis*; B7*: B. mycoides*; B8: *C. fimi*; B9*: Pantoea agglomerans*; B10: *Pantoea soilia*; B11: *P. vulgaris*; B12: *B. megaterium*; B13: *Agrobacterium radiobacter var. I*; B14*: Ac. calcoaceticus*; B15: *Micrococcus*; B16: *C. flavigena*; B17: *Ac. johnsonii*; B18: *Xanthobacter flavus*;

B19*: P. stutzeri*; B20: *B. cerecus*; B21: other bacteria; S1: *S. albus*; S2: *S. caeruleus*; S3: *S. chromfuscus*; S4: *Nocardia rubropertincta*; S5: *Nocardia convoluta*; S6: *S. roseoviolaceus*; S7: *S. canescens*; F1: *A. niger*; F2: *P. expansum*; F3: *P. nebulosa*; F4: *P. putaminum*; F5: *A. versicolor*; F6: *B. cinerea*; F7: *T. viriderma*; F8: *M. spinescens*; F9: *R. stolonifer*; F10: other fungi.

Methods: After added 2% (w/w) corn stalk powder in soil, fully mixed them together, and then put it (50 g) into a glass bottle with cotton plug, sterilized at 121°C, 2 hours, inoculated pure culture to each glass bottle, Water content: 30%, culture temperature: 25°C, 180 days, 3 times. Contrast soil samples without any microorganisms in soil. The method of Humic acid and fulvic acid isolation is according to (6).

3. RESULTS AND DISCUSSION

3.1. The results shown: After 180 d culture the amounts of soil humic acid in soil samples are substantially similar to each other, they concentrated in the center of coordinate axis, but not all the microbe participate in the transformation of soil humic acid. Among them, *Arthrobacter citreus*, *A. globiformis*, *N. convoluta*, *Aspergillus niger*, *Penicilium expansum*, *Phoma putaminum* and *Ph. nebulosa* take part in the transformation of soil humic acid. But *Cellulomonas fimi*, *S. chromfuscus*, *Nocardia rubropertincta*, *Bacillus subtilis* do not taken part in the formation and transformation of HA (Fig. 1, Fig. 2).

3.2. Although bacteria and fungi both participate in the transformation of humic acid, fungi may dominate the process of humic acid transformation (Fig. 1).

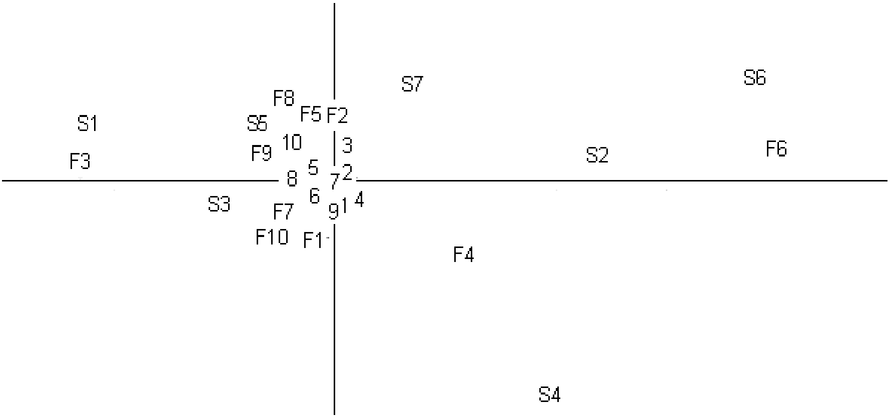

Figure 1. Relationship between streptomyces, fungi and HA.

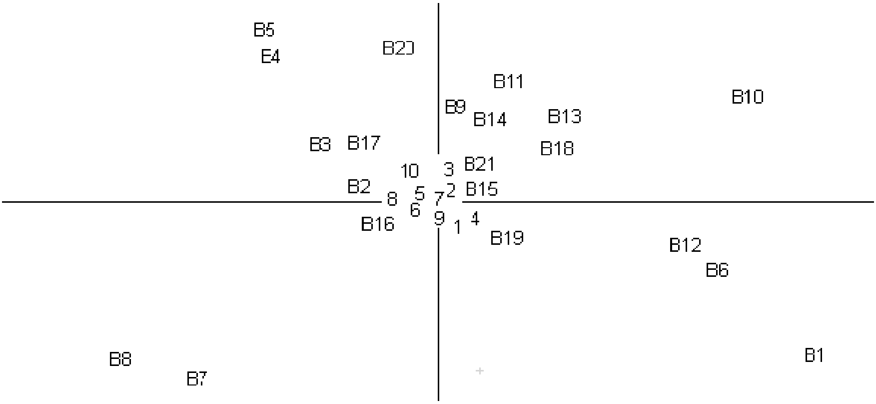

Figure 2. Relationship between bacteria and HA.

Note: the number 1-10 in Fig. 1-2 represent for soil HA at culture time is 1 d, 3 d, 5 d, 7 d, 15 d, 30 d, 60 d, 90 d, 120 d, 180 d, respectively.

4. CONCLUSIONS

Both bacteria and fungi participate in the transformation of humic acid, fungi may dominate the process of humic acid transformation.

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Testing Online Collaboration on Trace Metal-Humic Binding Modelling

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Keywords: humic, fulvic, natural organic matter, metal binding, complexation constants, models

1. INTRODUCTION

Ten years ago, W. Hummel, in one of the best reviews ever published on binding models for humic substances, described the situation faced by any modeller as a “veritable jungle” (1). The situation is even worse for any potential model user freshly embarking in the field or for any scientist who simply wishes to gain an understanding of the scientific literature. Reading the many reviews that exist on the subject may be very useful to all the potential ‘explorers’ of this jungle, whether modellers, users or ‘outsiders’, but these reviews are sometimes difficult to follow, often lack the practical details indispensable for practical applications, and are unavoidably biased by the subjective appreciation of the ‘expert’ who wrote them. Thus, review papers alone rarely provide the complete picture.

It is important to note that such a myriad of models exist not because of the caprices of model developers. Rather, this stems from the fact that the nature itself of the trace element binding properties of humic-type substances severely hampers description: they are undefined complex mixtures of organic compounds that defy the rules commonly applied to other types of chemical substances. This has the serious consequence that, in all probability, there will never be a universal, definitive model and that humic-binding models are ‘empirical’ by nature, their usefulness finally being directly related to their ability to solve particular problems. Scientists trained in disciplines where mechanistic models, based on first-principles, are highly praised find this fact difficult to accept.

Choosing the most appropriate model for solving a particular problem may be facilitated by taking an evidence-based approach, similar to those developed in the medical sciences (2, 3). This path has rarely been explored in environmental sciences. It requires clear information about the existing approaches and access to the models. The online collaboration described here will hopefully be a first step in this direction.

2. TRACKING AND ARCHIVING MODELS

Most of the models described in the literature require specific computer applications in order to be applied. In practice, however, most of these applications are now unavailable. Various factors have led to this situation:

- Although code obsolescence is a problem faced in many different environments (business, government, academia, etc), it is particularly serious in the academic world where the economic incentive to upgrade programs is lacking and where know-how is often lost due to the complexities of the academic world.

- Some models were intended to be used only once. A typical example would be the “oligotroelectrolyte model” developed by Bartschat and co-workers (4). This model has been abundantly cited (146 times according to the Web of Knowledge) and described in virtually every review on the subject but, to our knowledge, it was used only once to produce the original publication. It probably constitutes a perfect example of the halo effect of scientists working in high-prestige institutions (5, 6).

- Most of the models never had a finished, optimized version. Computer code is usually developed as part of a PhD thesis or project and used only by the developer and a limited number of collaborators. Often, user-friendly and well-documented copies are never produced and distributed.

The result is that (i) an incredible amount of scientific knowledge is in fact lost; (ii) only readily-available models are used in practice, irrespective of their actual value and suitability with regard to the problem that needs to be solved; (iii) significant amounts of time are lost reinventing the wheel.

The collaborative web portal being developed contains links to available code and may act as a repository for any existing program. An ’open source‘ approach whereby obsolete code is made available to be improved will be used, subject to the approval of the original developers.

3. A COLLABORATIVE WEB PORTAL THE WIKI WAY

The scientific literature describing and modelling trace element binding by NOM includes four types of publications: (i) original publications where the model is initially described by the developers and possibly modified later; (ii) applications of the models by end-users; (iii) papers comparing models; (iv) review papers. As mentioned above, finding one’s way through this ‘jungle’ is far from straightforward. Many traps are lying in wait for the unwary:

- Often models are published without having undergone the necessary validation process. Over time, mistakes are discovered (or the repeated use of the code reveals some shortcomings) and, hopefully, corrected. This process produces a saga of articles where the logical thread is difficult to follow and from which misunderstandings easily arise. For instance, just such a situation recently prompted the developers of a popular model, the NICCA-DONAN model, to devote an entire paper to clarifying the situation (7).

- In the cases where the code is available, the developers' help is needed to apply it correctly. At least, a quick review of the applications of Model VI-WHAM (8) and the NICCA-DONAN model (7) in a well-known journal, Environmental Science and Technology (years 2002-2007) shows that in a significant percentage of the articles where these models are applied, either the developer appears as an author or the study has been performed in close connection with his lab.
- By nature, model developers always think that their own models are the best and an objective exchange of experiences becomes difficult.

The above considerations mean that an online portal where information is contributed and updated by the developers themselves and the users would be extremely valuable. However, this is difficult to achieve through the traditional channels of scientific communication (conferences, peer-reviewed articles) because of their lack of 'reactivity' and limited audience. New collaborative tools such as wikis may provide a better platform. A wiki is a type of website that can be directly edited by visitors using only their web browser. Wikis are useful for collaborative document creation because document viewing and editing are integrated on-line. The changes to the wiki are stored in a database on a web server and are immediately visible to visitors. A record of all changes is also stored in the database so that older versions of the site's pages can be restored. The construction of a wiki on humic binding modelling may help to establish a neutral, permanently updated reference.

4. FOLLOW-UP OF THE LITERATURE THE BLOG WAY

New applications of existing models continuously appear in the scientific literature. Comprehensive follow-up is difficult and, in all probability, not worthwhile. Publication of reviews and compilations is time-consuming and relatively slow. Moreover, personal opinions and experiences cannot always be adequately reflected in such publications. An experiment is underway whereby new publications will be spotted and referenced and, if deemed interesting, commented on in a blog. The very nature of such coverage means it makes no claim to being comprehensive and the contents will be, to a certain extent, subjective. Information on 'buried' ideas and papers will also occasionally be commented on. The overall objective is to stimulate feedback and the exchange of experiences in the 'humic binding community'.

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Influence of Sorption to Dissolved Humic Substances on Transformation Reactions of Organic Compounds in Water

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Keywords: dissolved humic substances, hydrolysis, chlorination, catalysis

1. INTRODUCTION

Dissolved humic substances (DOM) as ubiquitous components of natural waters can sorb hydrophobic organic xenobiotics and thereby affect their fate in the environment. Sorption phenomena on DOM have been extensively studied in recent decades. Several models allowing a quantitative prediction of the extent of sorption depending on sorbate and DOM properties have been proposed. However, the effect of sorption on transformation and degradation reactions of the sorbates is less well understood. In the present study the influence of DOM on two types of chemical reactions is investigated: (i) hydrolysis under alkaline and acidic conditions, and (ii) chlorination of polycyclic aromatic hydrocarbons (PAHs).

2. MATERIALS AND METHODS

Typically batch experiments were conducted with aqueous solutions of two commercial humic acids (Roth-HA from Carl Roth GmbH, Karlsruhe, Germany and Aldrich-HA from Sigma-Aldrich Chemie GmbH, Muenchen, Germany) as well as one natural aquatic HA from a brown-water lake (Hohlohsee, Germany) were used. This HA is a reference sample of the German research project ROSIG. Measurement of reaction kinetics and identification of products were conducted by GC-MS analyses, combined with headspace sampling or liquid-liquid extraction. Sorption coefficients were determined by means of solid-phase microextraction (SPME). Details of the applied methods are given in (1-3).

3. RESULTS AND DISCUSSION

The effect of DOM on two types of hydrolysis reactions was investigated: (i) dehydrochlorination of γ-hexachlorocyclohexane (HCH) and 1,1,2,2-tetrachloroethane (TeCA) as reactions involving OH^- and (ii) hydrolysis of 1-octyl acetate (OA) which is catalyzed by H^+ at the applied pH value (pH 4.5). The rate of TeCA hydrolysis was not affected by addition of up to 2 g L^{-1} of HA at pH 10 ($k = 0.33\ h^{-1}$) but HCH hydrolysis was significantly inhibited ($k = 4.6 \cdot 10^{-3}\ h^{-1}$ without HA and $2.8 \cdot 10^{-3}\ h^{-1}$ at 2 g L^{-1} HA). HCH is sorbed to 51% whereas TeCA sorption is insignificant at this HA concentration. Apparently, sorbed HCH molecules are effectively protected due to electrostatic repulsion of OH^- by the net negative charge of the HA molecules. In contrast, OA hydrolysis at pH 4.5 ($k = 1.6 \cdot 10^{-5}$ h^{-1}) was drastically accelerated after addition of 2 g L^{-1} HA ($k = 1.1 \cdot 10^{-3}$ h^{-1}). The ratio of the pseudo-first-order rate constants of the sorbed and the freely dissolved ester fractions is about 70. This rate enhancement can be explained by H^+ accumulation in the microenvironment of the negatively charged HA molecules.

The addition of DOM accelerated the chlorination of fluoranthene and naphthalene as two representatives of the class of PAHs in hypochlorite solutions at pH 5. The stronger effect was observed for fluoranthene that is sorbed to a higher extent than naphthalene. The observed rate constant for fluoranthene chlorination is larger by a factor of 5 in the presence of 10 mg L^{-1} of an aquatic HA as compared to HA-free solution. While Cl_2 is the dominant reactive species in pure aqueous solution for both PAHs, the reaction of fluoranthene seems to involve an additional pathway of chlorination by HOCl in the presence of HA. It was found that not only HA did not protect PAHs from the electrophilic attack - as could be expected - but sorption of PAHs on the hydrophobic domains of the HA favoured instead the extent of the chlorination reaction.

4. CONCLUSIONS

In order to understand the effects of DOM on different chemical reactions it is necessary to consider the nature of the reacting species and of the microenvironment of sorbed molecules. The net effect can be a shielding of the sorbed substrate as well as an acceleration of its transformation.

Hydrolysis is not only an important mechanism for the transformation of xenobiotics in the environment but also for the degradation and transformation of 'biogenic waste'. The finding that DOM is able to accelerate acid-catalyzed hydrolysis reactions brings up the interesting question of whether humic substances might themselves act as catalysts of the humification process by means of this mechanism.

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Ultrafiltration Behaviour of Nitrophenols in the Presence of Humic Substances

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Keywords: nitroaromatic compounds, ultrafiltration, humic substances

1. INTRODUCTION

Nitrophenols (NP) and 2,4-dinitrophenol (DNP) in particular, are listed as priority pollutants by the US Environmental Protection Agency (EPA) because of their strong phytotoxic activity which is enhanced by their long-term chemical and photochemical stability. These compounds are used as reagents in the manufacture of dyes, drugs, fungicides and pesticides. As pollutants, the nitrophenols are almost entirely attributed to anthropogenic sources, however they are also formed in the atmosphere by OH-initiated photo-oxidation of aromatic hydrocarbons (1) and are therefore largely diffuse in the environment. The use of ultrafiltration for water treatment is nowadays growing rapidly and membrane processes, have received considerable attention because of the lower costs, simplicity of operation and addition of fewer chemicals. The retention of solutes depends on the size and shape of the solutes relative to the pore sizes of the membrane and is influenced by interactions, not only between membrane and solute but also by chemical properties of the solution and interactions among solutes (3). It is therefore important to understand the effect of common components of dissolved natural organic matter, such as humic substances on the ultrafiltration behaviour of these compounds. In this work we examined a series of NP of different acidity and hydrophobicity and studied whether their ultrafiltration behaviour was affected by the presence of dissolved humic acids.

2. MATERIALS AND METHODS

Ultrafiltrations of 4 nitrophenol, 2,4 dinitrophenol, 3 methyl 2,4 dinitrophenol, dinitrosalycilic acid and 4 nitro phenilphosphate solutions (0.5 mM, pH=7.0 and pH=4.5) were carried out with Amicon YM3 ultrafiltration membranes (d=62 mm, cut off for proteins 3000 daltons). The flux was set at 1 ml/minute by adjusting the pressure of N2 gas connected to the ultrafiltration cell. At the beginning of each experiment, the volume inside the cell was 200 ml and ultrafiltration was ended when the volume had decreased to about 40 ml. Fixed volume fractions (2 ml) were collected with an automated fraction collector.

Humic acids (HA), extracted from the Ao horizon of a forest soil (Entisol) were fractionated by ultrafiltration on a YM 10 membrane till the effluent coming out of the cell was colorless and its conductivity was below 3 mS/m. The retentate HA fraction with apparent MW larger than 10000 was used throughout this work. This fraction was completely retained by the YM3 membrane and allowed direct spectrophotometric determination of NP in the effluent.

3. RESULTS AND DISCUSSION

Neither NP, nor the HA used in this work were adsorbed by the membranes and did not affect fluxes. Rejection of NP decreased with the pKa_1 of the compound: the effect was enhanced in the presence of HA (increased slope) at pH 7, but was much less evident at pH 4.5. Increasing HA concentration resulted unexpectedly, in most cases, in a larger effect on rejection: again the action of HA was more evident at pH 7 than at acid pH. However, when compared to UF of NAC alone the effect was significant only at concentrations of HA>3 mg/ml. These changes are coherent with decreased ionization at lower pH and suggest that the UF behaviour of nitrophenols is for most NP, except 4 nitrophenol, affected only through some kind of charge shielding action by HA, whereas 4 nitrophenol shows an opposite behaviour (increased rejection with increasing HA concentration) as well as spectroscopic evidence of direct interaction.

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Interaction Selectivity between Lignin and Phenolic Compounds

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Keywords: lignin, phenolics, sorption

1. INTRODUCTION

Sorption of phenolic compounds onto wood actually occurs in several different situations, which go from environmental processes such as water decontamination (1,2) to food processes such as barrel aging of wines (3,4). Although the composition of the liquid medium differs between these systems, sorption mechanisms appear to rely on some common features, one of which being that among wood macromolecules, lignin seems to be responsable for the selective sorption of aromatic hydrocarbons (1,2,5,6). Here we report a study of the sorption of phenolic compounds onto oak wood or lignin, either in the vapour phase or in an acidic liquid medium, with the aim of discriminating and characterizing different possible mechanisms according to the nature of the phenolics.

2. MATERIALS AND METHODS

Phenolics were purchased from Sigma- Aldrich (4-propylguaiacol, 4-vinylguaiacol, resveratrol) or TCI-EP (4-methylguaicol, 4-ethylguaiacol, 4-ethylphenol), all with a minimum purity of 98%, and used as received.

Wood plates (2x10x20 mm) were donated by the Office National des Forêts (ONF, France) and taken from trees of *Quercus robur* oak from the Cîteaux forest (France). Lignin has been extracted from similar wood samples using HCl-acidified dioxane (6).

Sorption isotherms in the liquid phase were recorded after immersing for 10 days at 10°C, either a wood plate or lignin powder in a buffer (12 % v/v hydroalcoolic solution, pH 3.5) containing phenolics in mixture or alone. Concentration measurements in the liquid phase were done in triplicate after liquid-liquid extraction with dichloromethane, by gas chromatography (GC TRACE ULTRA,Thermo Electron Corporation with a flame ionisation detector; CP-WAX 57CB column: 25 m x 0.25 id; 0.2 µm bonded phase; 3,4-dimethylphenol was added as an internal standard) or by HPLC (resveratrol).

Isothermal adsorption experiments in vapor phase have been performed using a home-made McBain type thermobalance, at 298 K, on sample weights of about 20 ± 0.02 mg. The saturated vapor pressure was recorded with an MKS Baratron gauge with a relative precision of 1%. The experimental accuracy is ±0.02 mg for the adsorbate weight. Investigated pressures ranged from 10^{-5} hPa to a value close to the saturated vapour pressure of the adsorptive fluid at 298 K.

3. RESULTS AND DISCUSSION

Whether the sorbent was wood or extracted lignin, essentially two kinds of sorption isotherms were recorded for guaiacol-related phenolics within the 0 – 35 mg/kg range of concentrations at equilibrium (Figure 1). Linear isotherms were observed for all compounds except for 4-vinylguaiacol where a continuous non-linear increase was apparent.

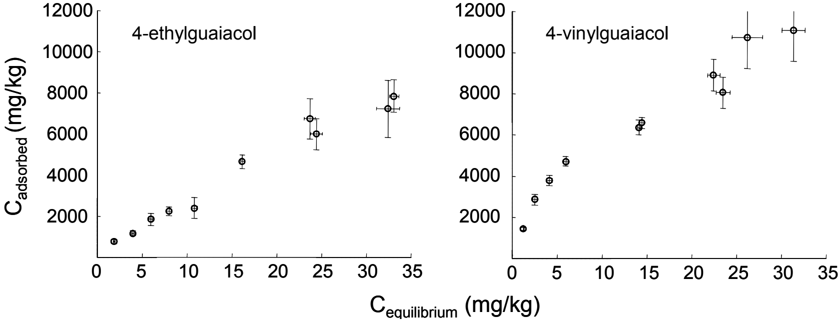

Figure 1. Sorption isotherms of 4-ethylguaiacol and 4-vinylguaiacol onto lignin at 10°C.

From the initial linear part of these isotherms, partition coefficients (K_w so-called Henry constants) were measured. As can be seen from figure 2, including the value for resveratrol, we have determined that, except for 4-vinylguaiacol, there is a good correlation between the wood-acidic buffer partition coefficient of a given compound and its hydrophobicity (logP), whereas partition coefficients are inversely correlated to solubilities (S) of compounds in the acidic buffer. This trend is particularly well illustrated by the homologous series of alkylguaiacols, where partition coefficients linearly increase with the length of the para side chain. In agreement with related studies in the literature, we postulated that lignin, which is the constitutive wood macromolecule which exhibits the strongest hydrophobic character was responsible for this selective sorption behaviour. Indeed, we have observed that neither cellulose nor xylan lead to any sorption selectivity among phenolics (6).

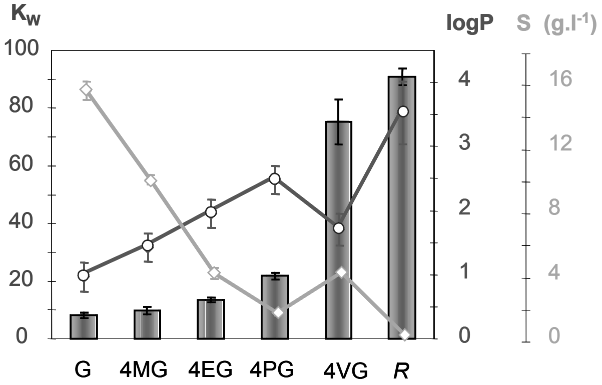

Figure 2. Plot of the wood-acidic buffer partition coefficients (K_w, vertical bars), the hydrophobicities (logP, open circles) and the solubilities (S, open diamonds) of the different phenolics; G: Guaiacol, 4MG: 4-methylguaiacol, 4EG: 4-ethylguaiacol, 4PG: 4-propylguaiacol, 4VG: 4-vinylguaiacol, R: resveratrol; Note the deviations observed for 4-vinylguaiacol, for which logP and S values are not correlated to the high K_w value.

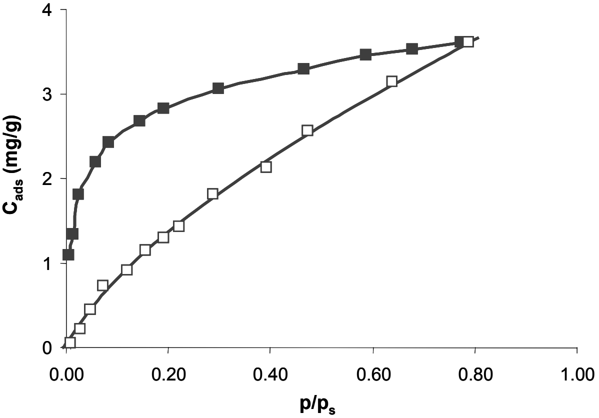

Figure 3. Adsorption-desorption isotherm of 4-vinylguaiacol in vapour phase on lignin.

Our results lead us to hypothesize that the sorption of 4-vinylguaiacol rely on a different mechanism than that of the other phenolics. In order to further characterise this particular behaviour, the adsorption-desorption isotherm of 4-vinylguaiacol in vapour phase was recorded (Figure 3). Upon desorprtion, along with a strong hysterisis, these curves indicated that even at $p/p_s = 0$, a complete desorption could not be achieved, suggesting that chemisorption could have likely occured. Such a result led us to consider selective reactions between the conjugated para-double bond of 4-vinylguaiacol and reactive moieties of the lignin network. Since 4-vinylguaiacol had been shown to self-polymerise through acidic promotion by phenolic OH groups (7), we hypothesized that it could react with lignin OH groups through an acid-catalysed addition to the double bond to form aryl ether bridges. FTIR and NMR results supported this hypothesis (8).

4. CONCLUSIONS

Beyond the sorption selectivity exhibited by wood, that we mainly attributed to its lignin hydrophobic macromolecular network, our results bring insights into the ability of natural lignocellulosic materials to sorb targeted compounds. These can either be non-desirable pollutants or high-value nutrients such as the acclaimed antioxydant stilbene resveratrol. In the latter case, lignocellulosic materials could be considered as candidates for further controlled release applications.

ACKNOWLEDGEMENTS

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Experimental Analysis of Sorption of Environmental Chemicals to Model Soil Organic Matter

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Keywords: soil sorption, pH dependence, humic substances, HPLC

1. INTRODUCTION

Sorption of environmental chemicals to soils and sediments affects their mobility and fate. The predominant sorbent phase for organic compounds is the organic carbon (OC) fraction (fOC > 0.001 g/g) of the solid matrix (1). The soil organic matter consists mainly of humic substances, proteins and carbohydrates. Humic substances are anionic polyelectrolytes which are very heterogeneous in terms of structure and physical and chemical properties. Instead of solubility they were divide in three groups humine, humic acids and fulvic acids. Humic substances Polar functional groups (amine, amid and carboxylic groups) and hydrophobic moieties are characteristic elements of humic substances, and form sites of interaction with environmental chemicals (2). Normalization of the solid-liquid partition coefficient of the solute, Kd, to the OC content of the matrix results in Koc = Kd / foc, a relation that is often used as quantitative measure of the sorption process. Sorption coefficients Koc of ionizable compounds depend on their degree of dissociation (α), the latter of which is governed by the pH of the matrix and the pKa of the solute.

2. MATERIALS AND METHODS

The compounds listed in Figure 1 were obtained from several companies as pure compounds. The RP-HPLC system used was a LaChrom Merck Hitachi system with L 4500 Diode Array Detector, tempered column oven at 25°C and auto sampler. The Nucleosil OH and Nucleosil NH columns (250 mm x 4.6 mm, 100 - 5 µm) were packed from Macherey-Nagel, Zorbax CN column from Agilent, and the humic acid immobilized Nucleosil column from MZ Analysentechnik. The eluent was an aqueous buffer, which was made of bidistilled water, solid phosphate tablets and hydrochloric acid. K_{oc} values of the compounds were determined through measuring their retention times, employing the column at 100 vol. % aqueous buffer. The capacity coefficients (k'_w) were obtained from retention and dead time. K_{oc} was calculated by the equation $K_{oc} = k'_w \cdot (V_M/V_{oc}) \cdot \rho_{sorb}$, where V_M is the volume of the mobile phase in the column, V_{oc} the organic carbon volume of the stationary phase, and ρ_{Sorb} the density of the column material. For weak organic acids and bases, K_{oc} was determined at pH 3, 5 and 7. Here, K_{oc} is given by $K_{oc} = K_{oc,un} \cdot (1-\alpha) + K_{oc,diss} \cdot \alpha$, keeping in mind that these compounds are (mainly) undissociated at pH ≤ 3, and (mainly) dissociated at pH ≥ 7, respectively (3).

3. RESULTS AND DISCUSSION

Fig. 1 shows that for weak organic acids (bases), log Koc (CN column) decreases (increases) with increasing pH. The results suggest that for the undissociated species, the interaction with hydrophobic stationary phases is stronger than for the respective ionized compound forms.

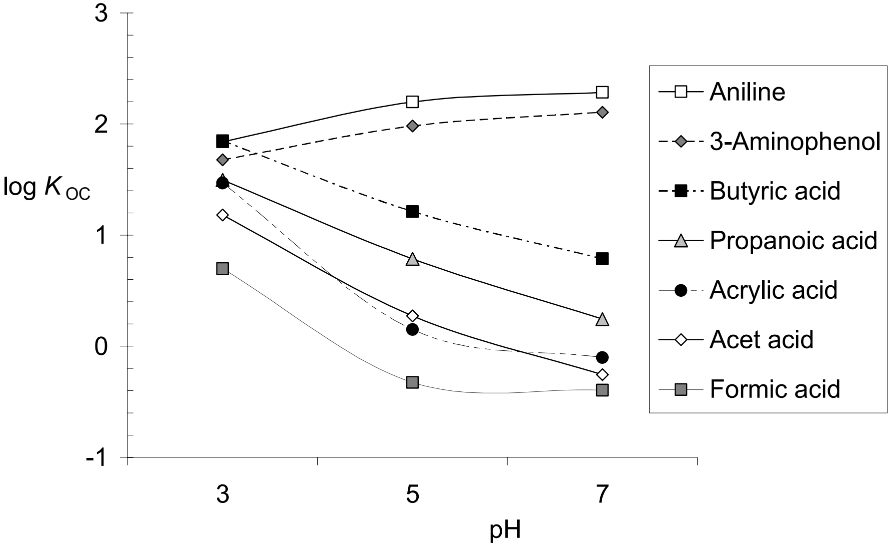

Figure 1. Log K_{oc} (CN column) vs. pH for weak organic acids and bases (pK_a 3.77…4.88).

In Fig. 2, the log Koc dependence on both the column type and pH is shown for acetic acid. As can be seen from the figure, the log Koc variation with pH is negligible when using polar stationary phases (OH and NH colums). Respective experiments with humic acid immobilized Nucleosil column are in progress, and will be compared with results using the other commercial columns. In addition, pH-dependent results from batch experiments with dissolved humic substances will be reported.

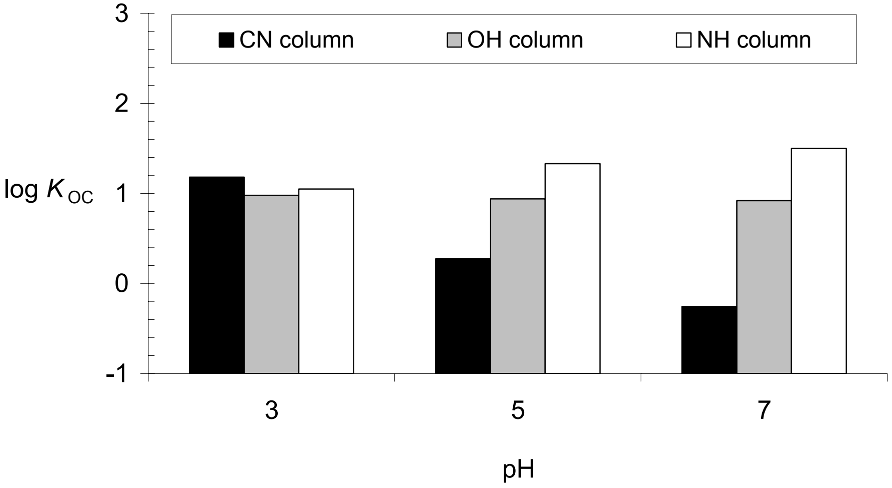

Figure 2. Log K_{OC} of acetic acid vs. pH and column type.

4. CONCLUSIONS

Sorption of weak organic acids and bases depends on the pH-dependent fractions of their neutral and ionized species. For the former, Koc is governed by hydrophobic interactions, while the latter interact more strongly with polar components of the matrix. It follows that indirect chromatographic methods calibrated for neutral organic compounds can not be employed directly to assess the sorption capacity of ionogenic compounds. Moreover, for ionogenic compounds the ambient pH triggers the hydrophobic and polar contributions to sorption. Accordingly, evaluation of the sorption capacity of such compounds should take into account its variation with pH.

ACKNOWLEDGEMENTS

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Influence of Suwannee River Fulvic Acid on the Speciation and Toxicity of Uranium, Aluminium and Arsenic to Australian Tropical Freshwater Species

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Keywords: fulvic acid, uranium, speciation, toxicity, tropical, freshwater

1. INTRODUCTION

Mining represents one of the threats to the quality and biodiversity of freshwater ecosystems in northern Australia. Uranium (U), aluminium (Al) and arsenic (As) are priority metals of ecotoxicological concern for the region's mining industry for which insufficient toxicity data exist (6). Few existing studies (see reviews 8, 3 and 4 for uranium, aluminium and arsenic, respectively) have incorporated dissolved organic carbon (DOC) as a variable, despite humic substances having been recognised as playing a major role in metal speciation for half a century (9). The few tropical freshwater toxicity studies that have investigated the influence of DOC on metal speciation and bioavailability, have demonstrated it is a key determinant of metal toxicity (7, 5). It is essential to build upon these studies in order to gain a comprehensive understanding of the role of DOC in metal bioavailability and toxicity in tropical freshwater ecosystems.

This study will quantify the influence of DOC on the toxicity of uranium, aluminium and arsenic to five tropical freshwater species, at a fixed pH, water hardness and alkalinity. The selected species, Northern Trout gudgeon, *Mogurnda mogurnda*, green hydra, *Hydra viridissima*, the unicellular alga, *Chlorella* sp. and two species of the unicellular flagellate, *Euglena* (*E. gracilis* and *E. mutabilis*) were chosen to cover a range of trophic levels. Laboratory toxicity testing is being conducted using a synthetic soft water and IHSS Suwannee River Fulvic Acid Standard I (SRFA). Tests will also be conducted using natural waters with a range of DOC concentrations. SRFA was selected as the organic carbon source for this study as it is a well characterised reference material (2) and the most biologically relevant standard for this study.

This study aims to: 1) quantify and improve the understanding of the relationship between dissolved organic carbon (SRFA) and the bioavailability and toxicity of U, Al and As to tropical freshwater biota, 2) use this relationship to enhance existing ecological risk assessment methodologies for metals in fresh surface waters; and 3) enhance future revisions of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (1) by incorporating information for bioavailable metal concentrations.

2. MATERIALS AND METHODS

Test organisms were exposed to a range of U concentrations (using uranyl sulphate) at a range of DOC concentrations (0, 1, 5, 10 and 20 mg/L) using SRFA in a standard synthetic water. The synthetic water used was of very low ionic strength (low hardness and alkalinity) and was slightly acidic (pH 6) - characteristic of sandy braided streams in tropical Australia. Test systems were static with 24 h renewal of test solutions (there was no renewal for the *Chlorella* test). Test temperatures were maintained at 27±1°C for *M. mogurnda* and *H. viridissima* and 28±1°C for *Chlorella* sp. Three tests were run for each species in order to fully characterise the concentration-response relationships.

The *M. mogurnda* test measured sac-fry survival over a 96-h exposure period. Each replicate consisted of 10 newly hatched (unfed) fry in 30 mL test solution. The *H. viridissima* test measured population growth rate over a 96-h exposure period. Each replicate consisted of 10 hydra in 30 mL test solution fed brine shrimp nauplii once daily. The *Chlorella* sp. test measured population growth rate, in doublings per day, over a 72-h exposure period. Each replicate consisted of an initial inoculation of 3×10^4 algal cells/mL in 30 mL test solution.

For all tests, water parameters (pH, DO, EC) were monitored daily. Water samples were taken for analyses of DOC (combustion catalytic oxidation), alkalinity (electro analysis), hardness (based on Ca and Mg concentration) and a standard suite of metals and major ions (ICP-MS, ICP-AES or ICP-OES). Physicochemical variables were used in the HARPHRQ geochemical speciation model to determine the effect of DOC on metal speciation. For each species, response data from three tests were pooled, and concentration–response relationships were determined (using both simple linear and multivariate logistic regression analyses).

3. RESULTS AND DISCUSSION

U toxicity was reduced with increasing SRFA for the three species tested. Concentration-response relationships, and associated linear regressions of toxicity (expressed as IC/LC50) against DOC concentration, are shown in Figure 1. Toxicity summary and regression equations are shown in Table 1. The decrease in toxicity of U in the presence of SRFA for all three species can be explained by a reduction in the free uranyl ion concentration due to its complexation by the fulvic acid.

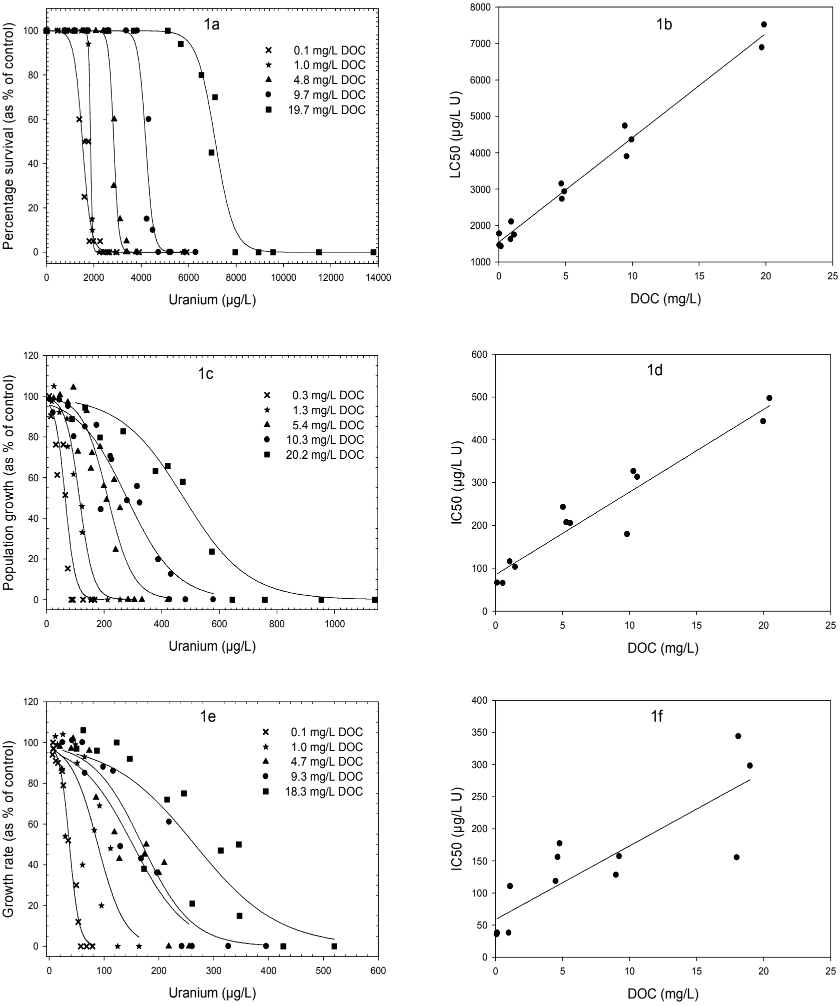

Figure 1. Effect of DOC on U toxicity to *Mogurnda mogurnda* (a & b), *Hydra viridissima* (c & d) and *Chlorella* sp. (e & f). Left: concentration-response relationships with curve fits based on a sigmoidal, 3-parameter model. Right: linear regressions of uranium toxicity (expressed as the IC/LC50) against DOC concentration.

Table 1. Results of uranium testing with three tropical freshwater species

Species	IC_{50} range (µg/L) from 0-20mg/L DOC	Reduction in toxicity	IC_{50} v DOC Regression equation, adjusted R^2, P value
Mogurnda mogurnda	1600-7200	4×	$IC_{50}=287[DOC]+1548$, $R^2=98\%$, $P<0.0001$
Hydra viridissima	65-470	7×	$IC_{50}=19[DOC]+85$, $R^2=91\%$, $P<0.0001$
Chlorella sp.	40-270	7×	$IC_{50}=7[DOC]+51$, $R^2=72\%$, $P=0.045$

4. CONCLUSIONS

The extent to which DOC ameliorated U toxicity differed for each species. DOC appeared to result in a more gradual (but higher overall) reduction in U toxicity for *H. viridissima* and *Chlorella* sp. than *M. mogurnda*. For *M. mogurnda*, there was an apparent increase in the threshold U concentration (the point at which survival dropped from 100%) with increasing DOC. However survival fell from 100% to 0% at a similar rate for all DOC concentrations. This may indicate a similar toxic response is occurring across DOC treatments once cation-binding sites of DOC are saturated with U.

Toxicity testing with SRFA for Al and As is still to be conducted. Short term exposures of all three metals to *Euglena* spp. will assess cell viability and morphology.

ACKNOWLEDGEMENTS

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Uptake of Humic Substances by Plants:
a Study Using Tritium Autoradiography and FTICR MS Analysis

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1. INTRODUCTION

Bioavailability of humic substances (HS) to plants is still questionable due to difficulties in HS determination in plant tissues. The most convenient way is a usage of labeled compounds, and the availability of isotope-labeled HS is an important prerequisite to elucidate the fate of the heterogeneous organic matter in complex environments. On the other hand, presence of the isotope does not allow identifying specific compounds of HS that entered a target object. To solve this problem, isotope-labeled HS together with Fourier Transformed Ion Cyclotron Resonance mass spectrometry (FTICR MS) approach could be applied. This study was aimed (1) to find out uptake and distribution of HS in wheat seedlings and (2) to identify humic-derived compounds that can found in plants.

2. MATERIALS AND METHODS

For this study humic acids (HA) derived from leonardite was used. To synthesize isotope-labeled HA, an approach developed in (1) for the preparation of tritium-labeled HS was used. The obtained ^{3}H-HA sample was dissolved in a phosphate buffer (0.028 M, pH 6.8) and purified by dialysis during a month at 4ºC. It allowed eliminating exchangeable tritium of OH^-, $COOH^-$, and NH_n groups of HA. Plants of wheat *Triticum aestivum* L. were used for the experiments. Wheat seeds were germinated at 24°C in the dark for 72 h followed by transferring seedlings into the 0.5 l tanks containing Knopp nutrition solution. After another 72 h plants were transferred into the vials containing HA at concentration 50 $mg \cdot l^{-1}$ with specific radioactivity 0.02 $Curie \cdot l^{-1}$. After 24 h plants were harvested and subjected to autoradiography. To perform FTICR MS analysis, shoots' tips were colleted and subjected to methanol extraction followed by FTICR MS. FTICR was conducted at

Apex Qe (Bruker Daltonics, Bremen, Germany) equipped with Apollo II electrospray ionization (ESI) source and 12 Tesla actively shielded superconductive magnet. Negative ionization mode was used with spray shield set to 3 kV and capillary voltage 3.5 kV. Flow rate was set to 120 mkl/hour. 100 scans in mass range 150…2000 Da were collected for each sample. To find tritium traces, FIRAN software (Kunenkov, 2006-2008) was used under the following considerations: a) there is only one tritium atom in the labeled ion; b) for each labeled ion there is ion without the label which consists only of ^{12}C, ^{1}H, ^{16}O and ^{14}N; c) for each labeled ion there is ion without the label but with one of its ^{12}C replaced by ^{13}C (this consideration were used to find ion charge by mass difference between two unlabeled ions); d) labeled ion present in mass spectrum of extract from shoots subjected to ^{3}H-HA must be absent in mass spectrum of extract from shoots subjected to non-modified HA.

3. RESULTS AND DISCUSSION

The obtained results revealed that coal HA were intensively adsorbed by wheat plants. The roots of the plants subjected to ^{3}H-HA were characterized with homogeneous distribution of signal intensity. Distribution of HA within the shoots was also relatively homogeneous except for the tips of the leaves where local increase of signal density was observed. That finding was evident for the fact that HA could be taken up through plant roots and moved al least in the xylem with transpiration stream to areas of new growth.

FTICR MS analysis of extract demonstrated presence of tritium-labelled fatty acids in the shoots of wheat plants treated with ^{3}H-HA. As fatty acids are important moieties for plant lipids biosynthesis, the obtained data demonstrated clearly that HA-derived compounds can be used for membrane formation.

4. CONCLUSIONS

Distribution of HA in plants was demonstrated to be relatively homogeneous except for the tips of both roots and leaves where increase in ^{3}H-HA concentration was observed. FTICR analysis of extract from tips leaves revealed presence of tritium-labelled fatty acids what was evident that HA-derived compounds could be used for membrane formation.

ACKNOWLEDGEMENTS

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Effect of Humic Substances on Metal Desorption From Sediments under Conditions of Experimental Simulation

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Keywords: humic substances, metals, sediments, desorption

1. INTODUCTION

Humic substances (HS) contained in surface waters are a complicated mixture of organic polyelectrolytes differed one from another by the molecular sizes, molecular structure and presence of diverse functional groups (1, 2). The sources of HS in surface waters are the soil humus and also terrestrial and aquatic plants. HS belong to the group of stable natural organic compounds and they are practically poorly decomposed (3).

HS, being an important source of organic matter in all terrigenous and aquatic ecosystems, are involved in a variety of biochemical and geochemical processes. It is evident that the role of HS in water bodies is regarded quite often from different standpoints, sometimes even diametrically opposite ones. HS are believed to be potential nutriments for plants, benthonic animals, and microorganisms (4). These compounds play an important role in the cycles of nutritive elements, such as phosphorus. Based on another point of view it is suggested that the bioproductivity of water bodies with high HS concentrations is lower substantially because of the increased water colority and binding of biogenic elements to their inaccessible forms.

Owing to the properties of complexation, HS are capable of extracting metals from sediments in water bodies, increasing thereby their migration mobility in the sediments – water system. As a result of this process, there rises a probability of a secondary pollution of aquatic environment with metal compounds that has to be considered as an undesirable phenomenon.

The aim of this paper is to assess the migration of metals in the bottom sediment/water zone under different HS concentrations.

2. MATERIALS AND METHODS

Experiments were conducted by stages in glass aquariums of volume 18 L at room temperature (20–22^{o}C). The samples of the sediments and natural water were taken in the upper part of the Kanev reservoir and loaded in the aquariums at ratio 1:10. Experiments were conducted under the static conditions without aeration and mixing. The first and second aquariums were taken as the control, where natural water only and silt and natural water were present. In the next 5 aquariums humic acids (HA) were introduced in the concentrations 2.5, 4.0, 5.0, 10.0, 20.0 mg$\cdot$L^{-1}, and in 3 aquariums fulvic acids (FA) were introduced in the concentrations 8.0, 16.0, 32.0 mg$\cdot$L^{-1} respectively. HA of the firm "Fluka" were used. Preparations of FA were obtained out of water of the upper part of the Kanev reservoir by sorption and purification.

The concentration of dissolved oxygen, pH, and the dissolved forms of metals were determined daily. Metal analysis involved chemiluminescence methods (Cu, Cr) (5) and anodic stripping voltammetry (Zn, Pb) (6).

Molecular weight distribution of metal complex compounds was studied by gel-permeation chromatography when using the column filled with TOYOPEARL HW-50 gel (Japan).

3. RESULTS AND DISCUSSION

Lowering in concentration of dissolved oxygen and pH occurs in the process of contact of natural water with silty sediments, because the oxygen expense for chemical and biochemical oxidation reactions of organic and inorganic substances contained in aquatic environment, and especially in sediments increases. Anaerobic conditions are among the main causes of increase in organic matter concentration, metals, and biogenic elements and so on in water.

Results of previous studies have shown that HS, FA chiefly, play the main role in complexation in the Dnieper reservoirs (7). It is also characteristic that the noted metal complexes are prevailing, too, in pore solutions of silty sediments. Therefore, it is sufficiently evident that the influence of HS on migration of metals in the sediment – water system may be rather appreciable.

Owing to HS reductive properties, they promote decrease of dissolved oxygen concentration and pH in the aquatic environment. The O_2 content in overlying water lowered to 0.9–1.3 mg$\cdot$L^{-1} and 0.2–0.5 mg$\cdot$L^{-1} at elevation of HA concentrations in natural water up to 10.0 mg$\cdot$L^{-1} and 20.0 mg$\cdot$L^{-1}, respectively. The analogous lowering in concentration of dissolved oxygen occurred at the additional concentrations of FA in natural water 16.0 and 32.0 mg$\cdot$L^{-1}.

It should be noted that under anaerobic conditions concentrations of metals in natural water contacting with sediments is always higher and these may rise 3–6 times in comparison with their initial contents in the same water. The presence of HS in the aquatic environment should lead to an additive extraction of metals from bottom sediments owing to formation of complexes by these ligands.

For comparison, Figures 1 and 2 demonstrate the data on the maximally possible quantity of metals that could be desorbed from sediments and pass in dissolved state into the aquatic environment under various experimental conditions, including influence of HA or FA.

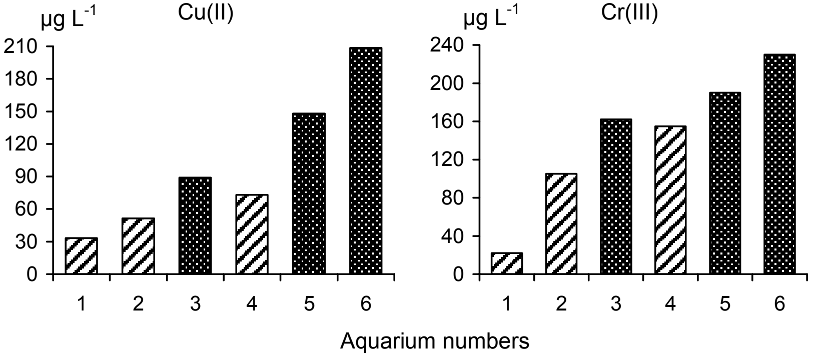

Figure 1. Maximal quantities of metals migrating from sediments into natural water under experimental aerobic (1, 2, 4) and anaerobic (3, 5. 6) conditions and at the presence of HA in contacting water: 1, initial natural water; 2, 3, sediments + natural water; 4, 5, 6, sediments + natural water + HA (3.8, 10.0 and 20.0 mg·L^{-1}respectively).

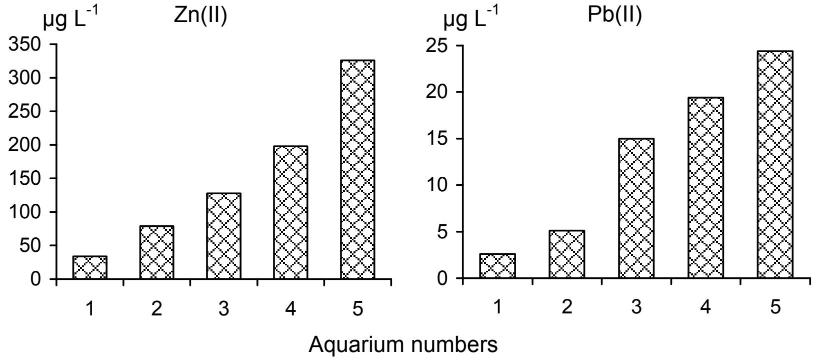

Figure 2. Maximal quantities of metals migrating from sediments into natural water in the absence and at the presence of additional concentrations of FA in contacting water: 1, initial natural water; 2, sediments + natural water; 3, 4, 5, sediments + natural water + FA (8.0, 16.0 and 32.0 mg·L^{-1} respectively).

It is very complicated to estimate quantitatively the influence of HS as natural high-molecular ligands upon migration of metals from sediments into the aquatic environment owing to concomitant process of oxidation of these organic compounds because this process promotes aggravation of the deficiency in dissolved oxygen. Nevertheless, it is clearly seen from the data presented in Figures 1 and 2 that maximal quantities of metals migrating from sediments are higher when water contains HS than it is under anaerobic conditions in the absence of additional concentrations of HS in overlying water.

The most part of metals migrated as the complexes with organic substances of interstitial solutions from the sediments and with the HA or FA added to natural water, which molecular weight did not exceed 2.0 kDa.

4. CONCLUSIONS

The results of experimental studies have shown that the migration intensity of metals in the sediments – water system, as well as the degree of secondary pollution of the aquatic phase by them, depends on a number of factors. Under anaerobic conditions of a simulation experiment, the quantities of metals desorbed from sediments and passed into contacting water are much higher than under aerobic conditions. The additions of HS to natural water influence significantly migration of metals from sediments; however, it is rather difficult to evaluate quantitatively this influence because of simultaneous effects of deficiency in dissolved oxygen and complexation at the participation of these natural organic ligands.

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Acid-Base Properties of Dissolved Humic Substances in Landscape Waters of the Southern Primorye (Russia)

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Keywords: humic, fulvic, potentiometric titration

1. INTRODUCTION

Due to the acidic non-saturated functional groups of dissolved organic matter (DOM), soil waters participate in weathering of mineral matrix and transportation processes of the metal-organic complexes through landscape. These processes are especially pronounced in the regions with a monsoon climate. Study of ecosystems in consecutively alternating altitudinal belt of vegetation enables one to reveal changes in characteristics of landscape components within relatively short distance. The aim of our study was to investigate the acid-base properties of soil water humic (HA) and fulvic (FA) acids, which are among the most active agents of pedogenesis and biogeochemical migration in mountain-taiga landscapes of the southern Primorye.

2. MATERIALS AND METHODS

The area under study is one of the southern slopes of the Sikhote-Aline mountain ridge, located in the South of the Russian Far East, southern part of Primorye. Soil waters were collected in zero-tension lysimeters below humus horizons of the main types of mountain-taiga brown soils: humic cambisol (750 and 950 m a.s.l.); and dystric cambisol (1250 and 1400 m a.s.l.). The characteristics of the soils and the composition of the seepage waters were published in (1).

HA were separated from FA by overnight precipitation at pH 1,5 followed by centrifugation of solutions for 30 min at 3000 rpm. HA were re-precipitated for purification. Acidic centrifugates were fractionated according to (2). The method is based on the Forsyth fractionation on the coal column followed by the separation of low-molecular-weight DOM. Solutions of HA and FA were dried at 30-40°C.

Potentiometric titrations of HA and FA were performed in a PC-controlled system under constant argon flow. 6-10 mg of air-dried sample was dissolved in 10 ml of 0.1M KCl adjusted to pH=10 by addition of 0,1M KOH to allow complete solubilization. Prior to titration the HA/FA solution was readjusted to pH=2,5 with 0,1M HCl to attain complete protonation of functional groups. Results of potentiometric titration were processed using the density function method (3) which yields pK-spectra of HA and FA.

3. RESULTS AND DISCUSSION

Potentiometric titration data indicated that HA and FA have different acidic characteristics determined by change of the ecological conditions of their synthesis. Total content of acidic groups was 6,9-9,3 mmol/g and 3,6-5,6 mmol/g for FA and HA of lysimetric waters, respectively (Table 1).

Table 1. Acid-base properties of lysimetric humic substances of mountain-taiga brown soils (humus horizons)

Sample	Total content of groups, mmol/g	pK	Content, mmol/g
FA, humic cambisol, 750 m a.s.l.	6,9	4,59	2,5
		6,82	0,6
		9,24	3,8
FA, humic cambisol, 950 m a.s.l.	9,3	4,0	5,1
		6,59	0,5
		9,35	3,6
FA, dystric cambisol, 1250 m a.s.l.	8,9	3,94	4,7
		6,18	0,8
		9,47	3,4
FA, dystric cambisol, 1400 m a.s.l.	5,6	4,10	3,1
		6,30	0,9
		8,50	1,6
HA, humic cambisol, 750 m a.s.l.	3,6	4,76	1,7
		6,94	0,7
		9,65	1,2
HA, humic cambisol, 950 m a.s.l.	4,9	4,53	2,2
		6,76	0,6
		9,41	2,1
HA, dystric cambisol, 1250 m a.s.l.	5,6	3,29	1,3
		4,71	2,5
		9,59	1,8
HA, dystric cambisol, 1400 m a.s.l.	4,6	4,41	1,9
		6,65	1,8
		9,41	0,9

FA of humic cambisol (950 m a.s.l.), whose DOM is characterized by the highest mobility in the soil profile, differ remarkably in total acidity (9,3 mmol/g). Another characteristic feature of this type of soil DOM was detected in FTIR-spectrum, which showed the highest intensity of the COOH groups stretching band (1720-1730 cm^{-1}).

In pK-spectra of HA and FA we distinguished three types of acidic functional groups with pK_1 = 3,94-4,59; pK_2= 6,18-6,82; and pK_3=8,5-9,47 for FA, and pK_1=3,29-4,76; pK_2= 4,71-6,94; and pK_3= 9,41-9,65 for HA. The results obtained reveal the tendency to increase of acidic strength of HA and FA functional groups with increase of the absolute altitude (Figure 1). However, this tendency is less explicit for weak functional groups that can be determined by the higher errors in evaluation of high pK-values.

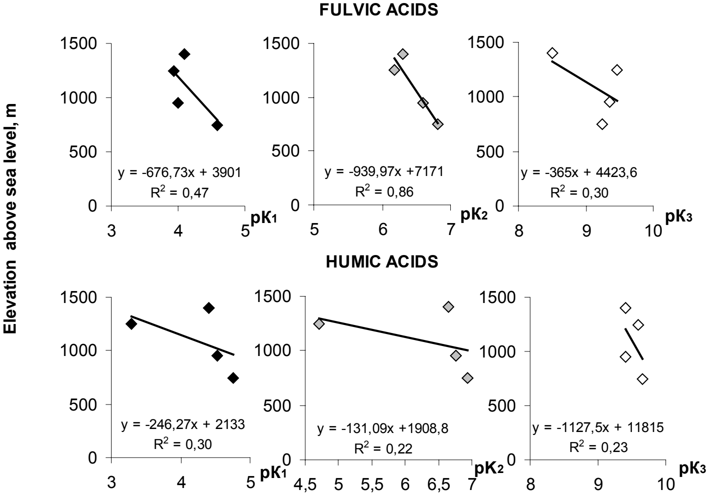

Figure 1. Trends of acidity of lyzimetric HS with the elevation.
pK_1 (black) - strong acidic functional groups, pK_2 (grey) - moderate strength groups, pK_3 (open) – weak functional groups.

It is important, that this altitude trend is broken by the lower values of pK for HA of dystric cambisol, (1250 m a.s.l.) formed under the coldest conditions among the studied soils of mountain pine formation on the slope of north exposition (1). Thus, the temperature trend in acidity of humic substances was more explicit than the altitude trend.

Lysimetric FA of humic cambisol (750 m a.s.l.) contain about 36% of acidic groups with pK_1 (pK=4.59), while about 50% of functional groups have pK>9, and are not ionized at environmental pH. At higher altitude of the macroslope with alternation of hydrothermal regime and increase of conifers share in vegetation composition (1), more than a half of FA acidic groups shows strongest acidity (pK_1=3,9-4,1). These groups are completely ionized at pH of lysimetric waters (4,0 – 6,5) and can interact with soil minerals.

Most likely, increase of acidic strength of HA and FA results from changes in the composition of community of organic substances destructors and humic substances producers under transition from warmer to colder forest ecosystems. Acidic conditions hamper the decomposition process of plant residues due to the depression of bacterial microflora activity and the stimulation of fungi development (4).

4. CONCLUSIONS

About 50% of functional groups of dissolved FA and HA of lysimetric waters are of the type with the strongest acidity, most likely, of carboxylic origin. These groups correspond to the most reactive type, which is completely ionized and can be involved in cation-exchange reactions at environmental pH of lysimetric waters (4,0 – 6,5). Substantial part of groups (30-50 % for FA, and 20-40 % - for HA) can be related to the phenolic type, which is unable to dissociate under environmental conditions, and, therefore, is geochemically inert. The most pronounced acidic properties (the high total content of acidic groups and the presence of groups with the lowest pK_1) were revealed for humic substances of dystric cambisol, (1250 m a.s.l.) formed under the coldest conditions of pedogenesis in mountain pine formation on the slope of the north exposition.

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Photochemical Reactivity of Australian Floodplain River and Billabong Fulvic Acids upon Exposure to UV-B Radiation

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1. INTRODUCTION

The importance of lateral connectivity between the main river channel and adjoining floodplain is well recognized, and is fundamental to the Flood Pulse Concept (1). Floodplain-river interactions are important for exchanging particulate and dissolved organic matter. Suspended sediments and nutrients are transported from the river to the floodplain, while organic detritus, algal and microbial biomass is returned to the river channel (3).

Billabongs, or meander cutoffs, are an integral part of Australian floodplain landscapes, and are thought to play an important role in the exchange of resources between the main river ecosystem and terrestrial floodplain ecosystem (2). Little is known, however, regarding the chemical characteristics or quality of dissolved organic carbon (DOC) contained in these systems. In addition, photochemical transformations can alter chemical properties, which may subsequently alter the quality (in terms of bioavailability) of DOC. Of particular interest is the effect of increased levels of UV radiation, due to the hole in the ozone layer. Fulvic acid, which comprises the majority of DOC, is chromophoric in nature and may be particularly susceptible to photochemical transformations. It is not yet clear whether or not different sources of organic matter yield different photoproducts or rates of photoproduct formation at various wavelengths. This research examines the photochemical transformations of fulvic acids from different environments.

2. MATERIALS AND METHODS

Sample Site

Sampling sites were located on the Murrumbidgee River (35°1’10”S, 147°3’38”E), and adjoining Berry Jerry Lagoon (35°0’60”S, 147°3’54”E), on the semi-arid Riverine Plain, approximately 30 km west of Wagga Wagga, Australia.

Isolation of fulvic acid

Fulvic acids were isolated using Supelite™ DAX-8 resin. Resin (50.0 g) was added to 4.0 L filtered water sample (adjusted to pH 2.0 with conc. HCl) in a glass beaker (5.0 L), and stirred for 30 min using a stirrer bar. After settling, the supernatant was decanted, and the slurry collected in a 10 mm diameter glass chromatography column fitted with a frit. Humic and fulvic acids were eluted from the resin with 0.1 M NaOH, and acidified to pH 2.0 immediately with conc. HCl. The humic substances were then re-concentrated onto DAX-8 resin, and again eluted with 0.1 M NaOH. Humic acid was separated from fulvic acid by lowering the pH to <1.0 (conc. HCl), and allowing the humic acid to aggregate overnight at 4°C in the dark. The humic and fulvic acid suspension was then centrifuged at 3000 rpm for 10 min, and filtered through a Whatman GF/C filter to remove the humic acid. The fulvic acid fraction was adjusted to pH 2.0 with 1.0 M aqueous NaOH and re-adsorbed onto the DAX-8 resin. The DAX-8 resin was then rinsed with one bed volume of Milli-Q water, and fulvic acid desorbed with 0.1 M NaOH. Fulvic acid was immediately pass thourh an Amberlite IR-120 (H) resin, frozen, and freeze-dried.

Sample preparation

Fulvic acid was re-dissolved in Milli-Q water to give a final concentration of approximately 20 mg L^{-1} carbon. Solutions were adjusted to pH 7 using NaOH (0.1 M) or HCl (1.0 M), and 10 mL placed into 20 mL glass scintillation vials. UV transparent film (Cast Away Premium) was placed over the top of the vials to prevent loss of water, but allow UV-B radiation to enter the vials.

Sample irradiation

Fulvic acid solutions were irradiated using a UVB-313 (40W Q-Panel, USA) lamp for 14 days to give a total dose of 234078 mJ cm^{-2}.

3. RESULTS AND DISCUSSION

The Murrumbidgee River differed in physico-chemical characteristics to Berry Jerry Lagoon, having a lower DOC content (3.3 -10.9 mg L^{-1} for the river, and 22.5 – 114 mg L^{-1} for the lagoon during 2004), and lower chlorophyll-*a* content (0.02-0.04 mg L^{-1} for river and 0.20 - 2.16 mg L^{-1} for lagoon) than the lagoon. Sunlight was also able to penetrate further through the water column of the river than the lagoon (River $K_d = 2.90 \pm 0.83$; Lagoon $K_d = 25.58 \pm 9.66$).

Fulvic acids from the Murrumbidgee River and Berry Jerry Lagoon differed in chemical characteristics, with the river fulvic acid being more aliphatic in nature, having a higher apparent molecular weight and polydispersity (Table 1). For comparison, Suwannee River fulvic acid had a higher molecular weight and aromaticity than Murrumbidgee River fulvic acid.

Table 1. Change in chemical characteristics of fulvic acids after exposure to UV-B radiation

	M_w (Da)	Polydispersity	a_{CDOM} (380) (m^{-1})	SUVA (UV_{254}/DOC; L mg C^{-1} m^{-1})
		Initial		
Berry Jerry Lagoon	986 (32)	1.9 (2x10^{-2})	3.1 (3.0 x 10^{-2})	2.8 (6.0x10^{-3})
Murrumbidgee River	1409 (9)	2.4 (3x10^{-2})	11.8 (0.2)	4.1 (5.2x10^{-3})
Suwannee River	2280 (128)	2.0 (3x10^{-2})	9.8 (0.1)	4.8 (2.2x10^{-2})
		% Decrease after irradiation		
Berry Jerry Lagoon	-22.1 (1.4)	-12.3 (1.0)	-47.9 (1.5)	-33.8 (1.5)
Murrumbidgee River	-61.7 (0.7)	-23.8 (0.2)	-51.3 (0.4)	-47.8 (1.1)
Suwannee River	-37.8 (2.6)	-10.9 (1.1)	-19.6 (3.6)	-16.0 (2.8)

Note: Errors are shown in brackets and are the standard deviation of triplicate analyses.

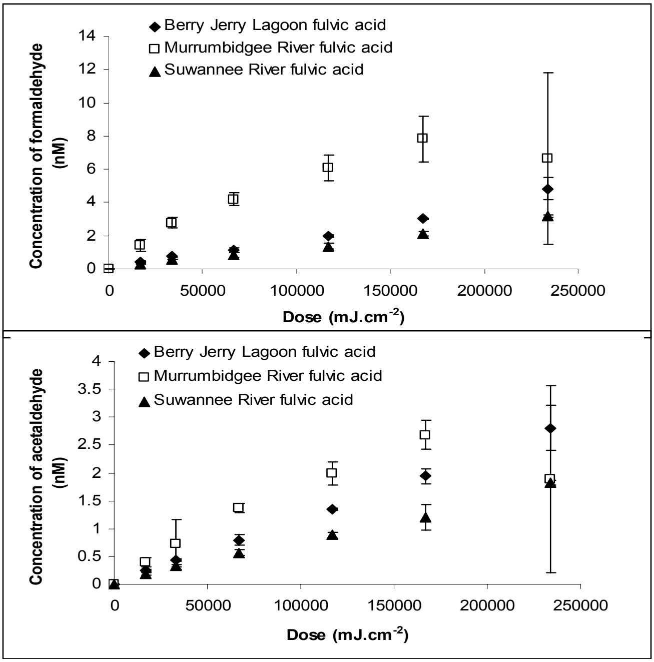

Figure 1. Formaldehyde and acetaldehyde formation from UV-B irradiated Berry Jerry Lagoon fulvic acid, Murrumbidgee River fulvic acid and Suwannee River fulvic acid. Error bars are standard deviations of triplicate analyses.

Exposure of fulvic acids to UV-B radiation resulted in a decrease in absorbing species, molecular weight, polydispersity and aromaticity. Typically Murrumbidgee River fulvic acid underwent greater change than Berry Jerry Lagoon fulvic acid (i.e. more photo-reactive). Upon exposure to UV-B radiation acetaldehyde and formaldehyde were produced in all samples (Figure 1), with Murrumbidgee River fulvic acid producing the highest concentration, followed by Berry Jerry Lagoon fulvic acid and Suwannee River fulvic acid.

4. CONCLUSIONS

Similar photochemical transformations were evident for fulvic acids from different environments, however, the degree of transformation varied. Murrumbidgee River fulvic acid typically underwent greater change due to UV-B radiation than Berry Jerry Lagoon fulvic acid. Photochemical transformations may alter the bioavailability of carbon, and therefore have implications for the quality of carbon between the two environments.

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Complexation of Mercury to Dissolved Organic Matter Isolated from an Anthropized Aquatic Ecosystem

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Keywords: mercury, complexation, humic/non-humic substances, speciation modeling

1. INTRODUCTION

The strong interactions of mercuric (Hg^{2+}) ions with dissolved organic matter (DOM) exert important controls on the mercury (Hg) fate, distribution and speciation in the aquatic systems. To obtain representative stability constants, we probed the binding affinity of Hg^{2+} with natural and urban DOM fractions of humic and non-humic substances.

2. MATERIALS AND METHODS

The competitive ligand exchange with solvent-solvent extraction (CLE-SSE) method was adapted for the determination of Hg^{2+} conditional stability constants (K'_{cond}) with the DOM fractions from the Seine Basin. The sampling sites consisted of the low urbanized Marne River (at Méry, upstream from Paris) and of the treated effluent of the Seine-Aval Wastewater Treatment Plant (~ 8 million equivalent inhabitants). The hydrophobic (HPO), transphilic (TPI) and hydrophilic (HPI) DOM fractions were isolated according to Croué (2004) by reverse osmosis concentration and DAX-8/XAD-4 resins adsorption. The CLE-SSE method utilizes two competitive ligands for Hg^{2+} binding, chlorides and DOM fraction, followed by water-methylene chloride extraction to separate the charged chloride Hg complexes from the neutral and organic Hg complexes (Hg-L). The concentrations of Hg^{2+} and chloride Hg complexes were assessed by applying the Winderemere Humic Aqueous Model (WHAM 6) to the measured concentrations of total dissolved Hg (HgT_D) in the aqueous phases. The K'_{cond} and L contents were determined using the Langmuir isotherm.

3. RESULTS AND DISCUSSION

At pH = 6.8, in the presence of 0.5 M chlorides and 1 µg L^{-1} DOM fraction, the inorganic Hg shifts from organic to aqueous phases with increasing $Hg(OH)_2$ additions (Figure 1). On this basis, the determined K'_{cond} values indicated that DOM fractions from the treated wastewater effluent contain extremely strong Hg-complexing ligands.

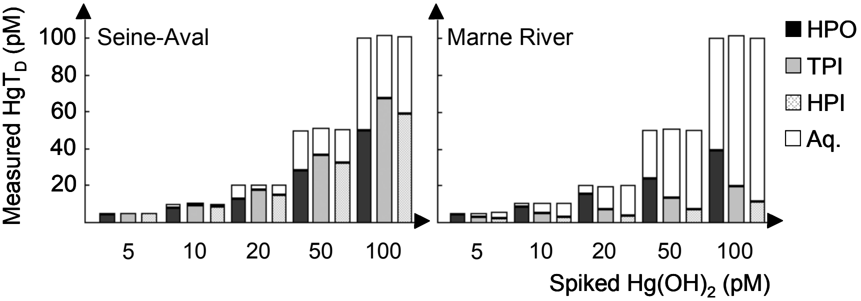

Figure 1. Water-methylene chloride Hg partitioning in the presence of hydrophobic (humic), transphilic and hydrophilic (non-humic) DOM fractions.

The non-humic DOM fractions (due to their high proportion in WWTP effluents and L contents) have a strong influence on the Hg-binding in the downstream from Paris. As a comparison, in the low urbanized area upstream from Paris, the DOM combined a stronger humic character and lower K'_{cond} values of the fractions (Table 1). The K'_{cond} values and L contents are consistent with sulfide and thiols containing ligands (as low-molecular weight compounds or as functional groups on DOM) that dominate the Hg^{2+} complexation.

Table 1. Conditional stability constants and Hg-binding ligand contents (nM mgC^{-1})

DOM origin	HPO (LogK'_{cond} ; L)	TPI (LogK'_{cond} ; L)	HPI (LogK'_{cond} ; L)
Seine-Aval	25.4 ± 0.1 ; 18.7 ± 0.2	25.1 ± 0.1 ; 50.1 ± 0.1	25.3 ± 0.1 ; 37.2 ± 0.4
Marne River	24.7 ± 0.1 ; 52.4 ± 0.3	24.4 ± 0.1 ; 19.2 ± 0.2	24.2 ± 0.1 ; 9.1 ± 0.1
Standards	SRHA (IHSS)	SRFA (IHSS)	Fluka Humic Acid
	24.4 ± 0.1 ; 61.2 ± 0.1	22.8 ± 0.3 ; 324.3 ± 1.2	24.7 ± 0.1 ; 8.3 ± 0.3

4. CONCLUSIONS

In the river Seine, a highly anthropized aquatic ecosystem, the DOM coming from urban discharge and particularly from WWTP in Paris conurbation is a source of non-humic strong Hg^{2+}-complexing ligands. These non-humic ligands can locally affect the Hg cycle in the Seine waters because ligands initially present in the upstream of Paris have lower affinity for Hg.

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Development and Validation of Antioxidant Capacity Assessment Protocol for Humic and Humic-Like Substances

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Keywords: antioxidant capacity, humic and humic-like substances

1. INTRODUCTION

Humic substances (HS) are considered to be involved in realization of various processes in soil and aquatic ecosystems (1, 2). The main feature of HS is their polyfunctionality as they can act as sorbents, soil conditioners, fertilizers, bioactive compounds etc. Due to their structure and functions HS have been widely explored for practical use. Nevertheless there is still little knowledge about quantitative characteristics of their biological activity. Therefore such parameters are of great importance. HS are known to take part in redox and radical processes and their antioxidant capacity (AOC) can be proposed as one of essential parameter for evaluation of their properties. Thus, AOC should be a quantitative characteristic of HS efficiency as reactive oxygen species scavenger or trap providing comparison of different HS preparations both for fundamental study and their application. The aim of this study was to develop standardized protocol for quantitative estimation of HS and humic-like substances antioxidant capacity.

2. MATERIALS AND METHODS

Standard and commercially available samples of HS including Suwannee River humic (SR-HA), fulvic acids (SR-FA) and dissolved organic matter (SR-DOM) and Aldrich coal humic acid (CHA-ALD) were chosen for development of antioxidant capacity measurement protocol. For verifying of the developed protocol two samples of humic-like substances (HLS) produced by basidiomycetes *Cerrena maxima 0275* cultivated on oat straw were used. HLS-45 and HLS-70 were isolated from nutritional media after 45 and 70 days of cultivation as described earlier (3).

Antioxidant capacity (AOC) of HS and HLS was measured as a decrease in the absorbance at 734 nm of radical-cation ABTS ((2,2-azyno-bis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt) in their presence. A water soluble analogue of vitamin E trolox (6-hydroxi-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a calibration standard. The obtained results were expressed in trolox molar equivalents per mg of sample that offers possibility of their easy comparison to AOC values for other objects (4). Measurements were carried out using Perkin Elmer lambda 25 UV-VIS spectrophotometer (USA). Generation of ABTS$^{\bullet+}$ radical-cation was made according to protocol of Re and co-workers (5).

HS and HLS AOC assessment was performed at three pH levels using 0.1M sodium citrate buffer solution (pH 3.75 and 4.25) and 0.1M potassium phosphate buffer (pH 6.80). The reaction was initiated by adding 100 μl of sample solution to 900 μl of ABTS$^{\bullet+}$ dissolved in suitable buffer followed by measurement of change of absorbance at 734 nm after 3 min. AOC for humics was measured over concentration range of 1-10 mg/l.

3. RESULTS AND DISCUSSION

Development of HS antioxidant capacity protocol requires determination of such conditions as pH of the reaction mixture and a range of HS concentrations where its relationship to AOC is linear. Also ABTS$^{\bullet+}$ stability should be determined in chosen matters.

Our findings showed that ABTS$^{\bullet+}$ exhibited maximal stability in deionized water. Increasing of reaction mixture pH led to gradual decrease of ABTS$^{\bullet+}$ concentration (Fig. 1).

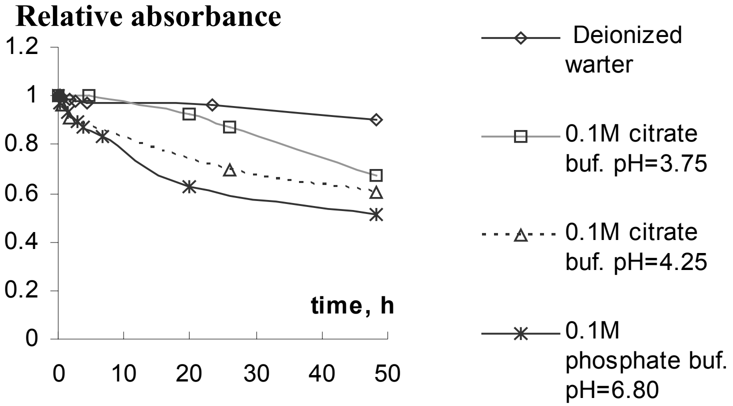

Figure 1. Stability of ABTS•+ radical-cation in different reaction mixtures. Relative absorbance is the ratio of optical density monitored in the course of experiment to initial value at 734 nm (D/D_0).

Concentration-dependent AOC over used concentration range was characterized by smooth, non saturating curves for all studied humics (Fig. 2A). At low concentrations of HS

(1-5 mg/l) AOC noticeably decreased along with increase in HS concentration followed by reaching plateau at about 6 mg/l. The exception was AOC measured at pH 6.8 where smooth decrease of that parameter was observed over concentration 1-10 mg/l.

Estimation of AOC at different pH demonstrated its increase with growing pH (Fig. 2B) what probably reflected processes of HS conformational rearrangements and dissociation. Besides mechanism of HS antioxidant action shifts from hydrogen atom transfer to electron transfer in neutral and alkaline pH could be proposed under such conditions (6).

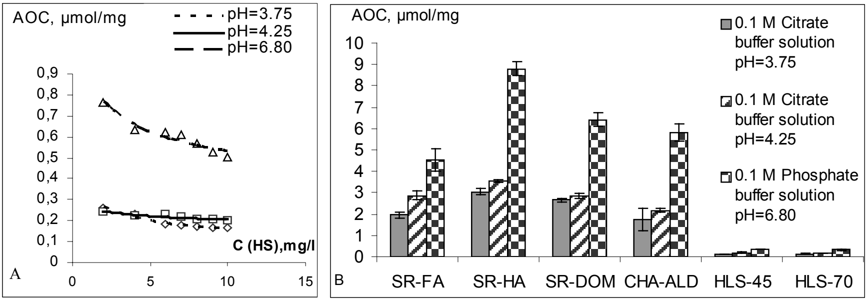

Figure 2. AOC of the studied HS and HLS at different concentrations (A; on the example of CHA-ALD) and pH level (B).

Values of specific AOC at pH 4.25 for HS varied from 2.18 to 3.56 μmol/mg what did not differ significantly from AOC of well known natural antioxidant dihidroquercetine (2.67 μmol/mg). At pH 6.80 those values increased to 4.54-8.79 μmol/mg that was corresponded to 6.77-12.29 μmol of trolox per mg of organic carbon (OC). The latter met well (7) where AOC range 4.38-26.57 μmol/mg of OC were reported for alkaline soil extracts. HLS have antioxidant capacity on the average 17-times lower in comparison to HS (Fig. 1B). This can be hypothesized by different functional groups content.

4. CONCLUSIONS

The trolox equivalent antioxidant capacity protocol has been developed for HS and HLS. The determined AOC was demonstrated to be concentration independent for both HS and HLS in the range of concentrations 4-10 mg/l. The results obtained at different pH levels showed significant increasing in HS and HLS antioxidant capacity with growing pH from 3.75 to 6.80. However, under neutral and particularly alkaline pH $ABTS^{\bullet+}$ was rather unstable, so mild acidic (pH 4.0-5.0) conditions could be recommended for HS and HLS antioxidant capacity determination.

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Dissolved Organic Matter in Urban Water: a Protective Role to Organisms

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Keywords: bioavailability, isolation, hydrophilic/hydrophobic organic matter, trace metals, speciation

1. INTRODUCTION

In aquatic systems, Dissolved Organic Matter (DOM) is an important component of the carbon cycle that controls the speciation, the bioavailability and the toxicity of trace metals (2). Studies dealing with DOM-metals interactions are mainly focused on Humic Substances (HS) (hydrophobic DOM). Nevertheless, the humic character of DOM is weaker in anthropized continental waters (about 30 % of DOC) because of various urban discharges (5; 7). Very few information are available concerning the Non Humic Substances (NHS)-metal interactions because of the very difficult isolation and purification.

The present study aims to evaluate the effect of DOM fractions particularly NHS isolated from Seine Basin: urbanized and low urbanized site (1) on the copper bioavailability and toxicity and (2) on the copper and lead speciation.

2. MATERIALS AND METHODS

DOM fractions were isolated from Seine-Basin following the reverse osmosis concentration and DAX-8/XAD-4 resins adsorption techniques (Figure 1) recently implemented (3; 6). The first site is the Marne River at Méry/Marne upstream from Paris which is considered as a slightly urbanized site. The treated effluent of Seine-Aval WasteWater Treatment Plant (WWTP) was also sampled. This WWTP collects more than 70% of dry weather flows (combined sewer) of Paris and its suburbs (≈ 8 million equivalent inhabitants) and contributes for 15% of the Seine River flow in dry weather. These effluents are treated in this WWTP by primary settling and aerobic activated sludge. The Seine River downstream from Paris was also sampled at Andrésy and Méricourt. One

IHSS standard fulvic acid (Suwannee River Fulvic Acid SRFA 1S101F) was used like “natural organic matter reference” for the following investigations.

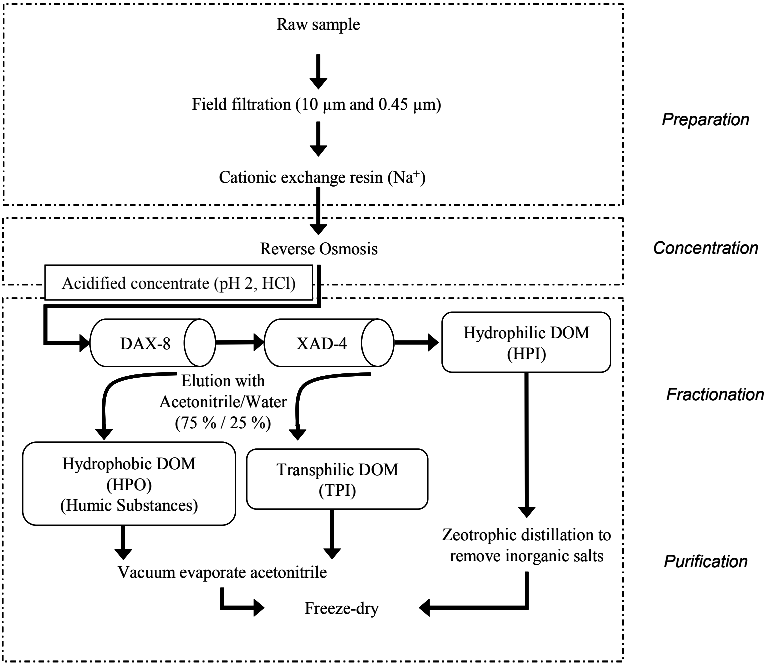

Figure 1. Comprehensive isolation protocol of DOM: The RO/DAX protocol.

Acute 24-h immobilization assays on a crustacean (*Daphnia magna)* were carried out according to the standard procedure ISO 6341 (International Standard Organization, 1993) in order to evaluate the effect of each isolated DOM fraction on copper toxicity. The results are expressed as EC50: the total copper concentration which immobilizes 50% of the Daphnia population. Copper bioavailability was also investigated by bioaccumulation tests on bryophytes (*Fontinalis antipyretica*) following the rate of disappearance of copper in solution in presence of each DOM fractions.

Copper titrations of each DOM fraction were performed by ionic selective electrode at three pHs and at constant ionic strength. Lead complexation was investigated by a new electroanalytical technique AGNES: Absence of Gradients and Nernstian Equilibrium Stripping (4) at three pH and constant ionic strength. Data were modeled by NICA-Donnan model (1) in order to determinate binding capacities and binding constants of each isolated DOM fraction.

3. RESULTS AND DISCUSSION

Concerning copper toxicity, results highlight a similar role of DOM fractions from treated wastewater compared to reference Suwannee River Fulvic Acid (SRFA) (Figure 2). The Non Humic Substances (transphilic and hydrophilic organic matter) from wastewater effluent present a protective role on copper toxicity to *Daphnia magna* because of their high proportion: 65% of DOC of raw sample (7). HS, e.g. hydrophobic organic matter (HPO) isolated from Méry/Marne (Marne River upstream from Paris), seem to have a significant low protective effect compared to DOM from effluent of wastewater.

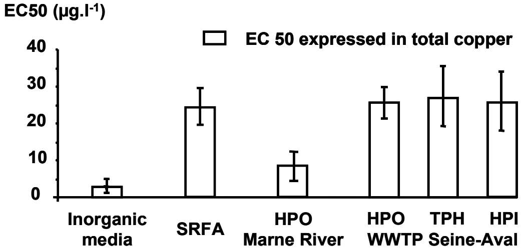

Figure 2. Copper EC50 obtained for each media.

Results of copper bioaccumulation on *Fontinalis antipyretica* (Table 1) reveal a low and similar bioaccumulation rate in presence of SRFA and treated wastewater DOM and higher rates in presence of DOM from Marne River. Copper bioaccumulation on *Fontinalis antipyretica* and copper toxicity on *Daphnia magna* present the same trends: a key and protective role of DOM from wastewater effluent particularly NHS (because of their high proportion) and a lower protective role of DOM (HPO + TPI) from Méry/Marne which is a slightly urbanized site.

Table 1. Influence of DOM fractions on copper bioaccumulation rate on *Fontinalis Antipyretica*

Sample	Fraction	Copper bioaccumulation rate ($\mu g.l^{-1}.h^{-1}$)
Inorganic media	-	4,30 ± 1,93
Wastewater effluent	HPO	0,27 ± 0,07
	TPI	0,28 ± 0,05
	HPI	0,28 ± 0,03
Marne River (upstream from Paris)	HPO	2,12 ± 0,21
	TPI	2,29 ± 0,44
Suwannee River	Fulvic Acid	0,56 ± 0,14

Concerning the trace metal (Cu, Zn and Cd) bindings, results revealed significant differences about binding site number and binding constants according to the DOM fraction nature and the sample origin.

4. CONCLUSIONS

In conclusions, Non Humic Substances in anthropized aquatic systems play a key and protective role on copper bioavailability to *Daphnia magna* and *Fontinalis antipyretica*. HPO and TPI fraction isolated from Marne River upstream from Paris seem to have a low protective effect compared to DOM from wastewater effluent. Binding capacity and stability constant vary significantly according to the DOM fraction nature and the sample origin.

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Physiological Activity of Humic Substances from Bark Compost

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Keywords: compost, humic, phytochrome

1. INTRODUCTION

It is well known that bark composts have a stimulating effect on growing plants. In a recent study, demonstrated that indoleacetic acid (IAA) is present in humic substances but its concentration is not sufficient to justify its biochemical activity in plant systems. The uncertainty about the mechanism by which humic substances stimulate plant biochemical activities is partly due to their chemical heterogenity and the difficulty of their characterization. Thus, the attempts to relate humus structure to biochemical activity have produced contradictory results (2).

The aim of this study is to determine the hormone and hormone-like effect of humic fractions with a standard seedling test using *Lactuca sativa* (evaluated with polyphenoloxidase, peroxidase, and dehydrogenase activities in humic extracts)

2. MATERIALS AND METHODS

The samples were prepared by composting for -14 days in a thermostat 25-mm-crushed barks added with 2g/dm^3 NPK (20:20:20) and various additives (Table 1). Microbial inoculation was done with conidia of *Trichoderma viride* - 5x10^9/100g*;and T.harzianum* 5x10^9/100g .The substrate humidity was 70% and the temperature was 28^{o}C in the first 3 days, 35^{o}C the second 3 days, and 38^{o}C until the end of the process.

The level of humification of bark was determined according the method of Kononova-Belchikova (1). The polyphenoloxidase, peroxidase, and dehydrogenase activities were measured in humic substances extracted with 0,1 N NaOH at a ratio extractant : substrate = 10:1. The physiological effect of the humic substances extracts was determined by a test using hypocotyls of lettuce on the controls: Barks 1; GA$_3$ and coffee compost with *T.harzianum*. “Magic Green” is a commercial product of ROMB Ltd, Bulgaria, proposed for increasing the enzyme activity in soilless substrates.

Table 1. List of additives used

No	Additive
1	Background
2	N-zeolite (10 g/dm^3)+*T.viride**
3	N-zeolite (10 g/dm^3)+*T.harzianum**
4	Biostabilized lignite with *T.viride* - 10 g/dm^3
5	Lignite – Stanyancy deposit - 10 g/dm^3
6	Poultry manure - 10 g/dm^3
7	Poultry manure - 10 g/dm^3+ *T.viride*
8	Poultry manure10 g/dm^3+ *T.viride* + bio-soluble lignite 10 g/dm^3
9	Control + Magic Green 80 mg/dm^3
10	N-zeolite (10 g/dm^3)+ *T.harzianum* +Magic Green 80 mg/dm^3
11	N-zeolite (10 g/dm^3)*T.harzianum**+ Magic Green 80 mg/dm^3+10ml/dm^3 5% extract from bio-soluble lignite

3. RESULTS AND DISCUSSION

The data presented in Table 2 show the level of humification of barks in the various treatments. Humification is better in the cases when lignite is present in the media; however this is an effect of the additional humic substances from coal. The composts loosed the acute pine odor.

Table 2. Humification degree of bark compost

№	Total org. C	Total N	C/N	Σ Ch+Cf	Ch	Cf	Ch+Cf % from total org. C	Ch	Cf
	% from the sample mass							% from Σ Ch+Cf	
1	34,40	0,85	40,47	2,63	1,14	1,49	7,65	43,35	56,65
2	29,20	0,96	30,42	2,26	1,31	0,95	7,74	57,96	42,04
3	28,99	0,82	35,35	2,16	1,18	0,98	7,45	54,63	45,37
4	31,32	0,89	35,52	2,59	1,54	1,05	8,27	59,46	40,54
5	34,43	0,98	35,13	3,46	2,28	1,18	10,05	65,90	34,10
6	33,45	0,95	35, 21	2,32	1,35	0,97	6,94	58,19	41,81
7	31,20	1,04	30,00	2,42	1,34	1,08	7,76	55,37	44,63
8	31,42	1,10	28,56	2,46	1,19	1,27	7,83	48,37	51,63
9	34,00	1,35	25,19	2,40	1,37	1,03	7,06	57,08	42,92
10	30,30	1,15	26,35	2,38	1,47	0,91	7,85	61,76	38,24
11	34,84	096	36, 29	2,76	1,55	1,21	7,92	56,16	43,84

Ch-organic carbon of humic acids; Cf-organic carbon of fulvic.

Table 3. Enzyme activity of compost

№	Polyphenoloxidase mgPPG/ 100g/30’	Peroxidase mgPPG/ 100g/30’	Dehydrogenase mg formazan/1g/ 24h
1	37	46	0,12197
2	-	70,8	0,06628
3	-	48,3	0,07823
4	7,2	97,4	0,09285
5	-	27,1	0,16457
6	8,1	69	0,18027
7	0,5	45,1	0,10124
8	0,5	32	0,03864
9	5,9	46,9	-
10	8,6	100,6	0,1166
11	11,3	114,6	0,148

The data presented in Table 3 show the influence of composting accelerators on enzyme activity of humic-like substances. Microfungal influence using the background of zeolite has no suitable effect, whereas in the presence of Magic Green or bio-soluble lignite *T.harzianum* increased almost twice the peroxidase activity of the extract.

Table 4. *Lactuca sativa* hypostyle test-mm of hypostyls after germination

<table>
<tr><td colspan="10">Dark D-mm of hypostyls after germination</td></tr>
<tr><td>D Control</td><td>D GA$_3^*$</td><td>D 1**</td><td>D 2</td><td>D 3</td><td>D 4</td><td>D 9</td><td>D 10</td><td>D 11</td><td>D Coffee</td></tr>
<tr><td>32,00</td><td>46,40</td><td>34,60</td><td>33,60</td><td>33,00</td><td>38,40</td><td>32,40</td><td>38,60</td><td>39,80</td><td>30,80</td></tr>
<tr><td colspan="10">Red light R-mm of hypostyls after germination</td></tr>
<tr><td>R Control</td><td>R GA$_3$</td><td>R 1</td><td>R 2</td><td>R 3</td><td>R 4</td><td>R 9</td><td>R 10</td><td>R 11</td><td>R Coffee</td></tr>
<tr><td>32,80</td><td>51,20</td><td>40,00</td><td>41,40</td><td>35,40</td><td>34,60</td><td>39,60</td><td>45,20</td><td>42,80</td><td>39,60</td></tr>
<tr><td colspan="10">Difference in mm of hypostyls after germination R-D-%</td></tr>
<tr><td>Control</td><td>GA$_3$</td><td>% 1</td><td>% 2</td><td>% 3</td><td>% 4</td><td>% 9</td><td>% 10</td><td>% 11</td><td>Coffee</td></tr>
<tr><td>1,01</td><td>10,29</td><td>16,33</td><td>25,53</td><td>9,64</td><td>-8,72</td><td>23,90</td><td>19,20</td><td>8,30</td><td>29,11</td></tr>
</table>

D+/ limits=5, 09; F= 7, 06; P=0,000; +/-limits=4, 64; F=11, 68; P=0,000

*20 ppm.

**All extracts are 1000 times water diluted.

The data presented in table 4 show that the humic-like extracts influence strongly the phytochrom activation with red light. *T. viride* and *T. harzianum* influence hypocotyls growth like gibberelic acid. Bio-soluble lignite forms humic substances with auxin-like effect. Magic Green promotes the activity of humic-like compounds and their effect on phytochrom activation.

4. CONCLUSIONS

The biotransformation of organic matter in bark composts was strongly affected by the microfungal treatment in combination with the commercial product Magic Green.

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The Probable Mechanism of Biological Effect of Humic Substances

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Keywords: humic, biological effect, biological activity

1. INTRODUCTION

Humic substances (HS) are main component of soil organic matter. One of major property of HS is their biological activity (1, 4, 6-9). There are most amounts of scientific publications, which are devoted HS biological effect on growing and development of green vascular plants. The HS biological effect on plants is connected with that that they, penetrating into plants (but unnecessary in cytoplasm), participate in different biochemical and biophysical processes (6-9, 12, 14, 17). Optimization of crops is an important direction of economic people activity. Understanding of biochemical ways of interplay between plant and soil, in which HS play a key role, will allow to develop effective crop technologies and to act upon agricultural plants under stress by non-root treatment of HS solution (3).

In this connection, the aim of this publication was to present a conceptual model, reflecting a HS biological effect on biochemical and biophysical plant processes. Model is built on the base of scientific literature analysis and own experimental data.

2. DESCRIPTION MODELS

Developing scientific positions, connected with this question, I worked up the new conceptual model of HS participation in biochemical and biophysical processes, occurring in green vascular plants (Fig. 1). In accordance with the proposed model, biological activity HS is determined by: 1) the presence of the varied functional groups, 2) the colloidal characteristic and 3) the material constitution.

Varied functional groups as positively (e. g. amides, amines, azogroups, imines etc.), so and negatively charged (e. g. carboxylic, carbonyl, hydroxyl groups etc.) are presented in humic substances (5, 9, 16), therefore HS – polyfunctional polyampholites. Although HS are polyampholites, they can be considered among acidosic colloidal micelles (10). Totality of functional groups determines the reactionary ability of these compounds a whole. Humic substances are donors and acceptors of electrons and hydrogen ions; therefore they can participate in different redox-reactions, including reversible ones too (18). Consequently HS inputting into plants can optimize a respiration and less photosynthesis as a result of that a biosynthesis is quickened (1, 4, 6-9, 12, 17).

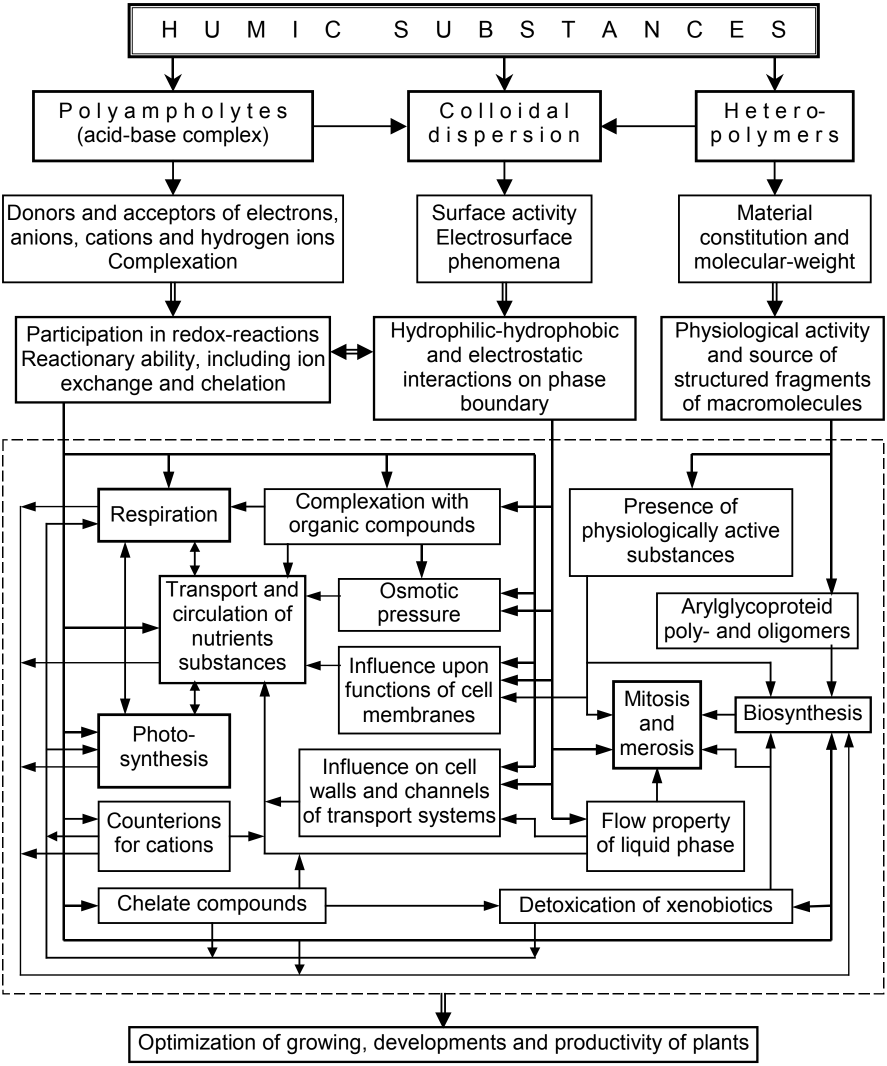

Figure 1. Participation of humic substances in plant metabolism.

Reactionary ability of HS is connected with possibility of complexation with macro- and microelements (1, 6, 7, 15). These compounds are easy transported both into plants, and inside ones. Besides reactionary ability of HS is showed by influence upon an osmotic pressure, ATPase activity, synthesizing of a transport proteins, bioelectrical reactions, intercellular diffusion relationship, counteraction to alkalization (in this case the decrease of absorption of NO_3^- and the increase of absorption of NH_4^+ can be observed), synergetic or antagonistic interactions with enzymes and some growth regulators of plants, detoxication of xenobiotics and etc. (4, 6, 12). Humic substances can lead to optimization of ratio between organic and inorganic anions and as a result of decrease a content of nitrates in plant product too. As well as HS can participate in some other processes.

From the position of colloid chemistry, HS are colloidal dispersions and so they have electrosurface and surface activity properties (10-11, 14). Electrosurface phenomena of HS are explained a double electrical layer on surface of dispersed phase. These phenomena of HS are realized in sorption properties, ion-exchange ability and electrostatic interactions. Humic substances as surfactant species can decrease a surface tension of water solution. Exactly this property of HS is indicative. The more surface activity properties of HS, the higher biological effect of ones. Humic substances inputting into plants make hydrophilic the walls of transport system channels and make easy a pinocytosis. They influence upon bioelectrical reactions, viscosity of protoplasm, surface tension of solution, properties of cell wall, penetrability of intercellular canaliculi, ionophore effect, selectivity of cell membrane and etc. (1, 3, 6, 7, 12-14). The higher a speed of transport and circulation of nutrients in plants, the more a speed of photosynthesis, the more an assimilation of mineral phytonutrients from soil. In result the growth and evolution of crops is improved, and the use factor of fertilizers is decreased.

From chemical point of view HS are heteropoly(oligo)mers, which are arylglycoproteid poly- or oligomers with the different molecular mass. That is why HS can be a source of structured fragments of organic macromolecules after the consumption and assimilation HS by plants. In this case plants "economize" energy by using the organic molecules (14). Biological effect of HS can be conditioned by the action like phytohormones and/or contents of physiology active compound in its composition (5-7, 12, 14) too. Besides HS can increase a value of mitotic index (6), influence on biosynthesis (2, 4, 12, 17). The biochemical composition of HS determines nutritive value, the molecular mass of components — food accessibility these compounds for plants. Factions of HS with the various molecular masses are differed by both properties and effects on plants (6, 12).

3. CONCLUSIONS

In the most general type a model is universal for green vascular plants and it illustrates that the biological effect of HS is integral representation of their properties. The biological activity HS is determined by: (i) presence the varied functional groups, (ii) colloidal characteristics and (iii) material constitution.

ACKNOWLEDGEMENTS

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Chemical and Optical Characteristics of HS Isolated from the South Moravian Soils

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Keywords: humic substances, synchronous fluorescence and energy dispersive X–ray spectroscopy

1. INTRODUCTION

The reactivity of humic substances (HS) is given by their functional group chemistry and microstructure, which are in turn influenced by the composition of the surrounding materials. They possess a highly complex and refractory character, and have the capacity for diverse chemical and physical interactions in the environment (1). Therefore determination of HS and humic acids (HA) spectral characteristics performed fast and convenient method for their evaluation. Synchronous fluorescence spectroscopy (SFS) can characterize content of condensed aromatic ring systems, bear electron-withdrawing substituents, such as carboxyl and carbonyl groups in order to investigate its function, origin and fate in soils (1) and (2).

Energy dispersive X–ray spectroscopy (EDXS) is capable to identify which elements are actually present under electron probe. The aim of our work was to compare humic substances quality in different soil types.

2. MATERIALS AND METHODS

Soil samples were collected from the topsoil of Modal Chernozem (locality Bratčice), Haplic Luvisol (locality Veverské Knínice), and Gleyic Stagnosol (locality Kameničky). We followed total carbon content (TOC) by oxidimetric method (3), fractional composition of HS according to Kononova-Belchikova method (4). Isolation of HA was made according to IHSS method (5). Elementary analysis of isolated HA was kindly made in Institute of Rock structure and Mechanics of the ASCR in Prague. Standard methods of Carlo Erba and elementary CHNS/O analyser - Thermo Finnigan were used. The non-destructive optical methods have been used for HA characterization. EDX spectra were measured using energy–dispersive X-ray spectrofluorimeter XEPOS to detect elements in situ. SFS spectra we measured by Spectrofluorimeter AMINCO BOWMAN Series 2, Thermospectronics, bandpass of both monochromators 4 nm, temperature 20°C, and constant difference ($\Delta\lambda=(\lambda_{em.}-\lambda_{ex.})$=20nm) between excitation and emission monochromators and were corrected at 845 V.

3. RESULTS AND DISCUSSION

Fractional composition of different soil types is listed at the Table 1. Results showed the highest amount of HS in Gleyic Stagnosol following by Modal Chernozem and Haplic Luvisol. Quality of HS was the highest in Modal Chernozem followed by Haplic Luvisol and Gleyic Stagnosol. Gleyic Stagnosol HS contained the highest amount of FA, which is typical for this soil type. Humification degree was middle for all samples (Table 1). Elemental composition of isolated HA is given at the Table 2. The highest carbon and oxygen content was found in Modal Chernozem HA, which corresponded with literature data (1). SFS spectra measured in mixture of 0.1M $Na_2P_4O_7$ and 0.1M NaOH are listed at Fig. 1. All samples exhibited the presence of five main spectral peaks at $\lambda_{ex.}$/ $\lambda_{em.}$: 467/487, 481/501, 492/512, 450/470, 339/359. Maximum relative fluorescence intensity gave the sample of Gleyic Stagnosol at 487 nm (the main peak). Lower relative fluorescence intensity gave Haplic Luvisol and Modal Chernozem at the same wavelength. We suppose that emission peaks at 359 nm and 419 nm were affected by the FA presence in the alkali extract. High amount of FA was typical for Gleyic Stagnosol (see Table 1, Fig. 1). The main peak (487 nm) was shifted to the longer wavelengths, which was caused by the polyaromatic moieties. Peaks at the lower wavelengths (<450nm) indicated the presence of simple phenolic compounds. Our results were compared with Elliot HA standard (Fig. 1) and the relative fluorescence intensity indexes were calculated. Correlation between optical indexes and fractional composition was found. EDX spectroscopy was used to investigate elemental composition of HA samples as a complex. Modal Chernozem HA and Haplic Luvisols HA had practically the same X - ray spectra (Fig. is not shown). Both samples contained by Fe, Ti, Al, Si, S and Cl. Gleyic Stagnosol HA contained much more Fe, Ti, Al, Cu, Zn, As, S and Cl (Fig. is not shown).

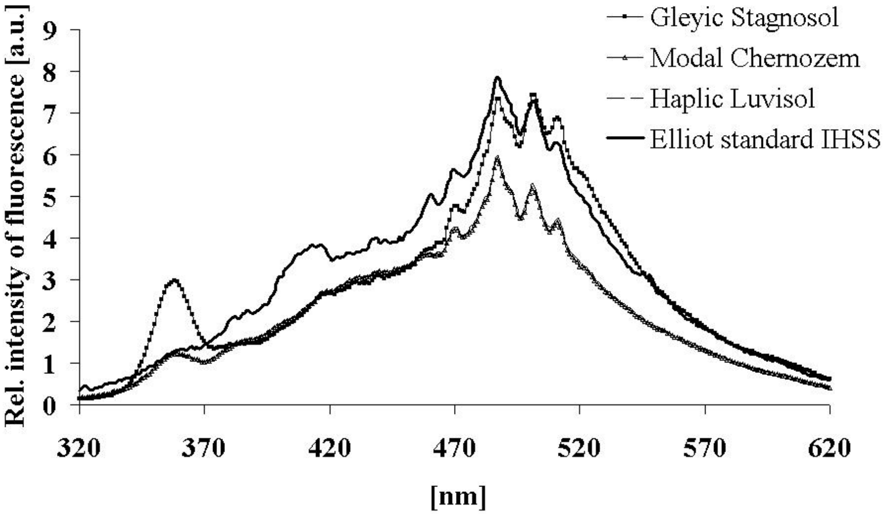

Figure 1. SFS spectra of HS isolated from different soil types.

Table 1. Total carbon content (TOC), fractional composition of HS and humification degree (HD) in selected soil types (numerator: in mg/kg, determinator: in % of TOC)

Soil Types	TOC (%)	HS sum (mg/kg)	HA sum (mg/kg)	FA sum (mg/kg)	HD (%)	HA/FA
Modal Chernozem	1.61	5.0 31.1	4.0 24.8	1.0 6.2	24.8	3
Gleyic Stagnosol	3.65	16.5 45.2	9.0 24.6	7.5 20.5	24.6	1.4
Haplic Luvisol	2.1	5.0 23.8	3.0 14.3	2.0 9.5	14.3	1.5

Table 2. Elemental composition of isolated HA in atomic %

SAMPLE	%C	%H	%N	%O
HA – Modal Chernozem	37.9	39	2.9	20
HA – Gleyic Stagnosol	36	42.4	3	18.4
HA – Haplic Luvisol	34.5	43	2.7	19.7
HA – Elliot standard	44.1	33.7	2.7	19.4

4. CONCLUSIONS

SFS spectra showed that the spectral behaviour (shape of curve) depends on fractional composition of HS. The relative fluorescence intensity indexes correlated with HA/FA ratio. Amount of HS was decreasing in order: Gleyic Stagnosol > Modal Chernozem > Haplic Luvisol. The relative fluorescence intensity suggested the highest values in Gleyic Stagnosol. EDX spectroscopy allowed us a quick, direct quantification of different elements in HA molecule.

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Relationship between Spectral and Photosensitizing Properties in Bulk and Fractionated Humic Substances

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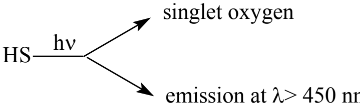
Keywords: humic acid, fractionation, fluorescence, photosensitizing, singlet oxygen

1. INTRODUCTION

Size fractionation of humic substances is often performed in order to study the change of one or other structural characteristics or properties as a function of the macromolecules weight and to have a better insight into the basic constituents or functional groups distribution. It may be also a way to draw a parallel between two properties or between structural characteristics and a property when the changes of both among fractions are similar. It was our purpose: we aimed to show that photosensitizing properties, i.e. the ability to generate singlet oxygen under light excitation, is linked to the emitting properties at long wavelength.



Two types of fractionation was used. A Chernozem HA was fractionated in three fractions using a two-step method: first size exclusion chromatography (SEC) in the presence of denaturing compounds and second polyacryacrylmide gel electrophoresis (PAGE). A peat HA was fractionated by tangential ultrafiltration into seven nominal molecular weight (NMW) fractions which were purified by dialysis (0.5 kDa) [1]. Separated fractions were further compared for their emitting properties and for their ability to generate singlet oxygen under light excitation. The photosensitizing properties were investigated using furfuryl alcohol as a probe molecule to scavenge singlet oxygen.

2. MATERIALS AND METHODS

Humic acids were extracted from the A horizon (10-20cm) of typical chernozem soil (Kursk region, Russia) and from an Irish peat (1). SEC-Page fractionation of Chernozem HA was described elsewhere (2). Three fractions were obtained: A, B and C+D showing distinct NMW and electrophoretic mobilities. Seven fractions with NMW using tangential ultrafiltration device with cellulose regenerated filters were obtained from peat (3). Finally, each fraction was dialysed (0.5 Da cut-off) against deionised water and freeze dried. We got the following NMW cut-offs: 0.5-5.0 Da ($HA_{0.5-5}$); 5.0-10 Da (HA_{5-10}); 10-20 Da (HA_{10-20}); 20-50 Da (HA_{20-50}); 50-100 Da (HA_{50-100}); 100-300 Da ($HA_{100-300}$); > 300 Da ($HA_{>300}$).

Furfuryl alcohol (FFA) was provided from Aldrich. Stock solutions (10^{-3} M) were stored at 4°C and their level in furaldehyde was checked every day. Water was purified using Millipore milli-Q system (Millipore αQ, resistivity 18MΩcm, DOC<0.1 mg/L).

Excitation Emission Matrix (EEM) spectra were recorded on a Perkin-Elmer LS-55 luminescence spectrometer that was equipped with a xenon excitation source. Excitation and emission slits were set to 10 and 5 nm band pass, respectively. Correction for instrumental configuration was made using the calibration data for excitation and emission factors provided by the manufacturer. In order to minimize the excitation inner filter effect, an absorbance of 0.100 ± 0.005 at 300 nm was chosen for all the samples solutions.

Irradiation experiments were conducted in a device equipped with polychromatic lamps simulating solar light, in a pyrex reactor. HLAs or fractions were irradiated with furfurylic alcohol (FFA) at an initial concentration of 10^{-4} M. All the solutions were buffered at pH 6.5. Photochemical reactions were investigated at a constant initial absorbance of 0.1 at 300 nm. FFA loss was monitored by HPLC. For all samples, FFA loss followed a first order kinetics. The rate coefficient was measured by plotting $\ln(c/c_0)$ vs t, where c and c_0 represent FFA concentrations at t and 0, respectively.

3. RESULTS AND DISCUSSION

In the case of Chernozem HA and SEC-PAGE fractions, the rate of FFA loss was significantly higher for C+D fraction than for A or B fractions (Table 1). Differences in rate coefficient values would be even more drastic if they would be corrected for the mass and normalized at a constant mass for all fractions. Indeed, fraction C+D was about twice more absorbing than fractions A and B (it means that when one works at a fixed absorbance, fraction C+D is less concentrated in organic carbon than other fractions). In fluorescence, important differences were also observed between fractions: only fraction C+D was emitting at $\lambda > 450$ nm (4).

Table 1. Rate coefficient, k. Chernozem HA and fractions

Sample	HA	Fraction A	Fraction B	Fraction C+D
$k/10^{-6}\ s^{-1}$	35	5.7	4.7	72

In the case of peat HA and its fractions, results are summarized in Table 2. The rate coefficient is the lowest for $HA_{>300}$ and the highest for $HA_{50\text{-}100}$. All the samples are emitting within the wavelength 398-540 nm, but again $HA_{>300}$ is the less emitting. We plotted k *vs.* I. The correlation coefficients were quite good for I_{498} and I_{540} (0.890 et 0.898, respectively) while fair for I_{398} and I_{430} (0.728 and 0.482, respectively).

Table 2. Rate coefficient, k, and emission intensities at several wavelengths for excitation at 300 nm. Peat HA and fractions

Sample	$k/10^{-6}\ s^{-1}$	I_{398}	I_{430}	I_{498}	I_{540}
HA	6.5	89.3	103.6	58.2	76.46
$HA_{0\text{-}5}$	8	131.6	121.5	62.5	78.2
$HA_{5\text{-}10}$	8.11	89.3	92.7	69	82.8
$HA_{10\text{-}20}$	6.94	107.6	95.7	63.5	71.6
$HA_{20\text{-}50}$	5.94	58.8	67.3	52.2	55.9
$HA_{50\text{-}100}$	9.38	87.5	113	86.8	102
$HA_{100\text{-}300}$	5.76	57.5	61	36	42
$HA_{>300}$	4.72	38.9	46.5	21.4	28.2

Thus, whatever the studied HA and the type of fractionation the capacities to generate singlet oxygen under light excitation and to emit at long wavelength are correlated. Either these properties arise from the same types of constituents, or they arise from different constituents linked together.

4. CONCLUSIONS

With both HAs and both types of fractionation, it appears that there is a good correlation between the ability of the fraction to generate singlet oxygen upon irradiation in polychromatic light and the property to emit at long wavelength (above 450 nm). This result will be discussed.

ACKNOWLEDGEMENTS

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Diffusivity of Cu^{2+} in the Humic Gel

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Keywords: humic, diffusion, sorption

1. INTRODUCTION

Sorption ability towards common groups of pollutants is one of most valuable and promising properties of humic substances. Many works concerning sorption capacities of solid humic acids and/or humic sols have been published (1). Recent papers of presenting authors focus on the study of HA in a form of gel (2). Sorption and complexation of different substances on humic acids influence their transport in humic gel, namely affect a value of effective diffusion coefficient (3).

The gel form of HA is easy to prepare, suitable for the exploration of transport phenomena (samples can be prepared in defined size and shape, which is necessary for mathematical modeling of observed processes) and mainly it simulates natural conditions well, because HA are usually found in highly humid environments (water sediments, peat etc.) and thus in the swollen form.

Cupric ion is well–known for its high affinity for humic substances (4). Besides this the HA–Cu^{2+} binding is among the highest strengths. Therefore and also because of easy quantification of copper content by means of spectroscopy, the cupric ions have been chosen as a model metal ion for this work.

2. MATERIALS AND METHODS

Methods of isolation and purification of humic acids from the South- Moravian lignite are listed in detail elsewhere[2].

For the preparation of humic gel, solid HA were dissolved in 0.5 M NaOH in solid-to-liquid ratio of 8 g/L. The gel was then acquired by a precipitation of sodium humate by means of an acidification with HCl up to pH below 1. The mixture was centrifuged, supernatant was discarded and gel was washed by deionized water and centrifuged repeatedly up to content of Cl^- in a supernatant undetectable by an addition of 1% $AgNO_3$. Resulting gel contained about 86% of water.

Several methods have been chosen to determine diffusion coefficient for Cu^{2+} diffusing in humic gels.

In Half-plugs method diffusion proceeded in a diffusion couple formed by 5 cm long cylindrical sample of humic gel with constant concentration of previously diffused Cu^{2+} connected with similar sample of Cu^{2+}– free humic gel. To stop a diffusion transport a diffusion couple was disconnected, gel samples were sliced by steel string and each slice was separately extracted by suitable agent and Cu^{2+} content in each extract was determined by means of UV–VIS spectroscopy.

An In-diffusion method focused on a time dependence of the Cu^{2+} diffusion flux from $CuCl_2$ solution with constant concentration (saturated solution with additional crystalline salt) into 5 cm long cylindrical gel sample.

The last method of Through-diffusion with constant concentrations in reservoirs was performed in following way: apparatus consisted of two horizontal cells separated by 3 cm long glass plug filled with humic gel. First cell was full of 1M $CuCl_2$ solution, the second contained deionized water. Both cells were continuously agitated with magnetic stirrers. In suitable time intervals, pH, specific conductivity and UV–VIS spectra of solution, initially containing pure water, were measured. After the end of experiment, the Cu^{2+} concentration profile in humic gel was determined by the same procedure as in previous methods.

3. RESULTS AND DISCUSSION

The initial conditions and corresponding solution of the Second Fick's law for all methods are listed elsewhere[4]. The result of this solution for the Half- plugs method and for in diffusion gives the equation for total amount of copper ions diffused across the border of unity area between half-plugs

$$m = 2\, c_0 \sqrt{\frac{D_{eff}\, t}{\pi}} ,$$

where c_0 stands for constant Cu^{2+} concentration at the plug interface (one half of the initial concentration in source plug) for the Half- Plug method, while it represents constant concentration at the solution/gel interface for the In- diffusion, t is time and D_{eff} represents the effective value of diffusivity.

As can be seen in following figures, according the theoretical equation, cumulative diffusion flux shows linear dependence on the square root of time for both methods. From the slope of linear regression, the value of D_{eff} was determined.

In the Through-diffusion method, the value of D_{eff}^{0} is determined, in which the reaction between Cu^{2+} and HA is not involved on the contrary of previous two experiments.

With the presumption of constant concentration gradient between cells (provided by high initial concentration difference) the steady- state diffusion occurs after an initial non-stationary period. The value of D_{eff}^{0} can be then calculated from an equation

$$D_{eff}^{0} = \frac{1}{A} \cdot \left(\frac{m}{t}\right) \cdot \left(\frac{l}{\Delta c}\right)$$

where ratio (*m/t*) is represented by slope of linear time dependence of cumulative diffusion flux, *l* is length of gel cylinder and Δc concentration difference between cells.

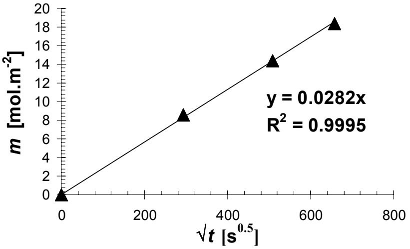

Figure 1. Time- dependence of the cumulative diffusion flux for the Half- plugs.

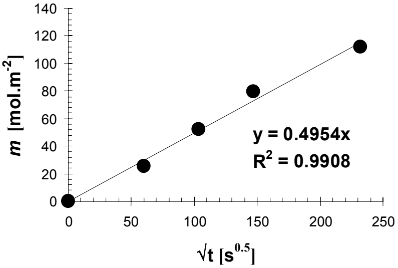

Figure 2. Time- dependence of the cumulative diffusion flux for the In- diffusion.

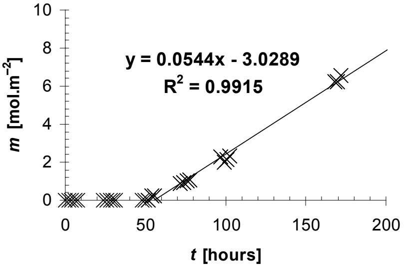

Figure 3. Time- dependence of the cumulative diffusion flux for the Through- diffusion

All calculated values of diffusivity are listed in Table 1 together with a value published for diffusion in water. It can be seen, that all determined values are of the same order with the value published for diffusion in water, which is expected for diffusion in hydrogels.

Table 1. Calculated values of Cu^{2+} diffusivity in the humic gel and a published value for the diffusion in water (5)

D_{eff}, Half-plug method	7.19×10^{-10} $m^2.s^{-1}$
D_{eff}, In- diffusion method	7.92×10^{-10} $m^2.s^{-1}$
D_{eff}^0, Through diffusion method	4.58×10^{-10} $m^2.s^{-1}$
D, diffusion in water	1.43×10^{-9} $m^2.s^{-1}$

4. CONCLUSIONS

All methods proved themselves valuable for simple laboratory determination of the diffusivity of cupric ions in the humic gel. Determined values of diffusivity were used in following diffusion experiments in humic gels.

ACKNOWLEDGEMENTS

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The Role of Hormonal System in Protection of Wheat Plants Induced by HUMI M

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Keywords: Humi M, salinity, abscisic acid, indoleacetic acid, cytokinins, mitotic index

1 INTRODUCTION

Salinity is one of the most world-widespread stress factors not only causing water deficit, but also having toxic effect on plants, which results in reduction in yield of agricultural crops (1). Growth regulators which are known to render growth stimulating and antistress effects may be used in order to increase stress-resistance and productivity of plants. Preparations created on the basis of humic acids are such growth regulators (2; 3). The present study is focused on the involvement of hormonal system in the protective effect of preparation of Humi M under salinity.

2. MATERIALS AND METHODS

The seeds of wheat (*Triticum aestivum* L., cv. Bashkirskaya 24) were treated with distilled water or Humi M (300 mg/kg of seeds) and grown for 3 days on filter paper moistened with distilled water under illumination of 400 μmol m^{-2} s^{-1} (PAR from mercury-arc luminous lamps) under 16-h photoperiod, at 21-23ºC. Humi M is a complex of sodium salts of humic acids and microelements (NVP “BashIncom”). Further experiments were carried out with 2-5-d-old seedlings grown hydroponically and their physiological and biochemical parameters were analyzed. Salinity was initiated by adding 2 % NaCl into the incubation medium containing 4-d-old seedlings. Plants pretreated by distilled water and incubated on the medium without 2 % NaCl served as control in all experiments. Mitotic index (MI) of root apical meristem was calculated as per cent of dividing cells (4). The contents of free abscisic acid (ABA), indoleacetic acid (IAA) and cytokinins (CKs) were measured in wheat plants by ELISA as described earlier (5). The analysis of lignin deposition in cell walls of basal region of roots was determined as describes (6).

3. RESULTS AND DISCUSSION

Humi M-pretreated wheat seedlings were characterized by significantly higher MI of wheat root cells during germination as illustrates in Figure 1a. This effect of Humi M on mitotic activity of root cells is likely to contribute to observed increase in plant length. Humic substances are known to possess auxin- and cytokinin-like activity (2; 3). It was important to study whether presowing treatment of wheat seeds by Humi M might change phytohormones content in wheat seedlings during germination. As seen in Figure 1b, Humi M-pretreated plants had 1.5 higher cytokinins concentration than control seedlings, but their IAA and ABA contents were almost the same as control.

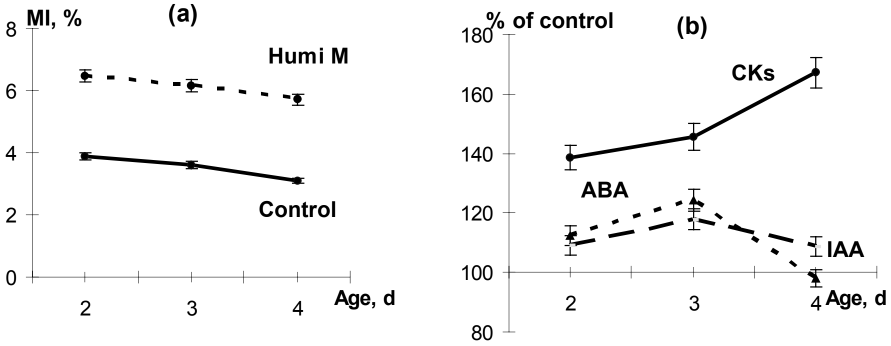

Figure 1. Dynamics of root apical meristem mitotic index (MI) (a) and abscisic acid (ABA), indoleacetic acid (IAA), cytokinins (CKs) concentration in untreated and Humi M-pretreated wheat seedlings during germination.

It is known that lignin deposition in cell walls of roots promotes strengthening of their barrier properties, which may reduce penetration of toxic sodium ions into the plants (7). Humi M increased lignin deposition in root cell walls of basal regions of 5-d-old plants after action of 2 % NaCl for 24 h indicating on protective effect of this regulator. Cytokinins have been shown to increase lignification (8) and this allows us to suggest that acceleration of lignification under salinity in plants pretreated with Humi M may be due to higher cytokinin content maintained in the plants in comparison with untreated plants. CKs are known to have a wide spectrum of effects, including growth activation and induction of plant stress-resistance (9; 10) and we suggested that both growth stimulation and protective effect of

Humi M on wheat seedlings under salinity is likely to be due to accumulation of endogenous CKs.

Protective effect of Humi M-pretreatment is also seen in Figure 2a, showing a decrease in detrimental effect caused by salinity on MI of wheat root apical meristem of plants pretreated with Humi M. MI of Humi M-pretreated 4-d-old wheat plants under salt stress was maintained close the control level. This was likely to result from ability of Humi M to diminish the degree of disturbance of hormonal balance caused by salinity. Figure 2 also shows that the balance of phytohormones of Humi M-pretreated wheat seedling reached the control level 24 h after withdrawal of salt unlike plants untreated with Humi M. Moreover, CKs concentration in Humi M-pretreated wheat seedlings during post-stress period exceeded the control level by 1.5-fold. All the data obtained allow to conclude that presowing treatment of wheat seeds by Humi M have an active effect on the hormonal system of plants due to regulation of CKs concentration in wheat seedlings.

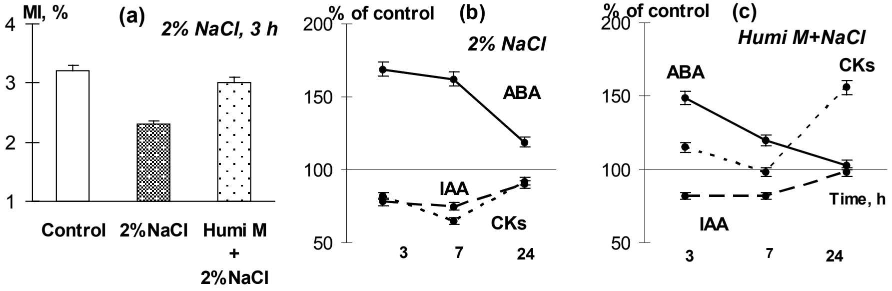

Figure 2. The effect of Humi M presowing treatment of wheat seeds and salinity on mitotic index (MI) of root apical meristem (a) and ABA, IAA and CKs contents (b, c) of 4-d-old wheat seedling under saline stress applied during 7 h and 24 h after stress.

4. CONCLUSIONS

The growth-stimulating and antistress effects of presowing treatment of wheat seeds by Humi M is caused by its ability to induce 1.5-fold CKs accumulation in plants. CKs, in turn, are likely to activate plant metabolism, which contributes to plant growth stimulation and development of protective reactions.

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Effects of Soil Organic Matter Conformation and Substrates Addition on the Fate of Xenobiotics in Soils

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Keywords: soil organic matter, sorption, bound residues

1. INTRODUCTION

Organic chemicals that are introduced into soils by human activities may persist, be translocated or are subject to complete mineralization or partial degradation in the pedosphere. For many chemicals, the parent compounds or their metabolites form nonextractable residues, mainly through interactions with the native soil organic matter (SOM). At present, there is only limited knowledge about the formation and binding mechanisms of these residues which in turn complicates their risk assessment.

There is evidence that the structural conformation of SOM is altered by cations at the exchange sites and that this affects sorption and desorption kinetics of xenobiotics and thus influences the formation of bound residues (1). The addition of substrates can enhance the biodegradation of xenobiotics, either through co-metabolism or through enhanced degradation of the sorbent (2). In an extensive preliminary study about the fate of polychlorinated biphenyls (PCBs) and PAHs in soils from sewage treatment farms, numerous experiments about the effects of pH, electrolytes and biological activity on the mobility or solubility on PCB 52 and on benzo(a)pyrene were conducted. The results showed that low pH and higher Ca-concentrations in the solute reduced the release of the two compounds from a spiked and aged soil, which was attributed to structural alterations of SOM (3, 4).

Our research is directed to investigate features of bioaccessibility and biodegradability of such xenobiotics as 17β-estradiol (E2), nonylphenol (NP) and phenanthrene (Phe). Especially research is developed to see the effects of SOM structural conformation on the mineralization and sorption of NP and Phe.

2. MATERIALS AND METHODS

Preliminary experiments with the steroid hormone 17β-estradiol (E2) were carried out with topsoil samples from various agricultural and forest sites. The soils were amended in the laboratory with different compounds to test their effects on E2 mineralization.

For the main experiments with altered soils the material with high organic content (peat soils and topsoil from agricultural fields) are used. Clay-rich soils were excluded to minimize alterations of the physical soil properties throughout chemical conformation of SOM. The structural modification of SOM was altered by changing the cation composition and by removing or adding specific cations from/to the SOM matrix. It was assumed that strongly hydrated monovalent cations (i.e. Na^+) cause SOM to expand and thus make it more accessible to xenobiotics. Polyvalent cations (i.e. Ca^{2+} or Al^{3+}) reduce the volume and flexibility of SOM through cation bridging, which can thus limit diffusion of xenobiotics into the matrix and back out again. Therefore, soil treatment was conducted to optimize the methodology with respect to minimizing SOM losses and achieving homogeneous cation saturation. The effects of sample treatment on SOM conformation are analyzed with differential scanning calorimetry by determining the glass-transition temperature (5).

Sterilized samples were used to perform short-term sorption studies in sterile batch assays. For investigation of bioaccessibility and biodegradability of ^{14}C-labelled xenobiotics the degradation experiment was carried out in Respicond-apparatus (Nordren Innovations) during 4 weeks.

3. RESULTS AND DISCUSSION

In most soils, the mineralization of E2 was in the range of 5-15 % within 4 weeks. Only in one acidic forest soil with high SOC content, E2 mineralization reached almost 50% although sorption was highest in this soil. Generally, no relationship between Kd and E2 mineralization was found for the 14 soils investigated, indicating that sorption was not the limiting factor for E2 degradation. The addition of glucose stimulated microbial activity in all soils and also the mineralization of E2 (Fig. 1), thus indicating co-metabolic degradation of E2. Again, the forest soil was an exception, because here, glucose decreased E2 mineralization despite its strong stimulatory effect on soil respiration. This shows that the E2 degraders in this soil are not operating co-metabolically and are out-competed by other microorganisms when easily degradable substrates become available.

In this soil, the addition of N-compounds (alanine or ammonium nitrate) also inhibited E2 mineralization, although it was expected that microbial activity is N-limited at the high C:N ratio of 23. Catechol and ground wood had similar effects on E2 mineralization, but this may be due to direct toxic effects (catechol) or to an increased availability of sorption sites (ground wood).

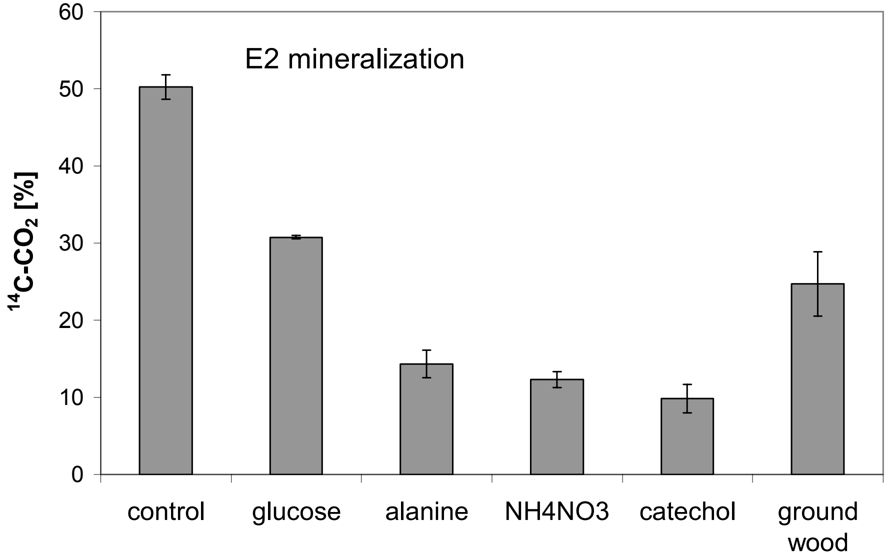

Figure 1. Effects of different compound additions on the mineralization of 17β-estradiol in a forest topsoil.

4. CONCLUSIONS

The differences in hormone mineralization in the test soils show that certain chemical or biological soil properties as well as land use seem to strongly affect the degradation processes, which still are poorly understood. It is expected that one factor influencing sorption and bioaccessibility of xenobiotics is the structural conformation of soil organic matter that can be altered by different cations. Results from the ongoing experiments are also presented.

ACKNOWLEDGEMENTS

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The Influence of Humic Acids on the Phototransformation of Organic Chemical Contaminants in Water

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Keywords: humic acids, ecotoxicants, phototransformation, water

1. INTRODUCTION

Aqueous photochemistry has recently received considerable attention because of the growing interest in processes involving photochemical transformations of organic pollutants in the natural environment. All natural waters contain dissolved humic substances. Humic substances representing the main fraction of organic matter receive increased attention because of their reactivity as light absorbers (1). Depending on their origin, humic substances have a remarkable ability to absorb light and transfer this energy to other substrates and in some cases strongly affect photolysis of xenobiotics. In water and in soils humic substances have been found to act as photosensitisers and they have also been reported to produce oxygen species upon irradiation, and be able to photoinduce the transformation of ecotoxicants. They could behave as quenchers or as photostabilizers. Humic acids (HAs), being the major component of peats of different structure, render unequal influence on natural ecosystems of biospheres. Excited singlet and triplet states of dissolved HAs are important players for the transformation of organic chemical contaminants in sunlit natural waters (2). Photochemical processes also play an essential role in engineering water treatment systems which use UV light for water purification and disinfection (3). In addition to direct phototransformations, which occur after absorption of light by a given chemical compound, a variety of processes take place as a result of absorption of light by natural water components, leading to the formation of transient species, which may react with this compound. This paper describes investigations into the photolysis of 2-methylphenol (2-MP) and 4-methylphenol (4-MP) mixture in distilled water and in water in the presence (HAs).

2. MATERIALS AND METHODS

Fractions of HAs were prepared from peat of Tomsk region (Klyukvennoe, Russia) as described elsewhere (4). The HAs pretreatment was made: HA-1 – from crushed peat; HA-2 – from mechanoactivated peat (5); HA-3 - from mechanoactivated peat with addition of fermented celloveridin (0.5%); HA-4 - from mechanoactivated peat with addition of NaOH (3%). The 2-MP and 4-MP mixture concentration («Aldrich») was $1{\cdot}10^{-4}$ mol/L. The aqueous MP solutions were irradiated with UV light in the presence and absence of HAs. As sources for photochemical investigations we used: 1) commercial mercury lamp high pressure OKN-11M; 2) exciplex U-type–barrier lamp on KrCl* working molecules with the parameters λ_{rad} ~ 222 nm, $\Delta\lambda$ = 5–10 nm, W_{max} = 18 MW/cm^2, f = 200 kHz, and 1-μs pulse duration; 3) exciplex U-type–barrier lamp on XeBr* working molecules with the parameters λ_{rad} ~ 283 nm, $\Delta\lambda$ = 5–10 nm, W_{max} = 18 MW/cm^2, f = 200 kHz, and 1-μs pulse duration; 4) exciplex U-type–barrier lamp on XeCl* working molecules with the parameters λ_{rad} ~ 308 nm, $\Delta\lambda$ = 5–10 nm, W_{max} = 18 MW/cm^2, f = 200 kHz, and 1-μs pulse duration (3). The irradiation time was varied from 1 min to 32 min. To prevent the solution from heating during the irradiation and to provide for stable operation of the lamp, the installation was air-cooled using a fan. The solutions were placed in a quartz cuvette (optical length =1 cm). Pre- and post-irradiation absorption and fluorescence spectra were recorded by a conventional procedure using a Specord M40 spectrophotometer and a CM2203 spectrofluorimeter, at 25 ± 1 °C in air equilibrated solutions.

3. RESULTS AND DISCUSSION

Photodegradation of MP mixture in aqueous solution in the absence of HAs has been carried out by UV-irradiation from different sources (Figure 1,a). After treatment of 222 nm excilamp irradiation the MP mixture was completely photodegraded. The transformation from the highest electronic excited states is the most probable route of MP decomposition by irradiation by 222 nm. With the increase in the energy of the photoexciting quantum, the probability of the population of the photodissociative triplet state increases and OH bond rupture in the MP molecule may occur (6). The worst MP degradation was observed under exposure to XeCl* excilamp.

Due to the complex nature of HAs, the absorption spectra of the HA solution shows a continuous absorption without typical peaks, and absorbance increases with the decreasing wavelength. The fluorescence of the HA solution is around 450-480 nm. The HAs pretreatment has an influence on the phototransformation of methylphenols. The most effective MP degradation in humic water was observed under exposure to KrCl* or mercury lamp, but it is lower than MP degradation in water (Figure 1,b). Contrariwise, the XeBr* excilamp irradiation decreased the photodegradation appreciably in the presence of HAs.

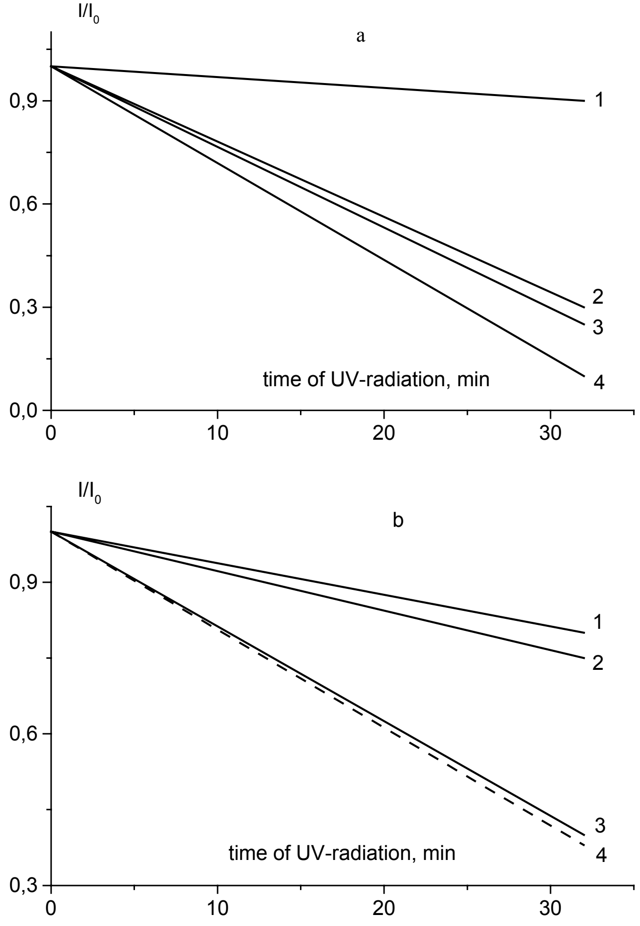

Figure 1. Change of the relative fluorescence intensity in its maximum for MP mixture in water solution (a) and in the presence of HA-1 (b) after UV irradiation:
1 - XeCl* excilamp; 2 - XeBr* excilamp; 3 – the polychromatic light of mercury lamp (290-600 nm); 4 - KrCl* excilamp.

Our experiments show that different samples of HAs lead to the appearance of various photoproducts. In the case of HA-2 and HA-4 presence were detected the products with the fluorescent spectra at 365 and 410 nm.

Table 1. The fluorescence characteristics of the aqueous MP mixture in the presence of HAs after irradiation

UV irradiation	Humic acids				
	–	HA-1	HA-2	HA-3	HA-4
	Fluorescence maximum of photoproducts (nm)				
KrCl	350, 410	325	325, 410	325	410
Hg		325	410	320	365, 410
XeBr	410	325	410	320	410
XeCl	365, 410	–	410	325	410

4. CONCLUSIONS

Humic acid did not increase the efficiency of MP photolysis contrary to earlier data for phenol at 222 nm irradiation. The results of our work have shown that HAs have photostabilizing properties with respect to methylphenols almost at all investigated excitation wavelengths.

ACKNOWLEDGEMENTS

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Potentiometric and IR Quantification of Metal Binding in Humic Substances

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Keywords: humic acids, potentiometric titration, metal complexation, IR

1. INTRODUCTION

HAs are a complex mixture of different compounds, with different sizes and molar masses and several functional groups. The major groups are: carboxyl, phenol, catechol, phthalic and salicylic. Knowledge of the amount of each group present in this mixture is important in order to understand the interaction of humic substances (HS) with metal ions in the environment (1, 2, 3). Different models and methods have been employed to calculate HA metal ion complexation. In this study, the interactions of Cu(II) and Zn(II) were characterized by potentiometric titration and IR spectroscopy.

2. MATERIALS AND METHODS

The potentiometric titration of HA (from Aldrich) in the absence and presence of copper(II) and zinc(II) were carried out with a Corning pH/ion analyzer 350 fitted with blue-glass and Ag-AgCl reference electrodes. The potentiometric apparatus was calibrated with standard HCl and KOH solutions to read $-\log [H^+]$ directly, and the pKw for water at $\mu = 0.100$ M was 13.78. Samples of around 0.100 g of HA were diluted with 40 ml of bidistilled water ($KMnO_4$) in a sealed thermostated vessel at 25.0°C. Computations of the triplicate experiments were all carried out with the BEST7 program (4), and the species diagrams were obtained with the aid of SPECIES program.

Infrared spectra of the HA fractions were recorded using samples in KBr pellets (1 mg of Aldrich HA per 100 mg of KBr) with a SHIMADZU Resolution 1.04 FT-IR spectrometer. The KBr (FT-IR grade, Aldrich Co.) was dried by heating and was kept under vacuum in desiccators prior to use.

3. RESULTS AND DISCUSSION

The distribution curves of the binding sites of the Zn(II)-HA system are shown in Figure 1.

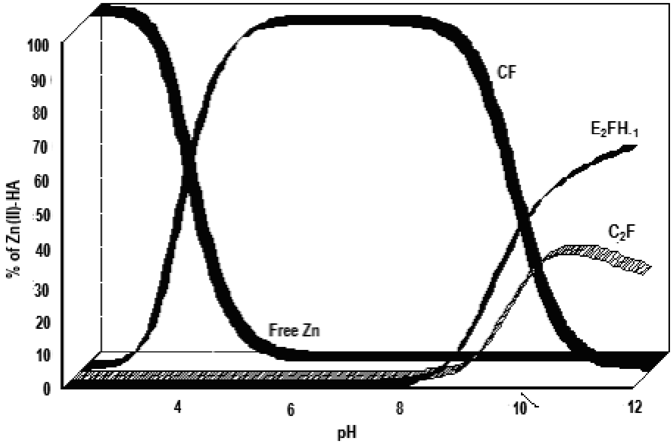


Figure1. Distribution curves of the binding sites of Zn(II) where C=Catechol, E=Salicylic, F=Zn^{2+}.

It can be seen that at pH 5.0, 98 % of Zn(II) is coordinated to the catechol group. At pH 7.0 the entire amount of the metal ion is coordinated to the catechol and at pH 9.0, 80% is coordinated to one catechol group, 6% to two catechol groups and 14% is coordinated to two salicylic sites.

The distribution curves of the binding sites of the Cu(II)-HA system are shown in Figure 2.

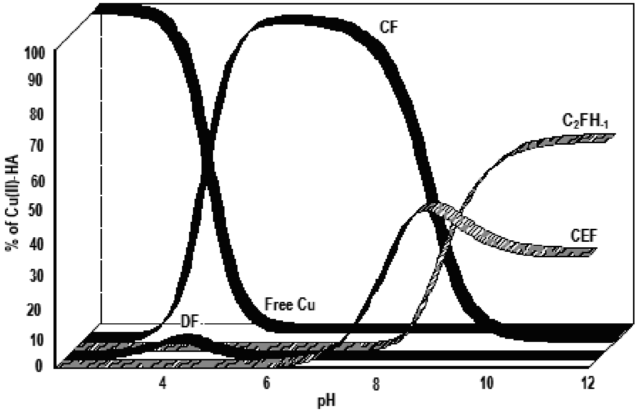
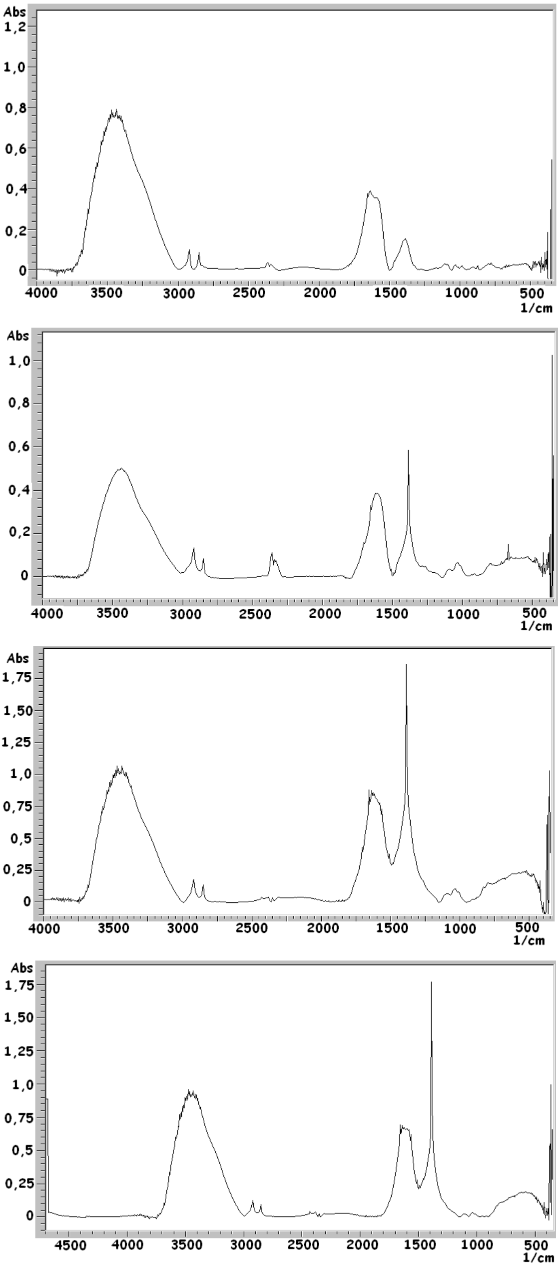


Figure 2. Distribution curves of the binding sites of Cu(II) where C=Catechol, D=Phthalic, E=Salicylic, F=Cu^{2+}.



a)
HA

b)
HA-Zn(II)
pH=5.0

c)
HA-Zn(II)
pH=7.0

d)
HA-Zn(II)
pH=9.0

Figure 3. IR spectra of HA-Zn(II) system.

It can be seen that at pH 5.0, 48% of copper(II) is coordinated to the catechol site, and at pH 7, all of the copper is bound to the catechol group. This situation changes at pH 9.0, where the metal ion is 81% bound to the catechol site, and 19% is bound to the salicylic site.

The IR studies show the complexation of the metal ions. On the IR spectrum of HA (Figure 3 for HA-Zn(II) system, not shown for HA-Cu(II) system) a broad peak can be seen at 3400 cm^{-1} due to the axial stretching of the carboxylic and catechol O-H groups. The peaks between 2915 cm^{-1} and 2851 cm^{-1} are due to the CH_2CH_3 stretching, and at 1680 cm^{-1} there is a peak due to the stretching of deprotonated C=O. The evidence of metal complexation can be seen by the increase in the intensity of the peaks at 1680 cm^{-1}, attributed to asymmetric stretching of the COO^- group (spectra b, c and d) and the broad peak lower than 800 cm^{-1}, due to C-O bonded to the metal ion. Also, at acid pH it can be seen that the peak at 1394 cm^{-1} is narrow due to the interaction with the metal ion and catechol group. At neutral pH it can be observed that there is complexation of the metal ion with the catechol, due to the increasing intensity of the peak at 1680 cm^{-1}. At alkaline pH values, the tuning of the peaks between 1680 cm^{-1} and 1394 cm^{-1} is attributed to the complexation of other oxygenated groups with the metal, as shown in the binding site distribution curves of HA-Zn(II) and HA-Cu(II) systems.

This study shows the binding sites where copper(II) and zinc(II) are coordinated to HA. The sites are characterized by potentiometric titration and IR spectroscopy. Copper and zinc show some similarities and some differences in the interactions with HA.

The major interaction for both metal ions at acidic and neutral pH values is at the catechol site, and at basic pH values, the metals interact with the catechol and salicylic sites.

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A Rapid Batch Procedure for Assessment of Humic Substances and Biodegradable Components of Organic Matter in Natural and Contaminated Materials

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Keywords: humic acid, fulvic acid, biodegradation

1. INTRODUCTION

Organic matter in sludge, contaminated soil and waste can lead to instability in landfilling due to gas formation and settling. As a result of biodegradation, dissolved organic matter (DOC) is generated. Depending on the nature of the organic matter, DOC can possibly mobilise heavy metals and organic micro pollutants (e.g. PAHs and PCBs). In the EU landfill directive (1), demanding targets have been set to reduce the amount of biodegradable organic matter on landfills. In addition, treatment techniques for organic materials (e.g. compost, sludge, source separated organic residues) require methods to assess the degree of organic matter degradation to judge treatment acceptability (2, 3).

Numerous test methods have been developed to test the degradability of organic matter in waste materials (3). Biological methods are promising to assess biodegradability of organic matter. However, these methods can be time consuming and are possibly affected by the presence of toxic contaminants, leading to underestimation of organic matter degradability (3). In addition, these methods are not suitable for estimation of the organic carbon fraction that is responsible for facilitated transport of metals and organic micro-pollutants from natural and contaminated materials. Particularly, relatively stable humic substances as humic (HA) and fulvic (FA) acids in the organic matter fraction can significantly enhance the solubility of these contaminants (4).

We have developed a new rapid batch method to measure the concentrations of HA, FA and the more hydrophilic organic acids (Hy) in both solid and aqueous samples (5). The method can be performed within 1.5-4 hours per sample.

Since HA and FA are only very slowly biodegradable (mean residence times can vary from hundreds to thousands of years), the Hy part of organic carbon can be regarded as the more biodegradable fraction. The new batch method for estimation of HA, FA and Hy concentrations in solid and aquatic samples may therefore contribute to the development of biodegradability assessment in the field of landfilling and for organic matter treatment technologies. In addition to the importance of estimating biodegradable carbon fractions, HA and FA concentrations are needed for proper estimation of the long-term environmental impact of materials in applications or under disposal conditions.

2. MATERIALS AND METHODS

Samples:

Samples from different origin, covering a wide range of natural and contaminated materials, were used and described elsewhere (6). Materials used are, amongst others, contaminated soil, contaminated harbour sediment, municipal solid waste (MSW), source separated organic-rich fraction of MSW (MSW-OR), compost and sewage sludge.

Extraction of actual soluble and potentially soluble organic matter:

Actual soluble organic matter was extracted by leaching with distilled water (L/S=10 L/kg) for 48 hours at pH 7.5±0.5. DOC was measured in the filtered (0.45 μm) eluates. Potentially soluble organic matter was extracted by an acid extraction (0.1 M HCl) followed by three sequential alkaline (0.1M NaOH) extractions at an L/S ratio of 10 (48 hours equilibration time. DOC and the fractionation of DOC in terms of HA, FA and Hy was analysed after filtration (0.45 μm) of the suspensions. All results are expressed as the total cumulative emission in mg/kg d.m.

Determination of HA, FA and Hy concentrations:

Concentrations of humic- (HA) and fulvic acids (FA) and the remaining more hydrophilic organic carbon (Hy) were measured in leachates at the native pH and in the total solid matrix of the samples. The organic matter was fractionated using a rapid batch procedure (5) based on generally applied isolation/purification procedures for HA and FA isolation from liquid samples (7) and solid source materials (8). In short, the rapid batch procedure involves isolation of HA at pH 1 and the subsequent adsorption of FA onto DAX-8 resin. The concentrations of HA, FA and Hy are either determined by direct DOC measurements (HA and Hy) or by the difference between two DOC measurements (FA).

3. RESULTS AND DISCUSSION

The actual and potentially soluble DOC results and the DOC fractionation (only potentially soluble DOC is shown) are given in Table 1. It appears that the fresh (highly) organic waste materials (e.g. fresh municipal solid waste -organic residue, sewage sludge and compost), generally have a relatively high concentration of hydrophilic organic carbon (Hy) due to the low degree of degradation. In addition, these materials have a relatively high DOC solubility at pH 7.5. This observation is consistent with findings in (9). Samples with a higher relative contribution of degraded organic matter (e.g. harbour sludge, soil samples and sieve sand) show a relatively low Hy contribution to the DOC concentration. We believe that this method forms a promising tool towards an improved understanding of organic matter stability in landfill scenarios. More examples and applications of the procedure will be shown in the presentation.

Table 1. Actual (pH 7.5) and potentially soluble DOC (g C/kg d.m.), fractionation in terms of humic and fulvic acids and hydrophilic organic carbon (potentially soluble DOC) and the percentage leached at pH 7.5 relative to the potentially soluble DOC for several soil and waste materials (6)

Sample	$HA_{pot.}$	$FA_{pot.}$	$Hy_{pot.}$	$DOC_{pot.}$	$DOC_{pH\ 7.5}$	%pH7.5
Sewage sludge (SEW)	105	28	44	177	35	20
Fresh MSW-OR 2	22	13	26	62	20	33
Fresh MSW-OR	18	18	20	57	26	46
Compost (CW5)	68	6	7	81	7	9
Degraded MSW-OR	15	6	6	27	3	11
predominantly inorganic waste	15	5	5	26	1	3
untreated landfilled MSW	7	3	4	15	3	19
Degraded landfilled MSW	10	4	3	17	1	4
Soil (SO24)	8	3	3	13	0.4	3
Harbour sludge Malburg	10	2	2	14	0.4	3
Sieve sand	0.1	1	1	2	0.3	14
MSWI bottom ash	0.0	0.2	1	1	0.4	55
Soil (BG)	1	1	0.3	2	0.3	13
Tar containing asphaltgranulate	0.0	0.5	0.0	1	0.1	20

4. CONCLUSIONS

The rapid batch method for HA, FA and Hy measurements significantly enhances the possibilities to include DOC fractionation in biodegradation studies. The results indicate the degradation stage of the samples and the ability of DOC (in terms of HA and FA) to bind contaminants (facilitated leaching/transport). The hydrophilic organic carbon fraction seems to represent the biodegradable part of DOC, although FA seems to be biodegradable to a certain extent in our samples. The extraction procedures for actual and potentially soluble organic matter and the batch method for quantification of humic substances are currently proposed for international standardization. Ongoing work focusses on linkage between organic matter fractionation and gas formation to gain more insight in changes in the organic matter species and properties during biodegradation.

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Natural Organic Matter in Some Tributaries of the Dnieper River and their Effect on Phytoplankton Growth

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Keywords: humic, fulvic, proteins, carbohydrates, river, phytoplancton

1. INTRODUCTION

Relationship between dynamics of the content of organic matter and the peculiarities of development of hydrobionts is of considerable importance in the process of formation of water quality in environment. Planktonic algae are involved in the process of formation of the composition and quantitative indices of dissolved organic matter (DOM). In its turn, organic matter depending on its chemical nature may serve as a source of organic compounds of nitrogen and phosphorus, and also as a potential reserve of various nutrients essential to the vital activity of hydrobionts [1, 2].

The main objective of the present work was to assess the composition and seasonal dynamics of DOM in some tributaries of the Dnieper depending on the peculiarities of development of planktonic algae.

2. MATERIALS AND METHODS

The samples of water from the Pripyat, Zdvizh and Irpen rivers were taken twice a month in 1998-1999, from another river in May, August, 2001, 2003, 2006. Suspended organic matter was separated from DOM by means of filtration through membranous filters with 0.40 µm sieve. The chemical nature of DOM was studied by the method of ion-exchange chromatography. Diethylaminoethylcellulose (DEAE-cellulose) was used for isolating DOM of the acid nature (humic substances) (HS) whereas carboxymethylcellulose (CM-cellulose) was used for isolating DOM of the alkaline nature (protein-like substances). The neutral group of DOM (carbohydrates) was concentrated by means of freezing. The molecular-weight distribution of the isolated groups of substances was investigated by the method of gel-penetrating chromatography using the G-25 and G-50 neutral Sephadexes. The content of humic and fulvic acids (HA and FA) was assessed by the method of spectrophotometry in terms of their own coloration with λ=400 nm in the concentrated samples. The numerical abundance of algal cells was determined by the sedimentation method by means of a direct microscopic count in a Najotte chamber. Biomass was calculated by the count cell-volume method.

3. RESULTS AND DISCUSSION

The surface runoff, water balance, and intra-waterbody processes are of considerable importance in the process of formation of organic matter. These factors are also responsible for dynamics of its content and composition. The obtained data suggest that HS prevailed amond DOM in the studied rivers (Table 1). In the Pripyat, Lva, Stvyga, Ubort rivers the HS concentration were assentially higher, than that observed in other rivers studied. The content of HS in the rivers with its high concentration consists 90–97% in total DOM.

Table 1. Changes of C_{org} and humic substances content in the rivers water during 1998–2006

River	C_{org}, mgC L^{-1}	HA, mg L^{-1}	FA, mg L^{-1}
Pripyat	14.36 –29.31	0.98 – 6.31	25.75 –51.61
Styr	8.13 – 30.41	0.29 – 4.22	11.97– 54.61
Goryn	6.21 – 8.01	0.47 – 1.11	9.74 – 12.91
Lva	23.69 – 43.05	1.92 – 6.98	40.77 – 76.90
Stvyga	29.50 – 37.40	2.54 – 5.47	54.09 – 65.55
Ubort	28.43 – 68.70	2.08 – 9.54	50.35 – 124.03
Slovechna	14.38 – 16.83	0.53 – 1.02	22.85 – 29.68
Nobel	13.42 – 17.33	1.02 – 2.49	22.81 – 28.20
Desna	9.63 – 10.54	0.48 – 0.65	14.15 – 17.30
Zdvizh	5.31 – 8.43	0.24 – 0.52	6.07 –13.91
Irpen	4.22 –7.92	0.15 – 0.41	4.73 – 10.51

The obtained data suggest that FA with the molecular weight > 1.0 kDa prevail mainly in the Pripyat river. In spring their relative amount in the Pripyat river accounted for 72.2%, in the Zdvizh river it was 30.4%, whereas in the Irpen river it accounted for 17.8%. It has been known that in spring the composition of DOM is formed mainly at the expense of DOM contained in the surface runoff. These HA are characterized by a high molecular weight. A relative amount of high-molecular FA in the Pripyat river gradually decreased from spring until winter. In this case, it was rather high throughout the year. In autumn in the Zdvizh and Irpen rivers, this index increased up to 40% (Figure 1).

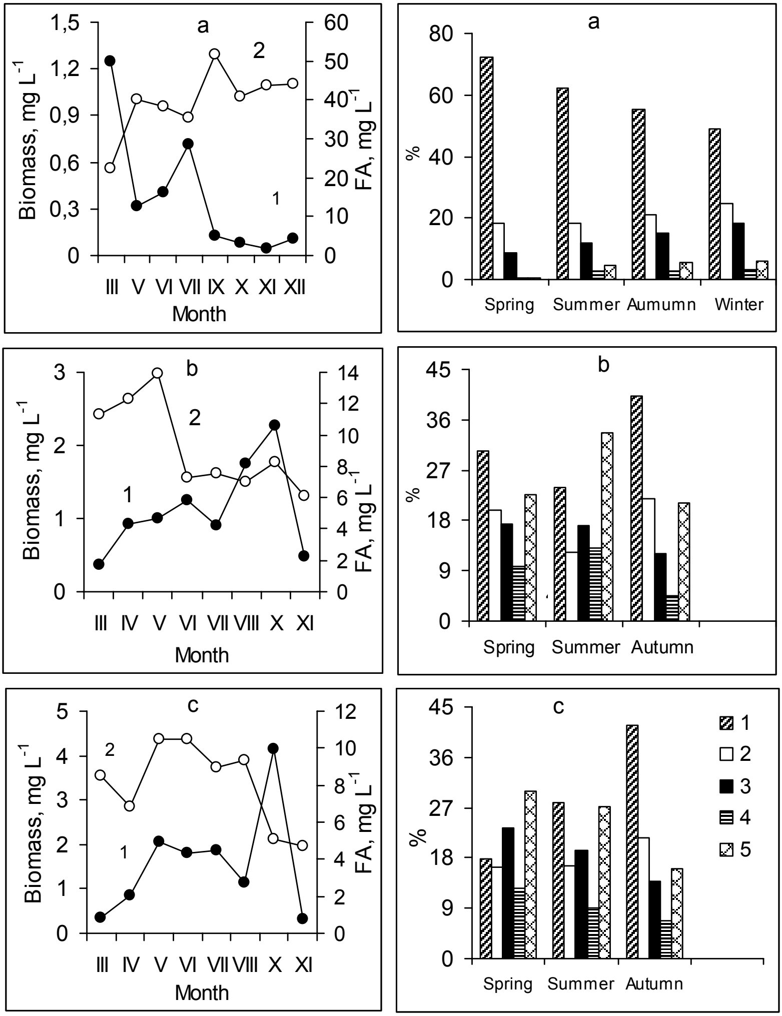

Figure 1. Dynamics of phytoplankton biomass (1) and FA (2) (I) and molecular-weight distribution of FA (II) in some tributaries of the Dnieper in various seasons.

a – Pripyat, *b* – Zdvizh, *c* – Irpen.

1 – >1.0 kDa, 2 – 0.8-0.6 kDa, 3 – 0.6-0.4 kDA, 4 – 0.4-0.2 kDa, 5 – < 0.2 kDa.

It has been known that the development of phytoplanton is conditioned by many factors, including chemical ones. The intensity of development of planktonic algae in water bodies of various types is determined mainly by the content of nutrients, including nitrogen and phosphorus. At the same time, HS are also of considerable importance in the formation of the hydrobiological regime of many water bodies. It is thought, that inhibition of the development of phytoplankton registered at a high concentration of HS conditioned by binding nutrients into the complexes.

The obtained data suggest that in the Pripyat river the biomass of phytoplankton inversely depended on the content of FA (r = -0.91). The same is true of the species diversity of phytoplankton. The number of algal species decreased with increasing the content of FA (r = -0.75). The biomass and species diversity of individual groups of planktonic algae occurring in the Pripyat river also inversely depended on the content of FA. Thus, the biomass of *Bacillariophyta* (r = -0.91) and their species diversity (r = -0.88) inversely depended on the FA concentration. *Cyanophyta* were observed in this river only at a low FA concentration.

In other studied rivers characterized by a lower content of HS,the biomass of phytoplancton was higher. It has been found that in the Zdvizh river the biomass of phytoplancton inversely depended on the FA content (r= -0.25). In particular, the biomass of *Cyanophyta* (r= -0.45) and their species diversity (r= -0.74) inversely depended on the FA content.

4. CONCLUSIONS

The obtained data suggest that in the studied rivers HS, primarily FA, are among the main components of DOM. Their content varied from season to season and depended on the peculiarities of the catchment area. The highest concentration of FA (up to 124 mg L^{-1}) was observed in the Pripyat, Stvyga, Ubort river in spring. In the Goryn, Slovechna, Desna, Zdvizh and Irpen rivers, the maximum content of these acids was essentially lower (10.51 –17,30 mg L^{-1}). In the Pripyat river, a relative content of FA with the molecular weight > 1.0 kDa accounted for 48.9–72.2%. In the Zdvizh river, it was 24.0–40.3%, whereas in the Irpen river it accounted for 17.8–41.7%. The concentration of FA in the studied rivers inversely depended on the biomass of phytoplankton.

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The Influence of Apparent Molecular Size of Aquatic Humic Substances during Coagulation with Ferric Chloride

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Keywords: aquatic humic substances, ferric chloride, coagulation, removal color

1. INTRODUCTION

Water sources that are used to supply the public frequently present high levels of true color due to the presence of aquatic humic substances, which contribute from 50 to 70% of dissolved carbon in such waters (1). The presence of aquatic humic substances in many water treatment plants can cause problems in the coagulation process.

Functionally, humic substances predominantly contain phenolic and carboxylic groups, but also contain alcohol, amine and ketone groups (2). Approximately 80-90% of the humic substances found in natural waters are fulvic acids, while humic acids constitute the remaining 10-20% (3).

Processes involving coagulation with ferric chloride have been studied for removal of humic substances in natural waters (4, 5, 6). The mechanism of coagulation with regard to the removal of aquatic humic substances is by charge neutralization for soluble colloidal compounds. The removal occurs due to adsorption on to precipitated flocs and metal hydroxides (7) and accordingly, optimization of the coagulation process occurs under acidic conditions, pH 4.5–5.5 (8). The aim of this study was to verify the influence of the apparent molecular size of aquatic humic substances on the effectiveness of coagulation with ferric chloride.

2. MATERIALS AND METHODS

The water samples were collected from the Itapanhaú river, Bertioga, São Paulo State, Brazil. The collected samples exhibited true color intensity in the order of 300 Hazen units. An XAD-8 resin was used to extract the aquatic humic substances. The extracted humic substances were filtered through a membrane with 0.45 μm pores (Millipore) and this fraction was denoted as F_1. Fraction F_1 was then separated by ultrafiltration using polyethersulfone membranes (Vivalflow50) into 2 apparent molecular size fractions of humic material: F_2 from 100 kDalton to 0.45 μm and F_3 from 10 to 30 kDalton. The fractions (F_1, F_2 and F_3) were characterized by elemental analysis (CE instruments EA 1110 CHNS-O, Fisons) and UV/Vis (Hitachi model U3501). Water from an artesian well was used to prepare 3 experimental samples of the following molecular size fractions of aquatic humic substances. F_1: 0.45 μm (water sample I), F_2 from 100 kDalton to 0.45 μm (water sample II) and F_3': < 30 kDalton (water sample III). Water sample color intensity was approximately 100 Hazen units, turbidity approximately 5.0 NTU with added kaolinite and temperature 20 ± 1°C. Solutions of NaOH (0.1M) and HCl (0.1M) were used to adjust the coagulation pH in the range of 3.4 to 7.0. Jar test equipment (ETICA), using direct filtration, was used to carry out the tests. The following parameters were adopted for the tests, rapid mixing time, 30 s; medium velocity, 1000 s^{-1}, filtration time was 20 min. The coagulant employed was commercial ferric chloride. Color measurements were based on the procedure recommended by the Standard Methods for the Examination of Water and Wastewater (9).

3. RESULTS AND DISCUSSION

In general, characterization results (Table 1 and Table 2) indicated that the smaller molecular-size fraction (F_3) had a higher proportion of aliphatic than aromatic carbon atoms and a relatively high percentage of oxygen atoms bonded to alkyl groups and carboxylic acids. Conversely, the apparently larger molecules (fractions F_1 and F_2) showed a higher content of aromatic groups this suggests an increase in the humification degree. This indicates that for the aquatic humic substances as a whole, there are a greater proportion of oxygenated groups and aliphatic carbon atoms.

Table 1. Elemental composition of aquatic humic substances

Fractions	In atom percentage				Molar ratio		
	C	H	N	O	H/C	C/N	N/O
F_1 (<0.45 μm)	37.35	3.34	1.44	57.86	1.07	1.16	30.23
F_2 (<0.45 μm - 100 kDalton)	36.01	3.32	1.29	59.38	1.10	1.24	32.57
F_3 (10 – 30 kDalton)	37.95	4.34	0.80	56.90	1.37	1.12	55.20

Table 2. Values of absorbance and absorbance ratios

Fractions	Absorbance (nm)		Ratio	Absorbance (nm)		Ratio	Aromatic character
	465	665	E_4/E_6	250	365	E_2/E_3	
F_1	0.314	0.037	8.40	0.866	0.274	3.16	31.07
F_2	0.044	0.007	6.07	0.449	0.146	3.06	31.75
F_3	0.331	0.031	10.52	0.680	0.198	3.43	29.24

Table 3 presents the best results for the removal of apparent color as a function of coagulant dosage for each water sample and pH range. The coagulation diagrams obtained with ferric chloride revealed the effects of the molecular size of the aquatic humic substances on the coagulation process. Based on the color removal observed in the coagulation diagram for each water sample, the region where the most efficient removal was obtained was selected for analysis in this paper: ≤ 5.0 Hazen units.

Table 3. Coagulation pH and dosage of commercial ferric chloride of the regions of apparent color in filtered water ≤ 5.0 Hazen units and turbidity ≤ 0.5 NTU

Water sample	Apparent color removal and turbidity	Coagulation pH	Dosage commercial ferric chloride (mg L^{-1})	Control parameters
I	Region 1	4.0 – 4.3	30 - 40	Apparent color
		3.9 – 6.0	From 30 to > 80	Turbidity
	Region 2	4.8 – 6.0	From 40 to > 80	Apparent color
II	Region 1	< 3.4 – 6.0	From 26 to > 132	Apparent color
		< 3.4 – 6.2	From 26 to > 132	Turbidity
III	Region 1	3.9 – 5.3	From 60 to > 100	Apparent color
		4.1 – 5.2	From 70 to > 100	Turbidity

It can be seen in Table 3 that for all doses of ferric chloride and pH ranges, the apparent molecular size had a significant influence on the color removal. The removal decreased with decreasing apparent molecular size according to: F1>F2> F3’. For fraction F3’, a greater dose of coagulant was needed to remove the apparent color and, even then, the amount removed was lower. F3 also presented a higher proportion of fulvic acids, which exhibit a larger number of negatively-charged groups. These smaller molecules are formed mainly by small, stable micelles, which remain dispersed due to the repulsion of the negative charge that originates from the dissociation of the large amount of groups with an oxygen in their structure (10,11,12). For these reasons, a high dose of coagulant is necessary to achieve efficient removal of apparent color. Charge neutralization seems to be the mechanism responsible for the removal of humic substances under the conditions employed in this study.

4. CONCLUSIONS

From the experimental results obtained the following conclusions can be made: i) Humic substances with different apparent molecular sizes behave differently during coagulation; ii) The higher the apparent molecular size of the humic substances used to prepare water samples (true color ~ 100 HU), the more efficient the removal of color; iii). The characterization results showed that for the fractions of aquatic humic substances used to prepare the water samples (F_3: < 30 kDa), which presented higher proportions of bonded oxygen groups, resulted in the lowest removal of apparent color.

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Influence of Tropical Humic Substances and Copper on the Survival and Reproduction of Tropical Cladocerans (*Daphnia similis and Ceriodaphnia silvestrii*)

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Keywords: humic substances, copper, *Daphnia similis*, *Ceriodaphnia silvestrii*

1. INTRODUCTION

Humic substances are recognized as influencing the toxicity of heavy metals (e.g. copper) towards the survival and growth of Cladocerans. However, information about the ecology of tropical humic acids is scarce when compared to those from other sources. In this work we evaluate the influence of tropical peat soil humic substances on the survival and growth of two Cladocerans species in presence of copper. The aim of this study was to investigate if tropical humic acid can reduce copper toxicity in both lethal and sub-lethal concentrations towards the Cladocerans *Daphnia similis and Ceriodaphnia silvestrii*, respectively. The influence of humic acids on survival and reproduction of these species was evaluated.

2. MATERIALS AND METHODS

Tropical humic substances were extracted from tropical humic soil (Mogi-Guaçu River basin, Brazil) using the recommendations of the IHSS protocol. The humic acids (HA) were purified using ion-exchange resins. All solutions were prepared using ASTM (1) soft water (hardness 40 mg L^{-1}). Copper solutions (Cu) were prepared using Sigma Aldrich AAS standard. A single concentration was used for both assays: 25 μg L^{-1}. The final assay combinations for both species were SH, SH+Cu, Cu and Control. For acute toxicity assays five concentrations of HA (0.3; 0.5; 1.0; 3.0 and 5.0 mg L^{-1}) were used and for chronic only 5.0 mg L^{-1} was used.

The organisms were cultured according to ABNT (2) (Brazilian standard) recommendations. The exposure time was 48h for acute toxicity assays using *Daphnia similis* and 192h for chronic assay using *Ceriodaphnia silvestrii*. All assays were conducted in triplicate. The results obtained were analyzed using statistical packages and the free copper the estimations were calculated using the BLM model from Hydroqual.

3. RESULTS AND DISCUSSION

The HA concentration is not observed to influence the survival and reproduction of both the Cladocerans investigated. For acute assays (Figure 1) the detoxification occurred at an AH concentration of 1.0 mg L^{-1}, which doesn't denote statistical difference in relation to the control (ANOVA $p< 0.05$). The simulations with the BLM model, show a significant negative correlation (r_S= -0.92 p= 0.008) between free copper concentration and survival. The fecundity of *C. silvestrii* was increased in treatment with copper in relation to AH only (means of 3.1 and 4.5 juv/female, respectively), but was not statistically significant.

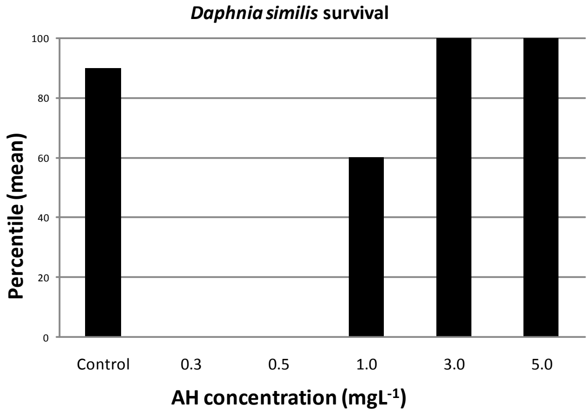

Figure 1. Survival of *D. similis* after acute exposure under 25 μg L^{-1} of Cu and several concentrations of tropical humic acids.

4. CONCLUSIONS

We can conclude is the AH can retard the acute and chronic toxic effects of copper in soft waters and can be predicted by BLM free metal estimations. However, the increase of fecundity in treatments of combined AH and copper, need more investigation.

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Buffer Properties of Humic Acids in the System with Phosphate Ions

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Keywords: buffer capacity, humic acids, potentiometric titration, phosphate ions

1. INTRODUCTION

Among many methods of getting information on properties of acidic groups, potentiometric titration is frequently used. A back titration method has been applied for the determination of qualitative and quantitative analysis: the dependence of variable surface charge Q versus pH, functions of apparent surface dissociation constants distribution versus pKapp and also quantity of particular functional groups. Electrochemical measurements can be also used to buffer properties analysis of soil components.

The issue of buffering in the system of soil organic matter – phosphates seems be interesting for ecological reasons. Humic acids and their salts fulfil function as natural soil buffers, whereas phosphates higher presence in the soil, which also have strong buffer properties, results from human intervention. Natural contents of phosphates in soils ranges between 40 and 1000 ppm (1). However increasing consumption of phosphate fertilizers in the last years produces the result of increasing phosphates presence in the soils and groundwater. Apart from great amount negative consequences which can be caused by phosphate ions, they can also in significant manner modify and increase of natural buffer properties of soils. It can be reason for change of acid – base equilibrium. Determination of buffer pH ranges of each investigated system can let define of component influence on environment and also enable calculating of increasing of natural environment buffer capacity after phosphates adding.

The aim of this study is to determine the buffer capacity of natural humic acids samples and to compare these characteristics with buffer capacity of commercial humic acid (Aldrich HA) as well as to investigate the influence of phosphate ions on buffer properties of studied humus extracts. In this case potentiometric titration method was used at a wide range of pH (3-10).

2. MATERIALS AND METHODS

The study was conducted on a few muck samples (Terric Histosols). The samples were collected at 5-20 cm depth from terrains located in a low moor area of the Wieprz-

Krzna Canal (Polesie Lubelskie) and Biebrza River in Poland. The selected properties of the investigated soil samples are presented in Table 1.

Table 1. Selected properties of the investigated mucks

Nr	W_1	Ash (% d.m)	Bulk density (g cm^{-3})	Porosity (% obj.)	pH H_2O	pH KCl
1	0,44	22,7	0,21	88,5	5,1	4,5
2	0,55	17,6	0,25	84,6	5,5	5,2
3	0,65	18,9	0,31	80,9	5,5	5,0
4	0,72	18,0	0,36	77,8	5,0	4,5
5	0,82	22,3	0,39	78,7	5,5	5,0

Within next step humic acids (HA) were extracted from mucks by Schnitzer method (2). From the obtained extracts, suspensions of 400 ppm of HA with different concentrations of phosphate (V) ions (0-600 ppm) were prepared on the basis of 1 M sodium chloride solution. High ionic strength gave lower electrostatic interactions in the solution and made the surface more easily charged. All investigations were carried out using Titrino 702 (Metrohm) apparatus. Samples were titrated using 0,1 M sodium hydroxide solution based on 1 M sodium chloride solution in wide range of pH (3-10). From the obtained potentiometric titration curves, buffer capacities of humic acids with different phosphate additions, were calculated using equation no. 1, where: Δn – amount of added moles of strong base and ΔpH – change of pH caused by strong base adding

$$\beta = \left|\frac{dn}{dpH}\right| \approx \left|\frac{\Delta n}{\Delta pH}\right| \qquad (1)$$

3. RESULTS AND DISCUSSION

Buffer capacity of investigated systems was described as function of the pH. Exemplary curves of β vs. pH were shown in figure 1 and 2. The buffering curves of particular soil humic acid extracts without phosphates addition, differ from each other (Figure 1). Significant discrepancies are clearly seen at the dissociation range of strong acid carboxylic groups. Buffer capacity at that pH range is the highest and reaches maximum at pH ~ 4.

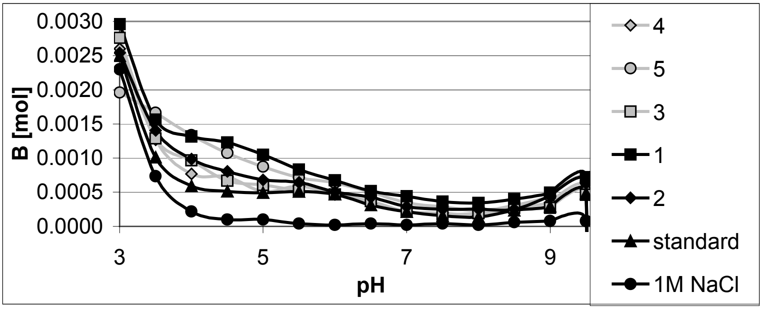

Figure 1. Exemplary curves of β vs. pH of humic acid extracts without phosphate (V) ions addition.

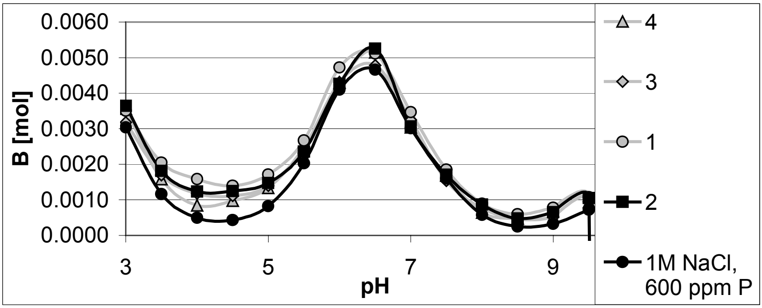

Figure 2. Exemplary curves of β vs. pH of humic acid extracts with phosphate (V) ions addition (600 ppm).

The discrepancies which occur at low pH values can be a result of different secondary transformation degree of organic matter (Table 1). Extract samples with the highest W_1 degree showed lower buffer capacity at low pH than buffer capacity of samples with lower W_1 degree. It is probably connected with altered oxidation and decomposition degree of investigated organic matter. Commercial humic acid had the lowest buffer capacity in comparison to natural extracts which can indicate high degree of secondary transformation or presence of ash in the commercial reagent, because the COOH groups may be complexed with silicate impurities of ash. Maximal β for Aldrich HA was wider and flatter than for the natural samples (pH ~ 4-6) which can denote vast influence either o-COOH type groups or simple carboxylic groups (COOH). For the natural extracts, o-carboxylic groups are predominating. The peaks observed at the pH range 8-10 are assigned to phenolic groups, eventually to secondary or tertiary amines which can be titrated together with phenols (4). It can be also referred to total content of nitrogen in the investigated material. The differences in buffer capacities at that pH range are not so significant. Addition of phosphates does not cause visible changes in structure of humic acids (It is noticeable in the functions of apparent surface dissociation constants distribution versus pKapp which were also obtained). Such behaviour is most probably caused by washing up multivalent cations (during Schnitzer extraction) which in natural conditions fulfil function of bridges connecting phosphates with humic acids. On the other hand, phosphates strongly buffer at the range of pH 5-8. Phosphate presence increases buffer capacity of natural humic acids at the range of pH ~ 5-7, so that in pH range of most soils. Functional groups from pH 5 to 7 are the most significant in soil because they are completely ionized and contribute to soil buffering capacity presence (3). Thus, increased presence of phosphates can have considerable impact on acid – base equilibrium and homeostasis state retaining.

4. CONCLUSIONS

On the base of above investigations their following conclusions may be drawn:

– humic acid shows the highest buffer properties at low pH, reaches maximum at pH ~ 4;

– phosphate ions reveal buffer properties at pH range 4,5-8,0 with maximum at pH ~ 6,8 increasing directly proportional with phosphate concentration;

– the discrepancies which occur on the β – curves at low pH values, can be a result of different secondary transformation degree of organic matter. With increasing W1, β decreases at low pH ranges;

– phosphate presence increases buffer capacity of natural humic acids at the range of pH ~ 5-7, so that in pH range of most soils;

– presence of phosphates can be significant for acid – base equilibrium and environment homeostasis retaining;

– phosphates in natural conditions apart from increasing of buffer capacity of soils, can also connect to humic acids by cation bridges and structure of humic acid can be changed.

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Population of Humic Acid Degrading Microorganisms under Different Soil Types and Vegetation Types

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Keywords: humic substances, degradation, decolorization, fungi, bacteria

1. INTRODUCTION

Humic substances participate in the global carbon cycle and are regarded as being rather resistant to biological influences, owing to their peculiar molecular structure; however, they are known to be degraded by microorganisms. Therefore, studies of the mechanisms by which humic biotransformation take place, and the organisms responsible, are important for understanding terrestrial carbon dynamics.

Since the 1960s, a number of microbial isolates that degrade humic substances have been obtained. The structural changes that occur during the transformation of these substances by microorganisms have also been investigated. However, no detailed study has been performed on the distribution and density of microorganisms capable of degrading humic substances in the environment. This information will be essential for developing a better understanding of humus biotransformation in various soil environments.

The purpose of this study was to examine the effect of soil types and vegetation type on the distribution of humus-degrading microorganisms. We measured the density of microbes that degrade humic substances using the decolorization of humic acid as an indicator of the ability to degrade humus.

2. MATERIALS AND METHODS

The study sites were three Andosol sites (GS, SN, TM) and three Cambisol sites (AJ, MY, YS) in western Japan (Table 1). Each Andosol site had three different vegetation types in adjacent locations: bamboo grassland (Gb), eulalia grassland (Ge), and coniferous plantations (F). The vegetation type of the Cambisol sites was broad-leaf forest. Soil samples were taken from each of the vegetation types at each site. Undisturbed soil core samples were collected using a stainless steel core sampler (100 mL). For soil samples at a depth of 0–2 cm, core samples were sliced off using a stainless steel knife just before they were studied.

Table 1. Description of the sampling sites and soil samples

Site code	Location	Soil type	Vegetation type	Sample code
AJ	Higashiura, Awaji, Hyogo prefecture	Cambisol	Broad-leaf forest	AJ
MY	Mt. Maya, Kobe, Hyogo prefecture	Cambisol	Broad-leaf forest	MY
YS	Yamashiro, Soraku, Kyoto prefecture	Cambisol	Broad-leaf forest	YS
GS	Mt. Gyosei, Kanzaki, Hyogo prefecture	Andosol	Coniferous plantation	GS-F
			Bamboo grassland	GS-Gb
			Eulalia grassland	GS-Ge
SN	Soni Highland, Uda, Nara prefecture	Andosol	Coniferous plantation	SN-F
			Bamboo grassland	SN-Gb
			Eulalia grassland	SN-Ge
TM	Tonomine Highland, Kanzaki, Hyogo prefecture	Andosol	Coniferous plantation	TM-F
			Bamboo grassland	TM-Gb
			Eulalia grassland	TM-Ge

The humic acid used in this study was prepared from a buried humus horizon of Andosol (50-80 cm, Mt. Oginosen, Yazu, Tottori prefecture, Japan). Sequential extraction of the humic acid using a pyrophosphate solution (pH 5.0) was carried out according to the method of Fujitake et al. (1).

A modified dilution plate technique was used for counting bacteria and fungi. For bacteria, 10^5 to 10^6 fold dilutions, and for fungi, 10^4 to 10^5 fold dilutions of the primary suspension were plated in Petri dishes containing nutrient agar medium. The number of colony forming units (CFU) was counted after 14 d growth at 25˚C on albumin medium containing humic acid (final concentration 8 g L^{-1}) for bacteria and on Martin medium containing humic acid (final concentration 8 g L^{-1}) and streptomycin (final concentration 100 μg L^{-1}) for fungi. The number of colony forming units of humic acid-degrading (HAD) microbes (DCFU) was determined by counting only colonies with a clear 'halo' surrounding them on the dark-brown agar. Five replicates were prepared for each treatment.

3. RESULTS AND DISCUSSION

The bacterial and fungal density data for nine Andosol samples and three Cambisol samples are shown in Table 2. The density of total bacteria varied, ranging from 30.0 to 214.9 × 10^6 cfu g^{-1} soil. The density of HAD bacteria ranged from 0.1 × 10^6 cfu g^{-1} soil for SN-Gb to 7.5× 10^6 cfu g^{-1} soil for AJ. AJ had significantly higher values for HAD bacterial density than the others. However, there seemed to be no clear relationship between the density of either total bacteria or HAD bacteria and vegetation type or soil type.

The total fungal density also varied, ranging from 63.9 to 362.3 × 10^4 cfu g^{-1} soil. The HAD fungal density ranged from 3.6× 10^4 cfu g^{-1} soil at SN-Gb to 24.8 × 10^4 cfu g^{-1} soil at MY and SN-Ge. The samples for Cambisol had significantly higher values for HAD fungal densities than the samples for Andosol except SN-Ge and TM-Ge. These two soils were maintained for grassland vegetation by annual burning in early spring.

Table 2. Total microbial density (CFU cfu g^{-1} soil) and density of humic acid degrading microorganisms (DCFU cfu g^{-1} soil) in different soil samples

Samples	Bacteria			Fungi		
	CFU ($\times 10^6$)	DCFU ($\times 10^6$)	(DCFU/CFU) ×100	CFU ($\times 10^4$)	DCFU ($\times 10^4$)	(DCFU/CFU) ×100
AJ	214.9	7.5	3.5	362.3	24.8	6.9
MY	22.9	0.3	1.4	101.5	16.2	15.9
YS	30.0	0.9	3.2	63.9	12.0	17.0
GS-F	50.4	1.9	3.5	77.8	4.5	6.0
GS-Gb	68.7	0.6	1.0	200.4	4.8	5.3
GS-Ge	82.6	0.7	0.8	217.3	5.9	4.9
SN-F	184.2	0.5	0.3	147.9	7.8	2.4
SN-Gb	61.8	0.1	0.2	109.0	3.6	3.3
SN-Ge	163.6	2.8	1.8	214.4	24.8	3.7
TM-F	157.4	2.1	1.4	160.2	7.6	2.8
TM-Gb	121.9	2.0	1.8	163.5	6.0	11.4
TM-Ge	38.9	0.3	0.6	117.8	14.7	12.5

The percentages of total bacteria or fungi that were able to degrade humic acid (i.e. (DCFU/CFU) × 100) at each site are also shown in Table 2. The percentages of HAD bacteria were rather low, and there were no significant differences between sites. There were greater percentages of HAD fungi than bacteria, with values ranging from 2.4 to 17.0%. The soil samples classified as Cambisol except AJ showed higher values than those for Andosol samples. Among Andisol samples, the percentages of HAD fungi were significantly higher for SN-Ge and TM-Ge than for the other sites (11.4% and 12.5%, respectively).

In our study, microorganisms that degrade humic acid were found in all soil samples. These results suggest that HAD microorganisms are widely distributed in surface soils. Low densities of HAD bacteria were detected at every site and for each soil type and vegetation type, and the overall percentages of bacteria able to degrade humic acid were low. In contrast to the bacteria, the densities of HAD fungi differed among the twelve soil samples. Greater densities and percentages of HAD fungi were detected in Cambisols than in Andosols, except for SN-Ge and TM-Ge. It is considered that these differences are probably related to the differences in soil type and vegetative cover. At the eulalia grasslands at SN and TM, which had higher percentages of HAD fungi than the other Andisol samples, annual burning is carried out in early spring to maintain the eulalia vegetation. At the eulalia grassland at GS, on the other hand, burning was previously applied, but unscheduled cutting is now used. The GS-Ge site had HAD fungal percentage similar to those at the bamboo grasslands sites, which were also maintained by cutting, but had considerably lower values than the SN-Ge and TM-Ge sites. Our results suggest that the proportion of fungi that degrade humic acid is also influenced by grassland management regimen, and that burning results in an increase in the proportion of HAD fungi.

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Section IV

Nature- and man-made design of humic materials and their properties

Radiochemical Approach for Studying Properties of Humic Substances

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Keywords: humic, radiolabeling, tritium, hydrophobicity, bacteria, wheat

1. INTRODUCTION

Study of physical and chemical properties of humic substances (HS) is a main question of modern investigations because they play a central role in industry, medicine and ecology. No doubt, that radioactive label might be a good instrument for the investigations of HS properties and their behavior in different systems.

Choice of radioactive isotope and radiolabeling technique is one of the main questions in such researches. Radiolabeled HS are required to be identical with the initial material. Thus, only limited number of elements is preferred for labeling. Previously 14C-labeled HS were produced by addition of a labeled precursor to a soil sample during composting or by the synthesis of model polymeric compounds under defined conditions. However, these techniques did not allow producing labeled HS identical to the native ones. Some methods of direct labeling of HS were therefore developed including labeling of HS with 125I, 99Tc and 111In. The main advantage of the direct labeling of HS is an opportunity to produce a broad spectrum of isotope-labeled native humics varying significantly in both their origin and properties.

Since hydrogen is one of the basic elements in HS, tritium is expected to be the most useful for the investigation of HS properties and HS behavior in different systems including biological ones. Among the methods of tritium introduction into organic molecules, tritium thermal activation method (the bombardment of solid targets by atomic tritium) is the only that allows introducing ^{3}H-label in any structural fragments of macromolecule. This work focuses on novel integration of several opportunities to advance studying of hydrophobicity and biological activity of humic materials by means of radioactive label. Thus, this work has two critical important goals. The first relates to introduction of radioisotope into HS molecule without lose of initial properties of HS. The second deals with the investigation HS behavior in different systems.

2. MATERIALS AND METHODS

Humic materials used in this study were humic (HA) and fulvic acids (FA) isolated from soil and peat and also included preparations of HA and hymatomelanic acids (HMA) from brown coal. All the HS were characterized by methods of elemental analysis, size-exclusion chromatography (SEC) and ^{13}C NMR spectroscopy.

Preparation of tritium-labeled HS. In the present study, we applied a new developed technique for radioactive labeling of HS using thermal activation method (1). The method implied bombardment of the target HS with atomized tritium followed by equilibrium dialysis to purify the labeled product from labile tritium and low molecular weight fractions. To prove the developed technique of HS labeling with tritium, parent HS and obtained samples of ^{3}H-HS was analyzed by SEC with radioactivity and UV detection.

Characterization of hydrophobicity and surface activity of HS. To characterized hydrophobic and surface activity properties of HS under study, the obtained ^{3}H-HS samples of corresponding initial humic materials were used. Hydrophobic properties of HS used were characterized using common logarithm of octanol-water coefficient $\lg K_{OW}$ determined directly following the distribution of HS between octanol and water phase by a scintillation phase method according to (2). To characterize surface activity of target HS, a parameter reflecting maximum adsorption of HS in the toluene-water interface Γ_{max} was used as described in (ibid.).

Study of HS interaction with bacteria. Experiments on ^{3}H-HS interaction with bacteria were conducted by growing *E. coli* cells overnight at 37°C in M9 medium supplied with labeled HS at concentrations of 5-50 mg l^{-1} After overnight cultivation, the cells were harvested by centrifuging (5000 rpm, 30 min), and the radioactivity of supernatant was measured. The value obtained was assigned to the equilibrium concentration of HS. Then the supernatant was replaced with the equal amount of M9 media, the cells were re-suspended, and radioactivity of the suspension was measured. That value corresponded to the amount of HS taken up by the cells, i.e. a sum of HS adsorbed on the surface of the cells and HS penetrated into the cells. To determine the amount of HS penetrated into the cells, cell lysis was performed using chloroform followed by centrifugation (5000 rpm, 30 min) to separate debris. The radioactivity of the supernatant was assigned to the amount of HS penetrated the cells. Bioconcentration factor (BCF) was calculated as a slope of the HS taken up by *E. coli* cells vs. HS equilibrium concentration.

Study ob HS interaction with higher plants. Plants of wheat *Triticum aestivum* L. (var. Inna) were used for the experiments. Wheat seeds were germinated at 24°C in the dark for

72 h followed by transferring seedlings into the 0.5 l tanks containing Knopp nutrition solution. After another 72 h plants were transferred into the vials containing HS at concentration 5-50 mg l^{-1}. After 24 h plants were harvested, weighted and subjected to autoradiography. To estimate HS uptake by plants, radioactivity of HS solutions before and 24 h of plant growing was measured using liquid scintillation method.

3. RESULTS AND DISCUSSION

Preparation of tritium-labeled HS and comparative analysis of parent and ^{3}H-HS. Obtained results demonstrated that developed approach allowed synthesis of ^{3}H-HS of high specific radioactivity and yield. For the studied HS value of specific radioactivity varied from 0.14 to 0.63 TBq g^{-1}; yield ranged from 23 to 87% depending HS labeled.

Parent HS and obtained samples of ^{3}H-HS were subjected to SEC analysis with radioactivity and UV detection. It was demonstrated that parent and labeled HS were characterized with the similar UV SEC profiles what was evident for the absence of significant alteration of HS structure due to either tritium introduction or dialysis. On the other hand, similarity of UV and radioactivity SEC profiles of ^{3}H-HS confirmed that introduced tritium was uniformly distributed among HS fractions of different molecular sizes. Therefore, the developed technique of the radioactive labeling of HS with tritium by thermal activation method can be used for the labeling of a broad spectrum of HS of different origin and composition.

Hydrophobicity and surface activity of HS. Determined values of partition coefficients of HS in octanol – water system lgKow for the studied HS varied from -2.98 to -1.95 indicating slight hydrophobicity of HS. At that soil FA were characterized with the lowest hydrophobic properties whereas peat HA possessed the highest values of lgKow. It should be noted that direct determination of HS hydrophobicity was performed for the first time.

Surface activity of HS also depended greatly on HS origin. Among studied HS the most surface active HS was obtained to be peat HA and FA whilst soil FA was showed to possessed negligible surface activity.

The preformed experiments demonstrated clearly that ^{3}H-HS are useful in study of both HS hydrophobicity and surface activity.

Study of HS interaction with bacteria. Obtained results indicated that HS adsorption onto bacterial surfaces can be extensive, and that the sorption behavior depended on a preparation used. HS adsorption onto bacterial surfaces was the highest in case of peat HA, and decreases to negligible amounts of adsorption in case of soil FA. Observed values of bioconcentration factor of HS by E. coli varied in the range 0.9-13.1 l kg^{-1}

depending considerably on the HS preparation. Performed experiments demonstrated also that HS could penetrate into the bacterial cells. Measured amounts of HS penetrated into the cells were 23-167 mg kg^{-1} what were at least 20% of totally sorbed HS and reached maximum of 100% in case with FA. Therefore, the obtained 3H-HS were demonstrated to be a promising approach for study of bacteria-HS interaction.

Study of HS interaction with higher plants. HS uptake by wheat seedlings was characterized by smooth, nonsaturating curves for all the studied HS. The obtained curves of concentration-dependent HS accumulation by wheat seedlings were fitted to Michaelis-Menten kinetics equation allowing determination of Michaelis constant Km and rate of accumulation Vmax. Among the HS studied, the highest Michaelis constant was observed for HMA derived from coal, which was characterized with the lowest molecular weight and the highest contents of quinoic and ketonic groups. On the other hand, a minimum value of Km was also recorded for coal HMA possessing the lowest C/N ratio. Rate of accumulation for the studied HS varied in the range 6-54 mg kg^{-1} h^{-1} and had its maximum in case of peat HA with the highest observed molecular weight.

Performed autoradiography analysis of wheat seedlings treated with ^{3}H-HS demonstrated that roots of the plants were characterized with homogeneous distribution of signal intensity. Distribution of HS within the shoots was also relatively homogeneous except for the tips of the leaves where local increase of signal density was observed. It could be concluded that HS could be taken up through plant roots and moved al least in the xylem with transpiration stream to areas of new growth.

4. CONCLUSIONS

The developed technique of the radioactive labeling of HS with tritium by thermal activation method can be used for the labeling of a broad spectrum of HS of different origin and composition. The obtained labeled HS may be useful for the study of HS behavior in the environment and biological systems.

ACKNOWLEDGEMENTS

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Oxidative Degradation of Chlorophenol by Iron-Porphyrin Catalyst Bound to Humic Acid via Formaldehyde Polycondensation

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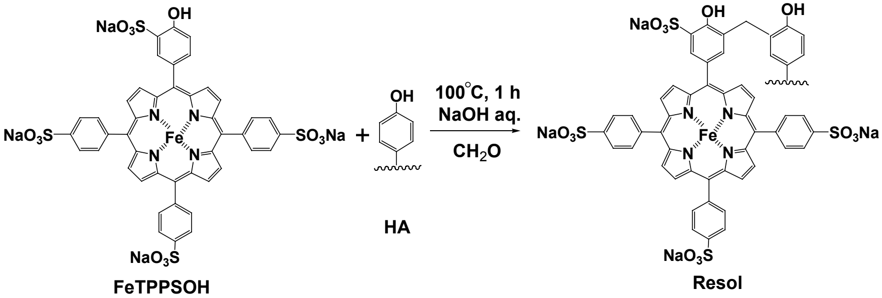
Keywords: humic acid, chlorophenol, catalytic oxidation, iron-porphyrin, polycondensation

1. INTRODUCTION

Iron(III)-porphyrin catalysts have been known to be inactivated by self-degradation in the presence of peroxides, such as $KHSO_5$. We reported that oxidative degradation of pentachlorophenol (PCP) by tetrakis(*p*-sulfonatophenyl)porphineiron(III) (FeTPPS) was enhanced in the presence of humic acids (HAs) (1). This effect can be attributed to the fact that self-degradation of FeTPPS in the presence of $KHSO_5$ is suppressed by the formation of supramolecular complexes via hydrophobic interactions between HA and the sulfonatophenyl groups in FeTPPS (2). However, the extent of supramolecular complex formation may be dependent on the type of HA, because the degree of hydrophobic interactions varies significantly, depending on the polar groups in HAs. Recently, hydroquinone groups were covalently introduced into HA using formaldehyde polycondensation to enhance the reducing abilities of HA (3). The introduction of functional groups into HA is a novel trend in current humic technology. Covalent binding of the iron(III)-porphyrin catalyst to HA would be advantageous, as it would prevent dissociation of the catalyst from the supramolecular complex. Thus, in the present study, FeTPPS was covalently introduced into HA via formaldehyde polycondensation. In addition, the self-degradation kinetics and catalytic activity of the prepared catalysts were examined.

2. MATERIALS AND METHODS

Synthesis of catalysts: An HA sample was isolated from a Shinshinotsu peat soil by a method approved by the International Humic Substances Society. After introducing a hydroxyl group into tetraphenyl porphyrin, this compound was sulfonated in the presence of concentrated H_2SO_4. FeTPPSOH in scheme 1 was obtained by the metalation of $FeSO_4$ in aqueous solution. The formaldehyde polycondensation reaction between HA and FeTPPSOH is shown in Scheme 1. In general, formaldehyde polycondensation using alkaline catalyst can produce the resol-type phenol resin. Thus, we call the FeTPPSOH-HA catalyst in Scheme 1, “resol”. The resol samples prepared were deionized by ultrafiltration (1000 Da), followed by dialysis (500 Da). Finally, powdered samples were obtained by freeze-drying. In the present study, three types of resol catalysts were prepared, and a control sample of HA (HA-RBL) without FeTPPSOH was also prepared. The quantities of FeTPPSOH, HA, and formaldehyde in the catalysts and control are summarized in Table 1.



Scheme 1. Introduction of FeTPPSOH into HA via formaldehyde polycondensation.

Table 1. Quantities of HA, FeTPPSOH and formaldehyde, and the results of elemental compositions for each sample

Samples	HA / FeTPPSOH (mg / mg)	CH_2O (mmol)	%C	%H	%N	%O	%S	%ash	C/N
HA (Untreated)			54.5	5.4	2.2	35.1	0.7	2.2	29.3
HA-RBL (Reaction BL)		6.9	51.3	5.3	2.2	34.9	1.4	4.9	27.3
Resol 1	60 / 30	1.0	41.8	4.4	2.7	34.5	6.8	9.1	18.0
Resol 2	60 / 30	6.9	44.9	4.6	2.6	35.1	5.3	7.6	20.2
Resol 3	20 / 30	6.9	40.5	4.0	3.2	35.4	7.9	8.9	14.7

Self-degradation kinetics of catalysts: Aqueous buffer solutions (pH 6, 0.02 M phosphate/citrate buffer) of FeTPPSOH or resols (10 μM) and $KHSO_5$ (1 mM) were mixed rapidly using a stopped-flow spectroscopic system (UNISOKU Co., Ltd., Osaka). The decrease of absorbance at 395 nm corresponding to the Soret band of FeTPPS and resols was then monitored for 2 s.

PCP degradation: A 2 ml aliquot of a 0.02 M phosphate/citrate buffer at pH 6 was placed in a 20-mL L-shaped test tube. A 2.5-10 μL aliquot of 10 mM PCP in acetonitrile and a 50 μL aliquot of aqueous FeTPPSOH or resols (200 μM) were then added to the buffer solution. Subsequently, a 10 μL aliquot of aqueous 0.1 M $KHSO_5$ was added, and the test tube was allowed to shake in a thermostatic shaking water bath at 25±0.1°C. After a 60 min reaction period, 1 mL of 2-propanol was added to the test solution. To analyze the level of PCP in the test solution, a 20 μL aliquot of the mixture was injected into a JASCO PU-980 type HPLC pumping system. Analytical conditions for HPLC were accord with a previous report (1).

3. RESULTS AND DISCUSSION

Characterization of samples: Table 1 shows the results of elemental analyses. Because of binding to porphyrin including pyrrole ring, nitrogen contents for resol catalysts may be larger than those for HA samples. Thus, C/N atomic ratios for resol catalysts significantly decreased, as compared to the untreated HA and HA-RBL.

Self-degradation kinetics: Figure 1 shows the kinetic curves for the self-degradation of FeTPPS, FeTPPSOH and the resol catalysts (5 μM) in the presence of $KHSO_5$ (500 μM) at pH 6. A_0 and A_t in y-axis represent the absorbance, which corresponds to the catalyst concentration, before and after the addition of $KHSO_5$, respectively. Pseudo-first-order kinetic constants (s^{-1}) were calculated from the kinetic curves: 14.1 for FeTPPS, 8.7 for FeTPPSOH, 6.9 for resol 1, 6.6 for resol 2, 7.8 for resol 3. The kinetic constant for FeTPPS was much larger than those for FeTPPSOH and resol catalysts, indicating that FeTPPS is less stable than the FeTPPSOH and resol catalysts. The kinetic constant for FeTPPSOH was larger than those for the resol catalysts, among which the kinetic constants for resols 1 and 2 were larger than that for resol 3. Thus, resols 1 and 2 appear to have higher catalytic activity than FeTPPS, FeTPPSOH and resol 3.

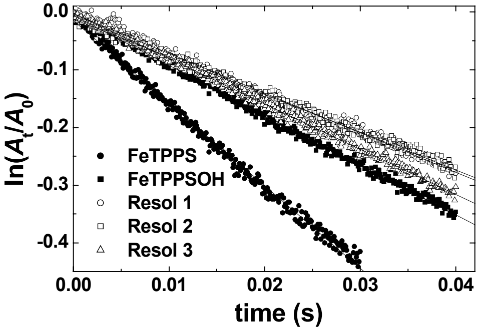


Figure 1. Self-degradation kinetic curves for FeTPPS, FeTPPSOH and the resol catalysts. [Catalysts], 5 μM; [$KHSO_5$], 500 μM; observed wavelength, 395 nm.

Catalytic activity: Figure 2 shows the influence of substrate concentration ($[PCP]_0$) on the percent PCP disappearance. For all catalysts, the percent PCP disappearance decreased with increasing $[PCP]_0$. At $[PCP]_0$ = 25, 37.5 and 50 μM, PCP disappearance was enhanced with the resol catalysts, although this effect was less with resol 3 than with resols 1 and 2. These results can be attributed to the fact that self-degradation of FeTPPSOH is retarded by binding to HA via formaldehyde polycondensation.

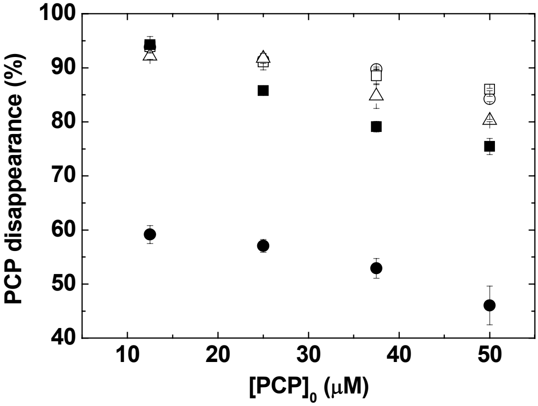


Figure 2. Influence of substrate concentration ($[PCP]_0$) on %PCP disappearance. [Catalysts], 5 μM; [$KHSO_5$], 500 μM; pH 6.0; and reaction time, 60 min. Symbols: ○ Resol 1, □ Resol 2, Δ Resol 3, ■ FeTPPSOH, ● FeTPPS

4. CONCLUSIONS

FeTPPSOH was introduced into HA via formaldehyde polycondensation. The prepared resol catalysts exhibited retarded self-degradation, compared with degradation in FeTPPSOH alone. Percent PCP disappearance, an index of catalytic activity, was 5-10% greater with the resol catalysts. Thus, resol catalysts effectively enhanced catalytic activity because of the reduced self-degradation.

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Biorefinery Chars as Potential Sources of Soil Humics

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Keywords: Biorefining, char, humified char, levulinic acid, formic acid, NMR

1. INTRODUCTION

The reliance of the transport sector on petroleum-derived fuels is well documented. However, the fact that more than 95% of all industrial chemicals are oil-sourced is of even greater importance since we have now passed ‘peak oil’. That is of particular relevance to Ireland whose modern economy has been driven by a thriving pharmaceutical sector. It is important therefore that we take advantage of our national assets, principally our soils and climatic conditions that have a huge potential for the production of the lignocellulose biomass that provides the raw materials for second generation biorefining technologies. Sugars, from carbohydrate components in woody plants (*ca* 45% cellulose and 25% hemicellulose), are liberated by these technologies and their fermentation, or their degradation as in the Biofine process (Biofine Technologies Inc, Walton, MA, USA), provides platform chemicals that serve as starting materials for the synthesis of fuel additives, and a wide range of industrial products such as furfural, hydroxymethyl furfural, levulinic acid (LA), formic acid, and solid biorefining residuals. This contribution focuses on biorefinery residuals that can give rise to chars and humified chars in soil.

The Biofine Process:

Biofine Inc. has developed a high temperature, dilute acid hydrolysis process for the fractionation of abundantly available lignocellulosic biomass into highly versatile platform chemicals (1). The primary “fractions” of the process are levulinic acid (LA) – a five carbon keto-acid [$CH_3C(O)CH_2CH_2COOH$], and formic acid ($HCOOH$). LA is a highly versatile platform chemical that can be converted to a wide range of environmentally acceptable chemicals and fuel additives. Its derivatives range from petroleum and diesel oxygenates to biodegradable pesticides, plastics and solvents, and solid carbonaceous residual materials (products of secondary reactions formed during the degradation processes and from the altered lignin, tannins, cutins, cutans, waxes and other non-carbohydrate components of plants) suitable for gasification to a high quality synthesis gas. The solid residual material from the gasification process will be a char product that can have soil ameliorating properties similar to those attributed to the chars responsable for the fertility associated with the Amazonian Dark Earths (ADE) or the Terra Preta (TP) soils of the Amazon region. The first commercial Biofine plant is operational at Caserta, Italy.

2. MATERIALS AND METHODS

Biofine biorefinery residuals from the processing of wheat straw (*Triticum aestivum* L) and of paper sludges were analyzed by solid-state CPMAS ^{13}C NMR spectroscopy, FT-IR, and thermogravimetric (TG) analyses. The NMR experiments used a VARIAN INOVA spectrometer at ^{13}C and ^{1}H frequencies of 100.5 and 400.0 MHz, respectively. Cross-polarization times of 1 ms, acquisition times of 13 ms, and recycle delays of 500 ms were used. and by thermogravimetric analyses. For comparisons, NMR spectra are given for charred mosses, and for deposits from the chimney of an oil fired domestic heater, and thermograms are presented also for lignin and miscanthus (a promising lignocellulosic biorefinery feedstock).

3. RESULTS AND DISCUSSION

Product yields from previous technologies for LA production were low (around 3% by mass). The Biofine process, with an efficient reactor system and the use of polymerisation inhibitors, achieves from cellulose LA yields of 70-80% of the theoretical maximum. That translates to the conversion of *ca* 50% of hexose sugars to LA, 20% to formic acid, and 30% to biorefinery residuals. The mass yield of furfural from pentose sugars is also ca 70% of the theoretical value of 72.7%, equivalent to 50% of the mass. The remainder is incorporated in the residuals.

The FT-IR spectra (not presented) showed that some features of lignin were preserved in the straw residuals, and these were absent from the products from paper sludge. The NMR spectrum of the residuals from the wheat straw confirm that some features of lignin structures survived the biorefining process (as evidenced by the resonances at *ca* 56 ppm – for methoxyl, and at *ca* 145 ppm – for O-aromatic functionality). The spectrum for the paper sludge residuals indicates that aromatic functionalities were formed in the biorefining process, and there is evidence for O-aromatic functionality. Surprisingly a considerable amount of aliphatic hydrocarbon was formed. The resonance at *ca* 75 ppm suggests that a small amount of carbohydrate survived the degradation.

The NMR spectrum for the hydrocarbon fuel clearly shows the symmetrical resonance peak characteristic of chars, as seen for the spectra for the coarse and fine moss chars.

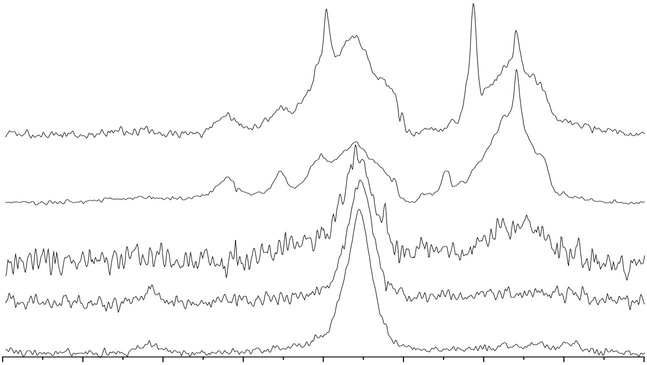

Figure 1. CPMAS ^{13}CNMR spectra of biorefinery residuals from wheat straw and paper sludge, of residues from domestic fuel burning, and of char from moss.

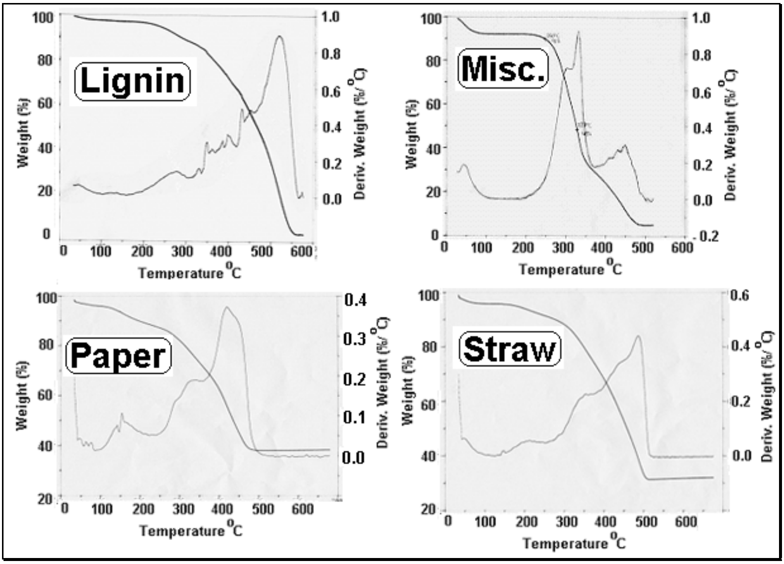

Figure 2. Thermograms and their first derivatives for lignin, miscanthus (Misc.), and biofinery residuals from paper and wheat straw feedstocks.

The broad resonance in the aliphatic hydrocarbon region indicates that the combustion was not efficient. The TG data, and especially the first derivatives, clearly show distinct differences between the thermograms for the lignin, and those for the miscanthus, and for the biorefinery residuals from the paper sludge and the wheat straw. The thermograms for the straw and paper show features in the 300-400°C range that correspond to those for the major components of miscanthus (with *ca* 70% carbohydrate), and the lesser feature at 400-500°C for miscanthus corresponds to the major peaks in the biorefinery products. That peak will have some characteristics of (altered) lignin, as indicated by the NMR spectrum for the straw residuals. However, it can be expected that, in the case of the residuals materials, it will include features for condensation products formed during the biorefining process.

4. CONCLUSIONS

It is likely that second generation biorefining technologies will give rise to major industries in the future as alternatives are sought for dwindling and increasingly expensive petrochemical resources. Very considerable amounts of biorefinery residuals will be generated in the biorefining processes, and these can be expected to have compositions similar to those obtained from the straw and paper sludge subjected to the Biofine process. These residuals when subjected to gasification processes can provide the energy required to run the biorefineries. The residual chars from gasification can be expected to have compositions similar to those from the moss chars shown. These chars will have very desirable environmental significances if they should have agronomic properties similar to those that were used as amenders to the TP soils in pre-Columbian times. Oxidation of peripheral aromatic groups in the fused aromatic structures can be expected to take place slowly in the soil to provide products that satisfy the operational definitions for humic substances. The chars and their humified products can be expected also to have value when considerations are given to carbon credits.

ACKNOWLEDGEMENTS

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Synthesis of Alkoxysilylated Humic Derivatives with Different Modification Rate Capable of Self-Adhering to Mineral Surfaces

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Keywords: organosilans, humic, silica gel, modification, barrier

1. INTRODUCTION

The effective method to purify ground water is installation of Permeable Reactive Barrier (PRB). A PRB is a subsurface wall of reactive permeable media emplaced across the flow path of a contaminant plume. The plume is allowed to migrate passively through the PRB and in the process contaminants are precipitated, sorbed, or degraded. A typical PRB is costly to install but economical to maintain. Much of the installation cost is related to the excavation of aquifer material that is then replaced with reactive porous media; furthermore, these systems are typically over-designed to address uncertainties in groundwater flow and to accommodate an anticipated loss of treatment efficiency with time. Humic-based materials show considerable promise as refractory and inexpensive reactive components for PRB. Due to a wide variety of functions, humic substances (HS) can bind both heavy metals and organic contaminants. The contaminants bound into humic complexes become non-toxic that causes a net reduction in toxicity of polluted environments. The humic-coatings immobilized on the surface of granular support could provide a very promising medium for sequestering contaminants dissolved in the ground water. However, the naturally occurring HS do not produce covalent bonding with granular aquifer support.

The goal of this work was to prepare the water-soluble derivatives of HS that have been specifically modified to adhere to the mineral matrices which would facilitate in-situ installation of humic barrier. A new approach was used for this purpose. It is aimed at modification of carboxyl groups available within HS with 3-amino-propyltrimethoxy-silane (APTS). To keep intact the intrinsic reactivity of HS with respect to metals, the derivatives with different modification rates of carboxyl groups were obtained. Their capability to adhere to the surface of silica gel was investigated.

2. MATERIALS AND METHODS

Leonardite humic acids – the main commercial source of humic acids - were used for all modifications. They were isolated from the commercial potassium humate (Powhumus, Humintech Ltd., Germany) and designated CHP. 3-amino-propyltrimethoxy-silane (APTS) was used for treatment of CHP. The choice of APTS was provided by the presence of reactive amino groups in its structure which can yield amide bonds upon reaction with carboxyl and carbonyl groups (1, 2).

A weight of HA (1 g) was stirred with dry dimethyl formamide (DMF) and the calculated amounts of silylation agent (APTS) were added to this mixture under continued stirring to yield the derivatives with 5, 10, 20, 50, 100 and 200% modification degree. The necessary amounts of APTS were calculated based on the content of carboxyl groups in the CHP, which accounted for 3.5 mmol/g. Given the molecular weight of APTS of 180 g/mol, to obtain the derivatives with different modification degree, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 g APTS per one gram of CHP were used. The reaction was carried out at 120°C for 20 hours. Then, DMF was evaporated and the derivative was dried in vacuum under heating at 40°C. The obtained derivatives were designated as CHP-APTS-X, where X – is a modification rate.

The sorption of modified HS on mineral support was studied using silica gel as example of mineral support. For this purpose, the solutions of obtained derivatives in water at concentration of 5 g/l were prepared and aliquots of 10 mL were added to 0.1 g of silica gel. A decrease in derivative concentration in solution was measured using UV-vis spectrophotometry. The carboxylic acidity was determined using calcium acetate method.

3. RESULTS AND DISCUSSION

The obtained derivatives were characterized using elemental analysis, titrimetric techniques, SEC, ^{13}C NMR and FTIR spectroscopy. FTIR spectra of the obtained derivatives are shown in Fig. 1. They were normalized to the intensity of $–CH_2$- peaks (2920 cm^{-1}) to provide for better comparison. It can be seen that along with an increase in the modification degree, the intensity of COOH-peaks (1720 cm^{-1}) decreased, whereas the intensity of amide bonds (1690 cm^{-1}) increased. This shows that the developed approach allows to incorporate into the HA structure controllable amount of the alkoxysilyl groups.

The content of COOH groups in the original and modified HS are presented in Fig. 2. The content of COOH groups is a particularly important parameter for APTS-modified humic materials as it provides a direct estimate for the amount of residual carboxyl groups non-reacted with APTS. Hence, it can be used to calculate the modification degree as a ratio of the carboxyl-group reacted with APTS to the total amount of carboxyl groups.

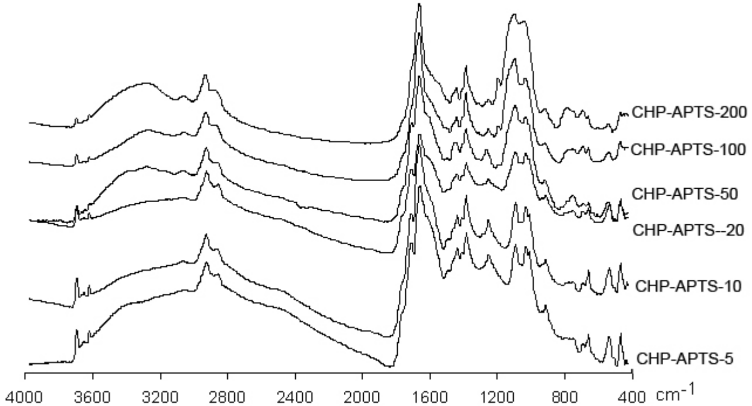

Figure 1. FTIR spectra of APTS modified CHP with different modification grade.

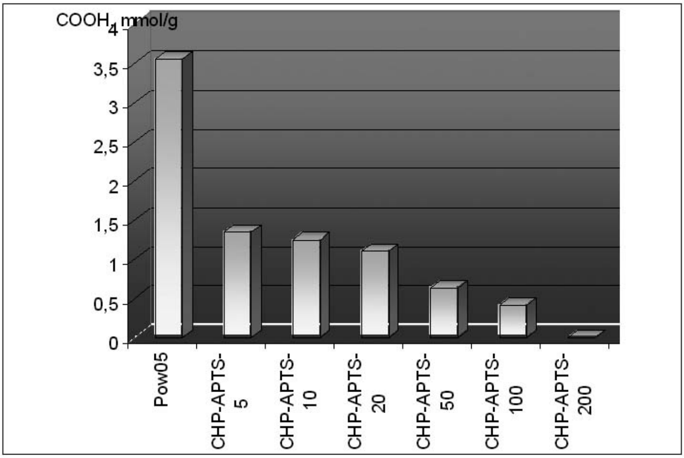

Figure 2. The concentration of COOH groups in original and modified HA.

The sorption of the obtained derivatives onto silica containing minerals was studied using batch experiments. It was shown that the sorption on silica gel depended on the modification rate of CHP-APTS. The amount of immobilized CHP (mg) on 1 g of SiO_2 is shown in Fig. 3. It can be seen that the sorption affinity of the derivatives increased drastically starting with the modification degree of 20% and reached its maximum value at the modification degree of 100%.

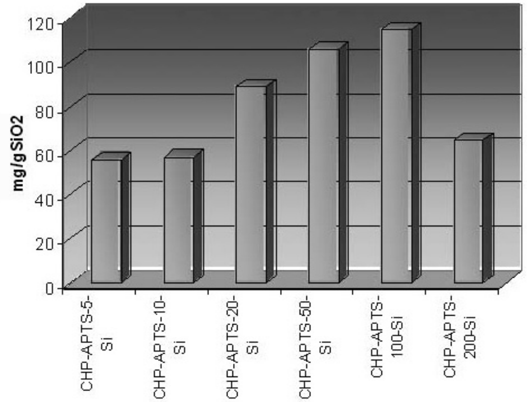

Figure 3. The amount of immobilized CHP (mg) on 1 g of SiO_2.

4. CONCLUSIONS

The dependence of adhering ability of the alkoxysilyl-derivatives of HS on the modification degree of carboxyl-groups was demonstrated. The maximum sorption affinity was observed for the derivatives with 100% modification, whereas starting with 20% modification the remarkable increase in adhering ability was observed as compared to the parent HS. The conclusion was made that 20%-modification degree can be considered as an optimum modification rater which provides for a reasonable adhering ability and is much closer to the parent HS by the properties as compared to 100% modified HS.

ACKNOWLEDGEMENTS

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Hybrid Sorbents on the Basis of Magneto-Active Nanoparticles and Humic Substances

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Keywords: humic acids, magneto-active nanoparticles, magnetite, sorbent

1. INTRODUCTION

Problem of selective binding chemical and radiochemical contaminations became especially urgent during last decades. Nowadays, the most efficient way of the binding exo-toxicants of different chemical origin and radio-nuclides is using selective sorbents of inorganic or polymeric origin. Introduction of inexpensive and active agents into the places of storage or formation the wastes, should be one of the methods for the cleaning of the environment. Lately, nano-structural materials find a wider application in the processes of waste processing and decontamination (1, 2). In order to develop the sorbents with the specified properties, it is rather prospective to use humic substances (HS) and their derivatives. The prospects for practical application of HS and their derivatives as sorbents is determined by huge resources of humus-containing materials, which include brown coal, peat, sapropel and other causto-biolytes. The aim of the work is to prepare and characterize of nanohybrid sorbents on the basis of HS and magneto-active nanoparticles.

2. MATERIALS AND METHODS

Commercially available potassium humate (Powhumus) produced by Humintech Ltd, Germany as well as sodium humate (from the Sakhalin coal, pH of water solution 9.5) and as humic acids after electrodialysis (nonsoluble in water) were used to obtain hybrid magnetic active nanocomposites.

Mossbauer spectra were recorded on a spectrometer (WISSEL) with a Co^{57}(Rh) source in the constant acceleration regime. The calibration by rates was performed relative to the spectrum of metallic α-Fe. The phase composition analysis of the nanocomposites obtained was performed using X-ray diffractometry on a DRON-UM-2 diffractometer using

$Cu(K_\alpha)$ radiation. Characterization of samples by transmission electron microscopy (TEM) was carried out by using a Hitachi H-7000 microscope.

3. RESULTS AND DISCUSSION

We have investigated several approaches for the preparation of magneto-active nano-hybrid composite, including *i*) *ex situ* method which consists of the precipitation of magnetic particles from Fe(II) and Fe(III) salts by addition of NH_4OH followed by their incorporation into the HS matrix and *ii*) a chemical precipitation method *in situ* when the magnetic particles are grown within the HS matrix. According to X-ray diffractometry studies the major phase formed during a chemical coprecipitation in the presence of humic acid in situ is a magnetite Fe_3O_4 (Fig. 1). As it is well-known HS have strong affinity to the surface of metal oxide particles (3). The absorption of humic acid on magnetic particles surface leads to an enhanced electrostatic and steric stabilization of particles due to the absorbed layer of highly charged macromolecular compounds. These syntheses reliably produced systems that were stable in dry and colloidal state over periods of more than 3 months as evidenced by the consistency in their magnetic properties and a lack of observable precipitation.

We have synthesized in the first time the Fe_3O_4 nanoparticles and humic acid hybrid nanocomposite using mechanochemical method. The advantage of such approach is that mechanochemical technique is particularly suitable for mass production because it is a simple and inexpensive process. The average crystal size of the nanoparticles calculated from diffraction peak half-widths according to Scherrer's equation for the Fe_3O_4 nanoparticles and nanocomposites obtained was 16 nm. These results were agreed with the Mossbauer data (Fig. 2) and electron microscopy studies (Fig. 3).

The humic acid macromolecule binds to the particles just after nucleation of the Fe_3O_4 nanoparticles preventing further growth. The small size of the nanoparticles results in a very large exposed surface area for heavy metals absorption that is achieved without use of porous materials that introduce a high mass transfer resistance.

Some magnetic characteristics for the Fe_3O_4 – HS hybrid nanocomposites are presented in Table 1. The hysteresis loops are closed and symmetrical versus origin of the coordinate system. Shape of the loops evidences the ferromagnetic character of the material desirable for their application to separation.

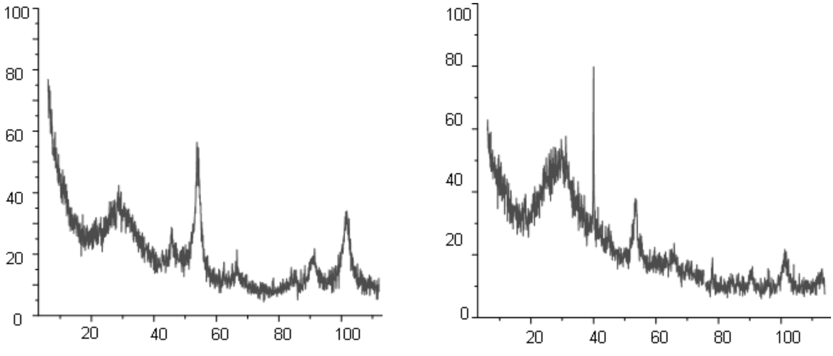

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Figure 1. XRD patterns of (a) the Fe_3O_4 nanoparticles-humic acids nanocomposite obtained by chemical coprecipitation method in situ and (b) the Fe_3O_4 nanoparticles-humic acids nanocomposite obtained by mechanochemical method.

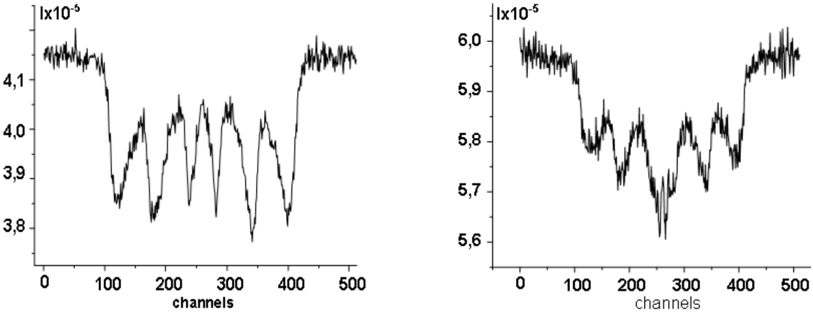

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Figure 2. Mossbauer spectra of (a) the Fe_3O_4 nanoparticles, (b) the Fe_3O_4 nanoparticles-humic acids nanocomposite obtained by mechanochemical method.

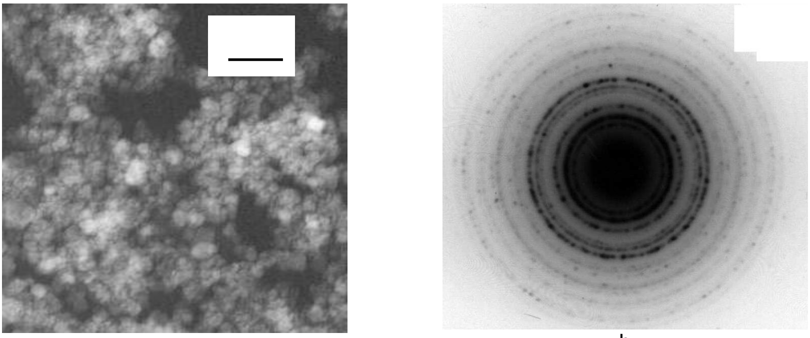

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Figure 3. TEM images of the humate – Fe_3O_4 hybrid nanocomposite obtained by a chemical precipitation (a) and electron diffraction (b).

Table 1. Magnetic properties of native magnetite and HS nanocomposites

Sample	Maximum magnetization, emu/g	Remanent magnetization, emu/g	Coercive force, Oe	Squareness of hysteresis loop
Fe_3O_4	33.6	4.14	86	0.12
Fe_3O_4 + potassium humate (*)	4.99	0.8	91.5	0.16
Fe_3O_4 + potassium humate (**)	6.81	1.1	89	0.16
Fe_3O_4 + humic acids (*)	3	0.39	90	0.13
Fe_3O_4 + humic acids (**)	7.28	1.21	91	0.17

(*) – mechanochemical method, (**) – co-precipitation

4. CONCLUSIONS

The methods for the preparation of new types of sorbents – resistant hybrid nanocomposites on the basis of magnetite and humic substances were proposed**.** Such sorbents of complex action give a possibility to bind radionuclides and heavy metals by different type of functional groups with the subsequent magnet separation and utilization.

ACKNOWLEDGEMENTS

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Preparation and Characterization of Biodiesel Industry Waste Partially Carbonized Material in Order to Produce an Organic Soil Conditioner

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Keywords: Biodiesel industry waste, partly carbonized material, soil organic conditioner

1. INTRODUCTION

This work seeks to determine the best methods of production of partly carbonized waste of biodiesel industry – castor (*Ricinus communis*) meal – aimed at obtaining material comparable to organic matter of soils *Terras Pretas de Índios* to be used as a soil conditioner. The subject *Terras Pretas de Índios* has aroused interest in the international scientific community that in recent years several articles and letters of Nature and Science were devoted to the subject (e.g. 1, 2, 3) and The American Association for the Advancement of Science (AAAS) organized a symposium in their annual meeting in 2006 with the title: Amazonian Dark Earths: New Discoveries.

This work corresponds to chemical studies in the sense of scientific knowledge and technological development and innovation in the use of organic by-products, especially from the biofuels industries, seeking the excellent performance of the so-called *Terras Pretas de Índios* of the Amazon (4). In these work the carbonized materials were obtained by controlled heating of different mixture of biodiesel industry tort with glycerol, starch as industrial Brazilian by-product, and potassium sulfate (K_2SO_4) as a macronutrient addictive. The obtained products were analyzed by EPR, NMR, and DRUV-Vis spectroscopy.

2. MATERIALS AND METHODS

Samples preparation. In a closed furnace the samples were heating from the room temperature (~300 K) to 773 K with the heating velocity of 1 $^0C\ min^{-1}$, and lifted at 773 K for two hours. After returned to the room temperature the samples were recovered, milled in a pot mill and sieved in an 80 meshes sieve. Details about the sample composition can be found in the Table 1.

NMR. Solid-state ^{13}C NMR experiments were carried out using a VARIAN INOVA spectrometer at ^{13}C and ^{1}H frequencies of 100.5 and 400.0 MHz, respectively. The techniques used were: Variable Amplitude Cross-Polarisation (VACP) and Recoupled Dipolar Dephasing (DD) experiments, both with Magic Angle Spinning (MAS) at spinning frequencies of 13 kHz. Typical cross-polarisation times of 1 ms, acquisition times of 13 ms, and recycle delays of 500 ms were used. The DD experiments were carried out with a dipolar dephasing time of 67 μs.

EPR. EPR spectra of the powdered samples were registered at room temperature (~300 K) in quartz tubes. A Bruker ESP 300E spectrophotometer (LABEPR/DQ/UFPR) was used, operating at a frequency of 9.7 GHz (X-band), with a 100 kHz modulation frequency, 2.024 G modulation amplitude and ~20 mW microwave power.

DRUV-Vis. The DRUV–Vis analyses were performed on solid samples contained in a 26-mm-diameter sample holder, operating in the range 190 – 900 nm, using a Shimadzu UV-2401 PC spectrophotometer equipped with a Model 240-52454-01 integration sphere.

3. RESULTS AND DISCUSSION

NMR spectra. The VACP ^{13}C NMR spectra (Figure 1) present the typical pyrogenic carbon signal (condensed aromatic signal at 127 ppm retained in the DD spectra), in the aromatic region have also a shoulder in the O-aryl region (152 ppm). This signal can arise from phenolic or aryl-ether, but in this case the aliphatic radical must be greater that methyl, because it don't have the corresponding methoxyl (~56 ppm) signal in DD spectra. Additionally some residual aliphatic signals (60-0 ppm) remain after the carbonization. The differences among the samples are very subtle, but the ones without castor cake present more methyl (18 ppm), methylene (36 ppm) and O-aryl (152 ppm) groups.

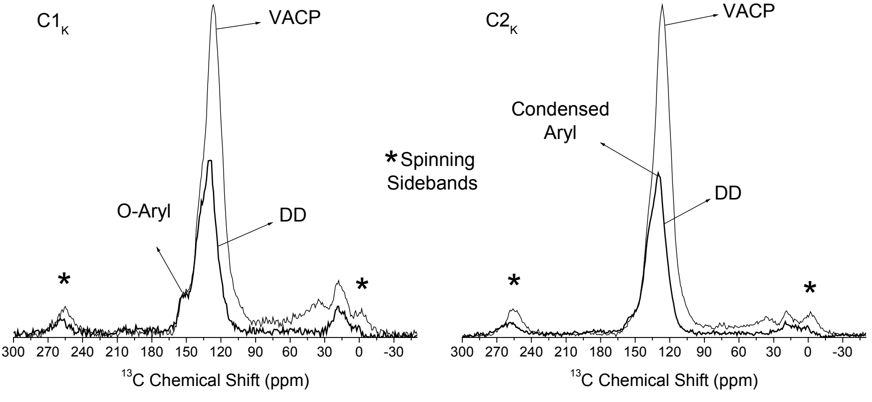

Figure 1. Full VACP and corresponding DD spectra of char samples.

Table 1. EPR parameters obtained of the studied samples

Sample	Spins g^{-1}	g-factor	ΔH (mT)
C1 (starch : glycerol, 7:3)	5.59×10^{18}	2.0040	0.54
C2 (starch : glycerol : castor cake, 7:3:1)	6.17×10^{18}	2.0044	0.43
$C1_K$ (starch : glycerol : K_2SO_4, 7:3:1)	2.95×10^{18}	2.0044	0.53
$C2_K$ (starch : glycerol : castor cake : K_2SO_4, 7:3:1:1)	5.05×10^{18}	2.0042	0.40
Castor cake	6.34×10^{15}	2.0049	0.70

The non charred castor cake sample presents a typical organic free radical signal (Figure 2) and the concentration of these organic free radicals, as determined by EPR analysis, increases sharply (three magnitude orders greater) with the carbonization (Table 1). The values obtained in the charred samples are in the range of those already reported for soil HA with large humification degree. On the other hand, the g-factor and peak-peak linewidth decrease with the carbonization. The greater linewidth of non charred sample is partially due to occurrence of two slightly different organic free radical centers (unsymmetrical signal and second derivative – Figure 2).

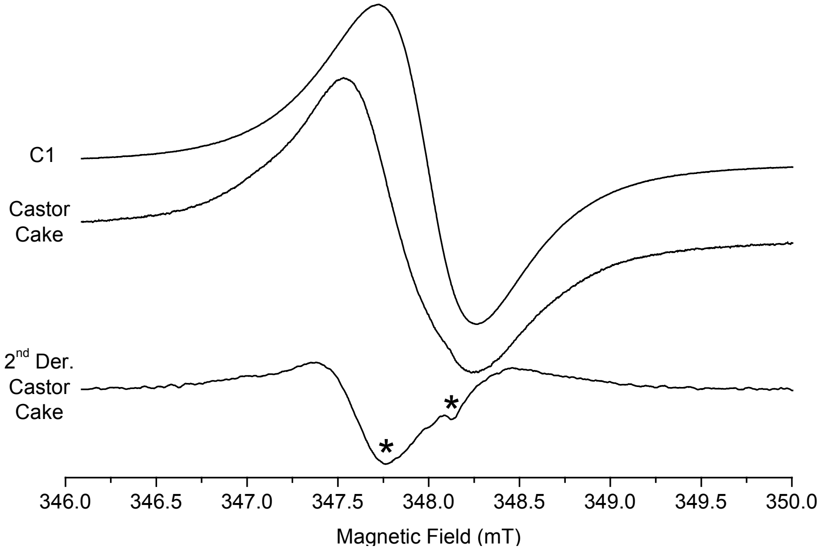

Figure 2. EPR spectra of castor cake and char samples. The two partially overlapped signals in the castor cake sample are star marked in the second derivative spectra.

The samples with K_2SO_4 ($C1_K$ and $C2_K$) presented a smaller organic free radical concentration than the corresponding samples without this salt; this could be explained by their greater ash content causing a diluting effect. On the other hand, the samples with castor cake in the mixture (C2 and $C2_K$) presented a greater organic free radical concentration.

The DRUV-Vis spectrum (data not showed) of the castor cake sample suggests the existence of aryl-hydroxyl pigments, such as anthocyanins (reflectance bands at 219 and 264 nm (5)). The carbonization produces a very black char, with an almost equivalent reflectance in the whole visible spectra. This feature can be attributed to the condensed aromatic structure of pyrogenic carbon, as indicated by the NMR data showed.

4. CONCLUSIONS

The controlled heating of the mixture of industrial Brazilian by-products, biodiesel industry castor cake, with glycerol and starch produced a material with some characteristics of organic soil conditioner. EPR, NMR and DRUV-Vis spectroscopy, as spectroscopic technique to analyze solid state samples, were vital to the obtained product characterization.

ACKNOWLEDGEMENTS

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Interaction of Modified Humic Substances with Np(V): Influence of Monomer Nature on Redox Properties of Humic Substances

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Keywords: neptunium, humic, hydroquinone, modification, reduction, kinetics

1. INTRODUCTION

The complexation of radionuclides with humic substances (HS), which are ubiquitous in the environment, is recognized as an important factor for the safety assessment of geological disposal of radioactive wastes. HS possess a whole variety of functional groups with prevailing contribution of carboxyls and hydroxyls. This provides for their redox and complexation properties. As a result, HS impact substantially speciation of heavy metals and radionuclides in the contaminated environments. Interactions of HS with neptunium (Np) are of particular concern due to high radiotoxicity and long half-life of this actinide. The most stable oxidation state of neptunium is (V) presented by dioxocation NpO_2^+. The latter has a low ion charge which results in high migration ability of these species. Interactions with mineral phases, complexation with different ligands and redox transformation can strongly affect transport of Np(V). Besides inorganic ligands such as carbonate, sulfate and phosphate, HS play an important role in speciation of Np in the environment. Most of the models of HS–metal ion interaction assume that carboxylic groups act as major complexing sites for metal ions (1, 2). At the same time, phenolic and quinonoid groups are considered to be responsible for redox properties of HS (3). In our previous publication (4) we have demonstrated that incorporation of additional hydroquinone groups into backbone of leonardite humic acids (HA) resulted to a substantial increase in reducing ability of the humic materials with respect to NpO_2^+.

The goal of this research was to find out if the kinetics of this reaction can be improved by changing structure of the quinonoid center, nominally, by using methyl-substituted hydroquinone.

The idea behind using the substituted hydroquinone for improving kinetics was the known fact that the presence of methyl-substituent in the hydrioquinone ring hinders formation of quinhydrone (5). The stability of quinhydrone slows down substantially reactions with participation of non-substituted hydroquinones.

2. MATERIALS AND METHODS

All experiments were performed in Milli-Q-water (Milli-RO/Milli-QSystem, Millipore, France) and in a glove box filed with nitrogen (99,9%). All reagents were of analytical grade. The methods of HS modification and further characterisation have been previously described in details by Perminova et al. (6). Oxidative polymerization with hydroquinone and 2-methyl-hydroquinone was used to prepare hydroquinone- and 2-methyl-hydroquinone derivatives of HS. Leonardite HA was used as parent humic material.

Study of Np(V) reduction under anoxic conditions was performed according to method published elsewhere (4). Np(V) stock solution was prepared by dilution of the NpO_2NO_3 solution (RIAR, Dimitrovgrad, Russia). Absorption spectra were collected using UV/Vis-NIR spectrophotometer (Cary-50, Varian) outside the glove box in the wavelength range between 950÷1030 nm. Absorption maxima for free neptunoyl and complexed form were detected at 980,9 ± 0.2 nm and 987 nm, respectively (7).

The experimental concentrations of Np(V) and HS were maintained constant $6{,}62 \cdot 10^{-5}$ M and 0,5 g/L, respectively; pH values was kept constant to 4.4±0.1.

3. RESULTS AND DISCUSSION

For the derivative characterization the methods of elemental analyses (C, H, N), acidic group analyses, capillary zone electrophoresis, IR- and NMR-spectroscopy were used. To quantify redox properties of HS redox capacities of HS were determined using ferricyanide as a oxidizing agent (8). Results of HS redox capacity determination are shown in Table 1. Among modified HS sample CHP – HQMe was of maximal redox capacity that decreases in the following order: CHP-HQMe > CHP-HQ > CHP. From this point of view we expected sample CHP-HQMe to be the most effective towards Np(V) reduction.

Table 1. Redox properties of HS

Humic Substances	Redox capacity, mmol/g	Residual of Np(V), %
CHP	0.20	70
CHP–HQ	1.32	35
CHP–HQMe	1.55	45

Figure 1 represents kinetic curves for Np(V) reduction by humic derivatives. Np(V) content reduces in time for all HS, even for parent HA. We tried to estimate the reaction order of Np(V) reduction. The best approximation has been got for the kinetics of first order reaction with respect to neptunoyl-cation. In frames of this approximation, concentration of

Np(V) should decrease in time according to logarithmic law. Correlation coefficient (r^2) for all cases of linearization ranged from 0.96 till 0.84.

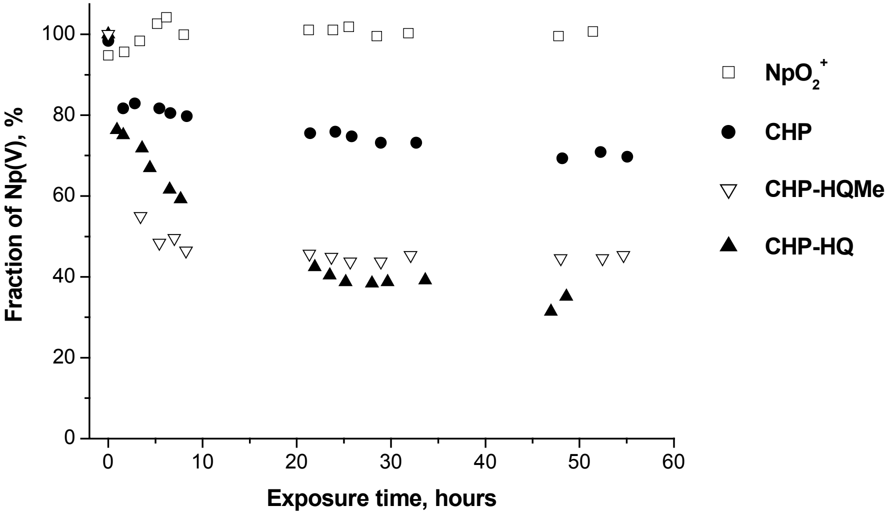

Figure 1. Np(V) reduction by modified HS; C(Np) = 6,62·10^{-5} M, C(HS) = 0,5 g/L, pH 4,4±0.1, I = 0 M (no background electrolyte was used).

One of the important factor in quantification of Np(V) reduction process is the residual of Np(V) in the system after equilibration. For all derivatives equilibration time was considered to be 25-30 hours. Table 1 summarizes final content of Np(V) fraction: sample CHP-HQ showed the most complete reduction of neptunium. It probably can be because the stability of hydroquinone gomopolymer is higher than that of substituted derivatives. Therefore kinetics of Np(V) reduction by CHP-HQMe is higher, but after equilibration residual of Np(V) in the system with CHP-HQ is lower.

4. CONCLUSIONS

Incorporation of 2-methyl-hydroquinone into humic backbone has improved substantially kinetics of Np(V) reduction as compare to hydroquinone-modified HS. Distinctive relationship between a value of the rate constant and structure of the quinonoid reaction center demonstrates that chemical modification is an excellent tool for preparing humic materials with the tailored properties. These results also show the important application of specifically customized HS as a redox-agents in remediation technologies concerning redox sensitive metal ions and organic contaminants.

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Microbial Oxidation of Humic Substances: Agricultural Consequences

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Keywords: redox, nitrate, bacteria

1. INTRODUCTION

Humic substances (HS) are a ubiquitous, recalcitrant, and structurally diverse class of organic material arising from degradation and condensation of plant and microbial biopolymers. Previous studies have discovered a number of bacteria capable of catalyzing the oxidation of reduced functional moieties within HS to their oxidized state, thereby providing electrons for anaerobic respiratory processes (1). Hydroquinone moieties, oxidized to their corresponding quinones, have been implicated as the main mediators of such reactions (1-3). Microbial oxidation of model hydroquinone-containing compounds and HS has been shown to support anaerobic respiratory processes such as reduction of nitrate to dinitrogen gas (denitrification) and reduction of perchlorate to chloride (1,2). In all cases, respiratory electrons are garnered from oxidation of hydroquinones to quinones, rather than degradation of organic molecules.

The redox interplay between reduced HS and microorganisms is likely important in agricultural soils where fixed nitrogen retention is critical and HS are prevalent. On the one hand, microbial oxidation of HS may support loss of nitrate from agricultural soils via respiratory denitrification reactions. On the other hand, microbial oxidation of HS impacts the oxidation state of the humic molecule, which in turn influences the ability of HS to bind chemicals of interest, such as organic pollutants and heavy metals (3). Given frequent treatment of agricultural soils with pesticides and herbicides, it may be important to study the redox transitions of HS in such environments, which may influence the fate, transport, bioavailability, and bioaccumulation of these common environmental pollutants. In this work, we illustrate the prevalence of HS-oxidizing, denitrifying bacteria in various agricultural soils. Dynamic, flow-through column systems were used to model microbial oxidation of HS under anaerobic, denitrifying conditions. Resulting geochemical data indicated that redox state of HS within columns could be tracked as a function of space and time, and corresponded stoichiometrically with observed levels of nitrate reduction performed by mixed microbial communities. Similar columns containing model hydroquinones as an electron donor indicate that the structure of microbial communities undergoing hydroquinone oxidation differs from those oxidizing electron donors such as acetate. This data suggests that HS oxidizing microbial communities may be distinct from communities oxidizing other electron donor sources.

2. MATERIALS AND METHODS

All media and media components were prepared as previously described (1). Column studies were performed with N_2-sparged, phosphate-buffered water as a mobile phase. For HS media, Aldrich humic acids were added to media in question, and chemically reduced where noted (1, 3). Hydroquinone-containing media were generated by chemical reduction of 2,6-anthraquinone disulfonate (AQDS) to the hydroquinone (AHDS) (1, 2). Most probable number (MPN) series for HS-oxidizing bacteria in agricultural soils were prepared as described in (1). All upflow columns were constructed with electron donor and acceptor containing media being continually injected. Anions, redox state of HS or AHDS (1-3), and other parameters were measured at sample ports spaced out along the column length (1 being closest, 4 being furthest away from donor point source). At the end of the experiment, columns were deconstructed and sectioned for subsequent microbial community analysis. AHDS columns constructed from a sand matrix and inoculated with 10% w/w Strawberry Creek water were provided with nitrate and perchlorate as respiratory electron acceptors. Electron acceptor utilization profiles were compared between acetate and AHDS electron donor treatments. Humic columns were constructed from a glass bead matrix, and inoculated 10% w/w with soil from a corn plot used in MPN analysis. Nitrate was the only amended electron acceptor. Columns treated with acetate, oxidized HS, reduced HS, and lacking electron donor amendment were compared.

3. RESULTS AND DISCUSSION

Soils collected from a Nebraska farm illustrated that hydroquinone-oxidizing, nitrate reducing bacteria were prevalent in soils collected from all crop treatments. Results from MPN analysis indicated that hydroquinone oxidizing, nitrate reducing bacteria were present in soils from bluegrass fields (10^4 cells^{-g}) and native wooded areas (10^6 cells^{-g}), as well as in plots of corn (10^6 cells^{-g}), and soybean treated (10^6 cells^{-g}) and un-treated (10^5 cells^{-g}) with pig slurry. HS-oxidizing microbial isolates were collected from the MPN series of each tested source soil, and were phylogenetically/physiologically characterized (data not shown). Upflow columns were constructed utilizing soil from the corn plot of the MPN series as inoculum. All columns were shown capable of nitrate reduction, including column treatments utilizing HS as the sole amended electron donor. In fact, chemically reduced humic acids enriched for reduced functional groups (hydroquinones) were shown more capable of supporting nitrate reduction than all other treatments along the column's length (Figure 1a), including oxidized humic acids which were not enriched for hydroquinones. The redox state of humic acids could be tracked based upon the difference between endogenous Fe^{2+} in a sample and Fe^{2+} generated when humic acids in filtered samples were allowed to reduce Fe(III)-citrate (Figure 1b). The redox state of humic acids, as well as the nitrate reduction profiles, could be used to correlate the enhanced nitrate reduction in the reduced HS treatment to the presence and subsequent oxidation of reduced functional groups. An electron balance was performed, indicating that reduced functional groups accounted for a near-stoichiometric 104% of the excess nitrate reduction occurring in the reduced HS treatment over the oxidized HS control (calculation not shown).

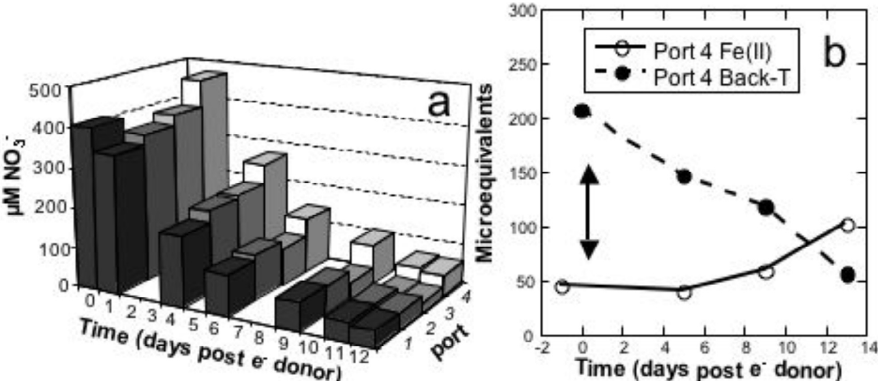

Figure 1. Nitrate levels and HS redox state in column studies. (a) Nitrate profile from point source of donor injection to the exit of column at port 4 in reduced HS treatment. b) Fe^{2+} (solid line) and Fe^{2+} generated from humic back-titration (dashed line) at port 4 in reduced HS column. The net difference between these lines represents redox state of the HS.

Upflow columns similar to those described above were constructed to compare AHDS and acetate as electron donor treatments in anaerobic systems containing both nitrate and perchlorate as potential electron acceptors. Both columns resulted in loss of nitrate and perchlorate from the mobile liquid phase, although the AHDS containing column was more stable in this capacity. This distinction is likely due to microbial overgrowth in the acetate-containing column, which resulted in loss of hydraulic conductivity (data not shown). AHDS and acetate were found to support the development of distinct microbial community structures (Figure 2a, b). In general, the AHDS treatment appeared to host a less diverse grouping of microorganisms as compared to the acetate treatment, including a strong enrichment of the *Dechloromonas* genera. Although acetate treatments supported development of communities of the delta sub-class proteobacteria, these were never present with AHDS as the electron donor. These results suggest that hydroquinone and acetate donor treatments support development of different overall microbial community structures, and that hydroquinones exhibit more selective pressure on the final community make-up.

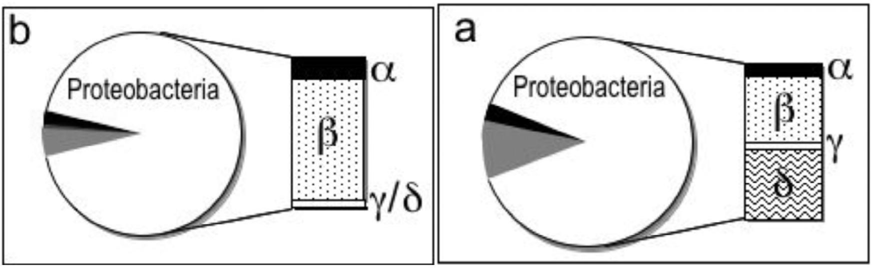

Figure 2. 16s rDNA clone libraries of microbial community structures near column exit ports in acetate oxidizing (a) and AHDS oxidizing columns (b).

4. CONCLUSIONS

Bacteria capable of oxidizing HS or model hydroquinones were found to be prevalent in agricultural soils. The activities of these microorganisms, including their oxidation of reduced humic functional groups, could be tracked in flow-through systems. Hydroquinones were furthermore found to support development of microbial community structures distinct from those developing from acetate treatments.

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TG Study of the γ-irradiation Effect on PVA with Addition of Sodium and Ammonium Salts of Humic Acids

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Keywords: lignite humic acids, antioxidants, stability

1. INTRODUCTION

Due to increasing price of oil and its limited deposits, the availability of synthetic antioxidants is becoming a potential barrier in development of products necessary for sustainable way of life. From this point of view, the technological potential of lignites (low rank coals with deposits all over the word and rich in humic substances) as a chemical raw material has been up to now rather overlooked. Lignite humic acids consist of a mixture of different organic molecules, while unsaturated fatty acids and polyphenols (having the profound antioxidant effect) represent their major part (1). Thus, industrial application of lignites or their humic components seems to be of a great interest in the near future.

Antioxidants in polymers can assure longer preservation of the initial structure by acting as oxygen scavengers. Proper dose is important, because over dosage can be detrimental. In our recent work we have demonstrated the antioxidant and pro-oxidant potential of humic acids and its salts (2). In fact, addition of HA and their ammonium salts in several concentrations caused an increase in polymer oxidation stability. In contrast, when the degradation processes have already started, the presence of humic acids supported faster degradation. As a result, using humic substances as additives, a material with specific properties and stability can be developed. Further, environmental or agriculture applications of humic substances in polymer materials seem to be promising with respect to their natural origin. Due to their profound influence on soil properties, they can serve as a kind of stable carbon-rich soil conditioner after the polymer life time expiration.

The thermo-analytical methods have been extensively used to study stability of various materials. They represent probably the easiest, most sophisticated and quickest way to evaluate the influence of additives on polymer matrix properties. Generally, the employment of thermo-analytical methods enables to predict stability and kinetics of degradation of (not only) polymer materials. By means of a mathematical treatment the non-isothermal data can be converted into isothermal values which can be simply used for estimation of material durability at specified conditions.

In this work the effect of γ-irradiation on stability and degradability of PVA with addition of sodium and ammonium lignite humates was investigated by TG measurements. The aim of this work was, using γ-radiation pre-treatment, to adjust the stability of humic/polymeric mixture to have required lifetime in natural system.

2. MATERIALS AND METHODS

Polymer/humic blends were prepared as reported earlier (2). Briefly, salts of lignite humic acids were mixed with polyvinyl alcohol. Polymer blends were prepared at final concentrations of humic acids sodium and ammonium salts (w/w) 0.5, 1, 1.5, 2, 5 and 10 %. γ-irradiation treatment was used to decrease the stability of polymer blends (exposure from 22 to 140 days). As a source of gamma rays ^{60}Co gamma-emitter with dose rate 0.26 kGy h^{-1} was employed. Thus, corresponding doses which were the samples exposed are 137, 524 and 873 kGy. The aim of this work was to monitor changes in stability of such pre-treated blends by means of thermogravimetry and evaluate potential antioxidant effect of humates after the γ-radiation treatment.

3. RESULTS AND DISCUSSION

Degradation of pure polyvinyl alcohol blend without addition of humates showed a dramatic decrease of stability after γ-irradiation pre-treatment. In fact, γ-irradiation with exposure dose 806 kGy caused the shift in the onset of degradation from 220°C to approximately 100°C.

Examples of thermogravimetric records of thermo-oxidative degradation of sample PVA enriched by 2% NH_4HA irradiated by doses 127, 484 and 806 kGy during 22, 84 and 140 days, respectively, are given in Fig.1. Basically, within 100-250°C, the temperature, at which a sudden decrease of weight was observed, was used as a measure of stability of PVA. As we reported earlier the 2% ammonium humate enriched PVA blend is stable up to 245°C, therefore, the γ-irradiation causes the decrease in stability of PVA blends already after 22 days (127 kGy) of exposure.

As reported earlier by Šimon (3) the onset temperature of degradation can be used for the calculation of material stability at required temperatures. As one can see, the sample which was exposed to lower dosage of γ-irradiation is stable up to 220°C, while that with higher dosage to 130°C. According to literature data, such significant shift in temperature to lower values means a dramatic decrease in durability of a material. In other words, in case of application of ammonium salt as an additive into polyvinyl alcohol matrix the γ-irradiation can be used to prepare a material with predictable properties and significantly lower stability. The second parameter which can be evaluated from thermoanalytical record is the degradability (rate of degradation) which is proportional to values of DTG (2nd Y axe in the Figure 1). Lower stability together with high degradability can play significant role in the selection of a proper material especially in case of environmental applications where the further material stability after expiration date is undesirable. Thus, obtained results indicate that although after irradiation the degradation started significantly earlier, however, when the degradation already started, the material which was exposed to longer γ-irradiation would degrade slowly than the original one (2). Other concentrations of NH_4HA in PVA measured gave similar results. It implies that ammonium humate still stabilizes PVA matrix also after 806 kGy dose of irradiation although in comparison with non-treated PVA a different mechanism can be expected. On the other hand, it is noteworthy, that pure lignite humic acids in all forms are rather resistant to γ-irradiation at relatively high exposure dose (4).

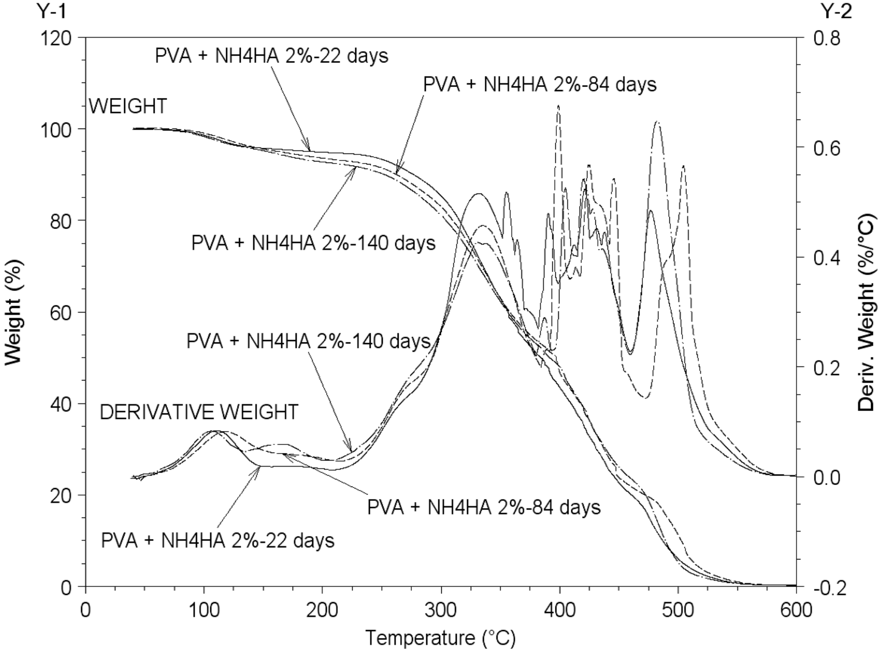

Figure 1. Comparison of thermogravimetric records of PVA + 2% NH_4HA irradiated for 22, 84 and 140 days, with respective dosages 127, 484 and 806 kGy.

In our previous work (2) we showed that at certain concentrations the presence of sodium humate can lead to the destabilization of PVA blend and increase the kinetics of degradation processes. In the presented work sodium humates behaved in the same way as ammonium humates did.

4. CONCLUSIONS

We demonstrated the potential of lignite humic acids salts to have an antioxidant effect in PVA stabilization after γ-irradiation in comparison with pure PVA. Obtained results indicate that humic acids salts were not influenced largely by the long term exposure of γ-irradiation and they can still, or at least partly, stabilize the polymer material. Changes in stability are promising with regards to the development of materials used in environment and agriculture with predictable life time durability and degradability.

ACKNOWLEDGEMENTS

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In vivo Algae Growth Monitoring in Sea Water Containing Production Water Treated with Humic Compounds

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Keywords: laser fluorescence, production water, image analysis.

1. INTRODUCTION

In Brazil the most oil production is offshore. In this activity one important environmental issue is the disposal of production water (PW). The PW is used to pump the oil from reservoirs and, at the end of the process, it contains many different organic compounds. More recently, the Brazilian Environmental Regulatory Agency has not allowed direct disposal of the PW at the ocean. Nowadays, it is necessary to bring a large amount of PW to a wastewater treatment plant on the coast, miles apart from the offshore platforms, increasing the whole production costs.

Years ago, in an environmental monitoring scientific expedition, an increase of fluorescence sign ($\propto$ concentration) of chlorophyll close to offshore facilities (1) was detected by a LIDAR (Light Detection and Ranging) system. This unexpected phenomenon could have different causes, including euthrophication impact on the analyzed region. To better understand it, a laboratorial study was proposed. This work intended to survey the growth of a particular marine microalgae population, which is frequently found in the Brazilian subtropical offshore ecosystems, in presence of production water and production water treated with humic compounds. The main goal was to evaluate the production water impact on marine water through microcosm simulations. We were also interested to study the viability of the use of humic compounds as a pre-treatment, before the final disposal of production water.

2. MATERIALS AND METHODS

A microcosm experiment was set up with the aim to monitor the growth of the chlorophycea *Tetraselmis gracilis* during 17 days in different water conditions.

2.1 – Experimental design

30L of open sea water were previously filtered at 0.45 μm, autoclaved by 15 minutes and leaved to cool down to room temperature. Supplemented sea water (SSW) medium was prepared adding growth factors (P, K, vitamins, metal traces and Si) for optimal algae growth.

Test tubes with 2L were used to support the different microcosm compositions as described bellow:

1- Blank sample (blank): supplemented sea water and the algae culture.
2- Sample 1 (PW): blank sample + production water 2% (v/v)
3- Sample 2 (PW+humics): blank sample + production water treated with humic substance 2% (v/v)

The microcosms were inoculated with a *Tetraselmis gracillis* culture, on exponential phase, diluting the initial aliquot to reach a concentration of 5.10E3 cells. Each microcosm was prepared in triplicate. All they were exposed to light 12hrs/day at 100±10 r and the room temperature was kept at 25^{o}C. The samples were constantly aerated and the test tubes were covered with sterile gauze to avoid any external contamination.

2.2 – *In vivo* growth measurement

A LIDAR system was used to measure the fluorescence of each sample once a day along the experiment. The LIDAR system consists of a Nd:YAG laser, with its 2nd harmonic (532nm) used as the emission line source. The fluorescence response is captured using a Newtonian telescope, which directs the light to a polychromator, followed by an image intensifier and a linear CCD. The obtained fluorescence spectrum is proportional to the chlorophyll a concentration.

2.3 – Cell density evaluation

At the beginning and at 8th day of the experiment, 1 mL of each sample was taken. These were fixed using a phormol solution. From this solution an aliquot of 0.1 mL was used for cell density evaluation with a fully motorized optical microscope. One hundred images of each sample were taken and, applying a homemade image analyzing (2) script in AxioVision software (3), the cells were counted and their size measured.

3. RESULTS AND DISCUSSION

The fluorescence measurements through LIDAR show an increase in chlorophyll a concentration along the first 7 days, the start of the senescence, for all samples as can be seen in Figure 1. It also can be noted that chlorophyll a concentration increases at higher rates for samples 1 and 2. Both samples achieve higher values at senescence, with the sample treated with humic substance presenting the highest concentration.

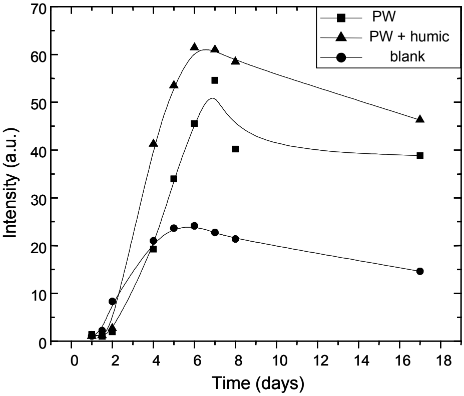

Figure 1. Fluorescence intensity of chlorophyll a of the samples.

By optical microscopy and image analysis, the number of cells per volume and the area of each cell were measured. In Figure 2 are shown images taken of (a) blank sample and (b) sample 2 and the corresponding results of image processing (c and d). In Table 1 are summarized the obtained results for the number of algae per volume and the mean area of each alga at the beginning and at the 8th day. Sample 2, which contained humic substance, showed a strong increase in the number of algae per volume and also in their size when compared with the blank, confirming the fluorescence results.

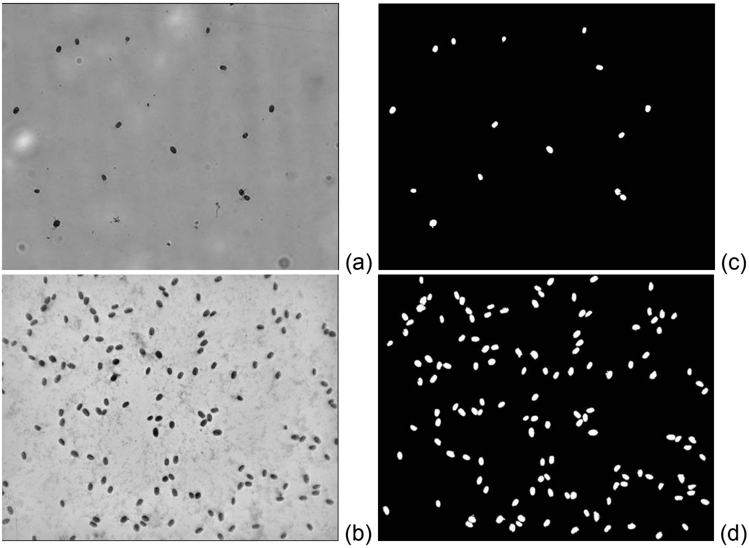

Figure 2. Typical images of Blank Sample (a) and Sample 2 (b) after 8 days; Binary mask images (c and d).resulting from image processing of original images.

Table 1. The number of algae and the mean area of a single alga at the beginning and at the 8th day, for the 3 samples

Sample	Density of algae(number of cells/volume)	Area (μm^2)
At start	2977	158±43
blank	1856	176±45
1	1803	236±67
2	4366	261±83

4. CONCLUSIONS

Both the fluorescence results and the microscopy analysis confirm an increase of the total biomass in the samples with production water (1) and with production water treated with humic substance (2) when compared with the sample only with supplemented sea water (BS). In the case of sample 2, an increase of almost three times in chlorophyll a concentration was determined. This can be explained by 50% increase of the algae population and an enlargement of 100% of the alga mean size observed by microscopy. So it becomes clear that the presence of humic substances aided significantly the algae growth.

Through these results, it might be possible to explain the phenomenon of concentration increase of chlorophyll a nearby the offshore platforms by the disposal of production water in the ocean. It is possible that the organic compounds, present in production water, increase the availability ov nutrients in the marine ecosystem.

ACKNOWLEDGEMENTS

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New Mechanochemical and Cavitations Technology and Products Deep Chemical Modification of Peat

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Keywords: peat, humic acids, fulvic acids, oxide humates, technology deep chemical modification

1. INTRODUCTION

Complex use of peat is one of urgent tasks of a modern peat industry. Optimal in this direction the way of deep chemical processing of all bioweight of peat is with the purpose of reception directly from him (without division into separate components) various polymeric materials (1). Significant interest in the applied plan represents oxide humates of substance, which are recommended for application in quality humus stimulators of growth, of fertilizers and is superficial - active substances. In work (2) it is offered to receive oxide humates from peat by his oxidation of hydrogen peroxide in alkaline environment in rather severe constraints at 125°C within 4 hours at the presence of salts cobalt and cuprum as catalysts. Such ways allow receiving humus of fertilizer with an output 75-93% from organic weight of peat containing connected cobalt and cuprum as microelements. One of perspective methods of activation of peat for his chemical modifying is cavitations the processing in water environment in cavitations devices (3). The peat subjected cavitations processing in various environments, changes the chemical structure that results in his activation.

Agrochemical the value of peat is determined in basic his organic part (humic and fulvic acids) and contents of nitrogen in his structure. However owing to small availability of organic substance the initial peat poorly shows properties of fertilizer. The activator of organic substance of peat can be water ammonia, which takes humus of substance as water solution humates of ammonium (2). The way of reception nitrogen contains of organic fertilizer - ammonization of peat is known by processing peat of the certain humidity by water ammonia. The basic lacks of a known way ammonization of peat are his duration, which results in losses of ammonia, and also low contents of the connected nitrogen in nitrogen contains of peat (1).

The way of reception stimulator of growth of plants from peat is developed by his processing of hydrogen peroxide in environment of water ammonia at the hydro module 9,5:1, at the contents of ammonia 20-50% and contents hydrogen peroxide 20-30% on absolutely dry weight of peat at 120°C during 4 h. This way has essential technological lacks: high temperature of realization of process (120°C), duration up to 4 h, two stages of process (4).

By us is developed new mechanochemical a way of reception nitrogen contains of organic fertilizers by oxidation of peat hydrogen peroxide in water-ammoniac environment with reception humus derivative with a quantitative output (5). However of works on regular study of oxidation and oxidizing ammonolyse of peat in various environments in conditions mechanochemical and cavitations of processing in the literature it is not revealed. In the present message we submit development new mechanochemical and cavitations of technologies of reception nitrogen contains humus of fertilizers and oxides humates from peat.

2. MATERIALS AND METHODS

As initial raw material for mechanochemical oxidizing ammonolyse used top peat a deposit of Tomsk Territory by humidity 60% containing 2.2% of general nitrogen, 13.8% humic and 18.5% fulvic acids. As initial raw material for cavitation oxidizing used bottom peat of a deposit of Altai Territory with a degree of decomposition 25%, zole 22.1%, humidity 50% containing 2.1% of general nitrogen, 24.6% humic and 23.4% fulvic acids.

Mechanochemical synthesis nitrogen contains polymeric humic of preparations from peat carried out by the following way. In cylindrical reactor vibro mill in capacity 300 cm^3 wich 15 Steel cores of 10×100 mm (industrial vibrator, frequency 2800 mines^{-1}) load of peat of weight 5.0 g, fill in 25% a water solution of ammonia (at the rate of 0.25-0.5 g NH_3/g peat), then add 30% a water solution of hydrogen peroxide (at the rate of 0.1-0.6 g H_2O_2/g peat) also subject to intensive mechanical crushing during 0.5-3 h. Temperature in the reactionary chamber –25°C. Further received products unload from a mill, separate from mill of bodies and dry up on air before disappearance of a smell of ammonia. The contents of general nitrogen and his forms defined on techniques given in a manual (6).

Cavitations processing of peat at the presence of an oxidizer (H_2O_2) and water alkali carried out as follows. Sample initial of peat by humidity 50% of weights 2.0 kg previously process 2-10% by a solution NaOH in cylindrical tubular reactor in capacity 10 litres, connected with rotor cavitator by the device (frequency of rotation of a rotor 3000 rev/min) at the hydromodule 1:2-4 during 15 mines at 60°C. Then in reactor add 50% a water solution of hydrogen pxeroxide (at the rate of 0.025-0.2 kg of H_2O_2/kg of absolutely dry peat) and received water suspense subject cavitations processing at temperature 60°C during 15-60 minutes. Further received suspense pour out in 10 liter polyethylene capacities. For the analyses received liquid himic of fertilizers take tests in capacity in volume 1 litres. A deposit fugation, separate a liquid phase (target product) and in it define the contents of general organic carbon, of carbon humic and fulvic acids by the modified method Turin (6).

3. RESULTS AND DISCUSSION

In the table 1 the data on influence of duration mechanochemical of synthesis on the contents of the connected nitrogen, humic and fulvic acids in products of oxidation top of peat of hydrogen peroxide in water-ammoniac environment are given.

Table 1. Influence of duration mechanochemical treatment on composition nitrogen contains of fertilizers from peat

Sample	Duration of crushing, h	The contents of general nitrogen, %	The contents, %	
			humic acids	fulvic acids
Initial top peat	-	2.2	13.8	18.5
1	0.5	2.9	15.3	22.9
2	1.0	3.8	18.3	25.4
3	1.5	4.7	-	-
4	2.0	4.9	18.2	27.1
5	2.5	5.1	-	-
6	3.0	5.3	18.7	31.1

The note: humidity of peat - 60%, weight peat - 5.0 g, quantity of hydrogen peroxide - 0.1 g/g peat, quantity of ammonia - 0.25 g/g peat.

As show results of the carried out experiments (tab. 1), to increase of duration of vibrating crushing of peat at the presence of ammonia and oxidizer (of hydrogen peroxide) there is a natural increase of the contents humic and fulvic acids in structure of peat. The increase of the contents of the connected nitrogen in 2.5 times for 3 h mechanochemical of synthesis testifies to course of reaction oxidizing ammonolyse of organic substance of peat.Influence of duration cavitations of processing also is investigated at 60^0C on an output water solution of organic substances from the bottom peat. The results of research are given in the table 2. As show results of the carried out experiments, at increase of duration cavitations of processing of peat at 60 ^{0}C at presence of hydrogen peroxide in water-alkaline environment, there is a natural increase of concentration water solution of organic substances in the received extracts from 22 up to 39 g/l. For 60 mines of oxidation of peat in conditions cavitations processing the output water solution of organic substances make 39 g/l.

Table 2. Influence of duration cavitations of processing on an output water solution of organic substances at oxidation of peat of hydrogen peroxide in a water solution NaOH*

Sample	Duration of oxidation in conditions cavitations of processing, mines	The contents of general carbon, g/l	The contents of general carbon humic acids, g/l	The contents of general carbon fulvic acids, g/l
Initial bottom peat	–	80.5	41.2	39.3
1	15	22.3	12.2	10.1
2	30	26.4	15.2	11.2
3	45	32.5	19.3	13.2
4	60	39.3	22.2	17.1

*Duration preliminary alkaline cavitations of processing - 15 mines, quantity H_2O_2 - 5% from weight of absolutely dry peat, concentration of a solution NaOH - 2%; temperature – 60°C.

4. CONCLUSIONS

Thus, the offered way for the account mechanochemical and cavitations of processing provides intensification of technological process, reception of the highest output water solution of organic substances in a target product with more simple and less power-intensive way in comparison with analogues. Thus, is established, that the received products of oxidation of peat of hydrogen peroxide in water-alkaline and ammonia environment in conditions cavitations of processing are effective stimulators of growth of plants.

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Effect of Humic and Fulvic Acids on the Anaerobic Hydrolysis of Lignocellulosic Biomass

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Keywords: humic, fulvic, extraction, manure, maize, ^{13}C CPMAS NMR, hydrolysis, inhibition.

1. INTRODUCTION

Lignocellulosic biomass, like agricultural wastes, energy crops and animal manure is considered as an important source for the production of bio-energy, viz. biogas, and methane (8). The production of biogas by anaerobically digesting lignocellulosic biomass is, for many years, applied throughout Europe. However, due to an incomplete understanding of its inhibition mechanisms, the design and operational conditions are still not optimized. For the anaerobic digestion of lignocellulosic biomass, the hydrolysis, which is the conversion of complex polymers, like carbohydrates, lipids and proteins, into soluble monomers, is considered as the rate limiting step (9). Consequently, the biogas production depends not only on the biodegradability of the biomass, i.e., how much methane a certain biomass can, potentially, produce, but also the hydrolysis rate (3).

Humic and fulvic acids are the end products of the biological decay of biota residues and they behave like weak acid polyelectrolytes (10). They can be extracted from lignocellulosic biomass based on their physical-chemical characteristics, since humic acids (HA) are soluble above pH 3,5 and fulvic acids (FA) are soluble at all pH values (2).

Humic and fulvic acids have been reported to have a scavenger effect on enzymes, inhibiting hydrolysis (1). The presence of Ca^{2+} was shown to reverse the inhibitory effect of humate (1), since the Ca^{2+} competes with the enzymes for cation-exchange on the humic and fulvic acids (6).

In the present research humic-like and fulvic-like acids are extracted from cow manure and energy maize and subsequently characterized. Furthermore, the inhibiting effects of humic-like and fulvic-like acids, from cow manure and energy maize, on the anaerobic hydrolysis process are looked at.

2. MATERIAL AND METHODS

Fresh cow manure and silage energy maize were air-dried at 40°C and grinded to particles smaller than 2 mm by a cutting mill (Retsch Muhle; type sm1). The samples were further used for humic and fulvic acids extraction according to the methods developed by the International Humic Substances Society (4). HA-like were sequentially extracted with HCl and NaOH under nitrogen atmosphere. After the last acidification the HA-like precipitate was dialyzed (Spectra/Por, MWCO 1000) against distilled water and finally freeze-dried. FA-like were extracted with XAD-8 resin, purified with H^+ saturated cation exchange resin (Bio-Rad AG-MP-50) and finally freeze dried. All extracted humic-like substances were stored in a desiccator.

Solid-state ^{13}C Nuclear Magnetic Resonance (NMR) spectra were recorded using Cross Polarization Magic Angle Spinning (CPMAS) on a wide-bore AMX 300 spectrometer (Brucker, Karlsruhe, Germany) operating at 75.47 MHz of ^{13}C frequency. The spinning speed was 5 kHz, the acquisition time was 33 ms, the ^{13}C 90° pulse length was 4 μs, the contact time was 0.8 ms, the recycle delay was 1 s and the line broadening was 50 Hz (11). For each spectrum, 800 to 1200 scans were recorded. The resulting spectra were analyzed quantitatively according to (11), where the NMR spectrum is divided into four types of carbon: alkyl (0-50 ppm), O-Alkyl (50-110 ppm), aromatic (110-160 ppm) and Carbonyl (160-220 ppm). From the four types of carbon relative areas, four bio-macromolecules were calculated: Aliphatics (lipids, biopolymers and fatty acids), polysaccharides (cellulose and hemicellulose), proteins and lignins. The results were presented in g bio-macromolecule per kg of volatile solids (VS). These four biomacromolecules represent most of the organic matter produced by plant material (7)

3. RESULTS AND DISCUSSIONS

The extraction yields of humic-lke acids from cow manure and energy maize were respectively 12,2 and 5,2% of VS and the extraction yields of fulvic-like acids from cow manure and energy maize were respectively 1,4 and 0,7% of VS. Manure showed to have higher yield of HA-like than maize. The relation between FA-like and HA-like remains analogous for both lignocellulosic biomasses, being 0.12 for cow manure and 0.14 for maize.

FA-like showed to have higher lignin content than HA-like from both manure and maize, as shown in Table 1. Conversely, the polysaccharides from the HA-like were almost four times higher than for the FA-like from both manure and maize. It is also noticeable that the polysaccharides from the HA-like from manure were almost half of the

HA-like from maize, probably indicating their anaerobic degradation in the digestive track of the cow. The content of aliphatics, viz., lipids and fatty acids, were low for the HA-like and inexistent for the FA-like from both manure and maize, confirming the lignocellulosic characteristics of this type of biomass.

Table 1. Lignin, polysaccharide, protein and aliphatic contents of HA-like and FA-like extracted from cow manure and maize in g.kg volatile solids (VS)$^{-1}$, obtained by ^{13}C CPMAS NMR. Data expressed as mean ± standard deviation

	Lignin	Polysaccharide	Protein	Aliphatic
HA-like from cow manure	353,8 ± 3,7	171,9 ± 2,0	450,9 ± 10,6	23,5 ± 6,3
HA-like from maize	233,2 ± 27,0	339,4 ± 73,9	345,3 ± 36,8	82,2 ± 10,3
FA-like from cow manure	406,5 ± 50,1	48,7 ± 21,8	591,9 ± 69,2	0
FA-like from maize[1]	553,6	86,6	425,8	0

[1] no duplicates analyzed.

Results of preliminary research show an increase of the hydrolysis rate of cellulose, in the liquid fraction of digested cow manure medium, at increasing calcium addition.

The inhibitory effect of oxygen containing functional groups, viz., COOH and OH, from extracted humic-like and fulvic-like acids of cow manure and energy maize, on the hydrolysis is being evaluated and results will be included in the presentation for this international meeting.

4. CONCLUSIONS

Cow manure has higher humic-like and fulvic-like acids than ensiled energy maize.

Cow manure has higher lignin content than ensiled energy maize.

Humic-like and fulvic-like acids might inhibit the anaerobic hydrolysis of lignocellulosic biomass.

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Pyrolysis of Biomass and the Genesis and Oxidations of the Chars

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Keywords: char, pyrolysis, biomass

1. INTRODUCTION

The thermal conversion of biomass, under the complete or partial exclusion of oxygen, gives rise to the production of biochar, bioenergy, and other bioproducts. Slow pyrolysis technologies tend to favour the formation of char from lignocellulosic biomass. Most of the remaining mass gives rise to non-condensable gases that are mostly utilised for heat. There is growing interest in the potential value of the char from such processes. The process of slow pyrolysis has been observed to give rise to desirable agronomic properties of the chars that are attributable, in part at least, to the char ultrastructure.

A survey of the literature indicates that applications of biochar improve soil fertility. This is partially attributable to the slow biological oxidation to carboxyl functionalities of peripheral aromatic groups of the fused aromatic structures. The resulting humic acid-type properties give rise to cation-exchange and moisture retention capabilities. Moreover, the transformation, through the formation of char, of labile plant organic matter into stable C pools reduces the release of greenhouse gas because charcoal is very slowly mineralized in the soil environment. Biochar from fallow vegetation and organic wastes can readily be produced by farmers and with economic benefits.

We are investigating experimentally the influences of various slow pyrolysis procedures on the compositions and properties of chars. Biochar is generated in a pyrolyser in oxygen restricted environments under controlled (time, moisture content, temperature and pressure) conditions.

2. MATERIALS AND METHODS

Biochar was formed in O_2 restricted environments under controlled temperatures using bamboo as the biomass substrate. The biochar (provided by Robert Flanagan, SAFFE, Hangzhou, China) was produced at temperatures of 400, 450, 500, 550 and 600°C. The reactions were carried slowly, and the temperature readings were taken using Raytek infrared rather then the conventional method of reactor wall temperature. During the course of the reaction the biomass was constantly moved around the reactor using a paddle, and so it was crushed as it was being charred. The product was a dark powder-like substance.

Three different agents, nitric acid, sodium hypochlorite, and hydrogen peroxide were used for controlled chemical oxidations of the chars.

Humic and fulvic-type acids have been isolated from the controlled oxidations of the char samples and the functional groups in these were characterized by Fourier transform infrared (FT-IR) and by solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Our FT-IR interpretations were based on contributions by Stevenson (1), and by MacCarthy and Rice (2). Potentiometric titrations were carried out on the char samples before and after the oxidations. The most comprehensive oxidations were obtained from the treatment with nitric acid. Only results obtained from nitric acid oxidation of the char obtained at 500°C are outlined in this communication.

3. RESULTS AND DISCUSSION

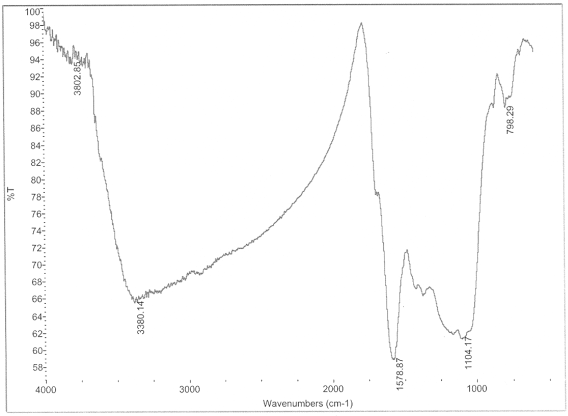

Figure 1. FT-IR of 500°C char before chemical oxidation.

The FT-IR range was 4000 to 400cm^{-1} and the scan rate was 11 scans per minute. The FT-IR spectra of all samples showed an intense broad band around 3450-3350cm^{-1} attributed to O-H stretching of various functional groups, but most likely from water that sorbed on the char surface. There is clear evidence for aromatic functionality in the cases of the oxidised and control chars (Figures 1 and 2). The band at ~1538cm^{-} in association with that at ~1342cm^{-1} indicates that nitration of the char structures (3) had occurred (Figure 2). There is clear evidence for the formation of carboxyl groups, based on the carbonyl band at 1717 cm^{-1}. This was also indicated by the titration data.

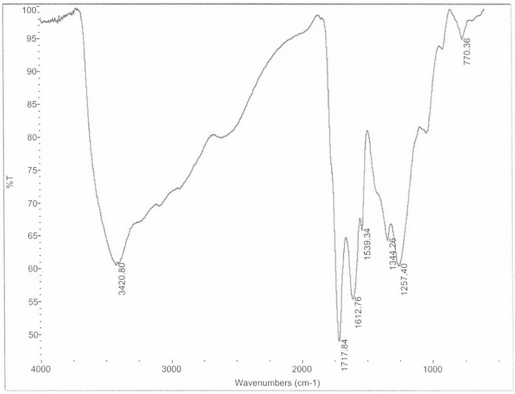

Figure 2. FT-IR of 500°C char after oxidation with HNO_3 (4 hr).

The NMR spectra, before and after oxidation, show the typical symmetrical aromatic resonances (centred around 125 ppm) characteristic of char material. The peak at 160-175 ppm is from carboxylic acids formed as the result of the oxidation that gave rise to the genesis of carboxylic acids. The shoulder at 145-150 ppm and the resonance between 50 and 60 ppm in the case of the char material before oxidation suggests that traces of lignin had survived the char-making process. However, any such materials were oxidized by the nitric acid treatment.

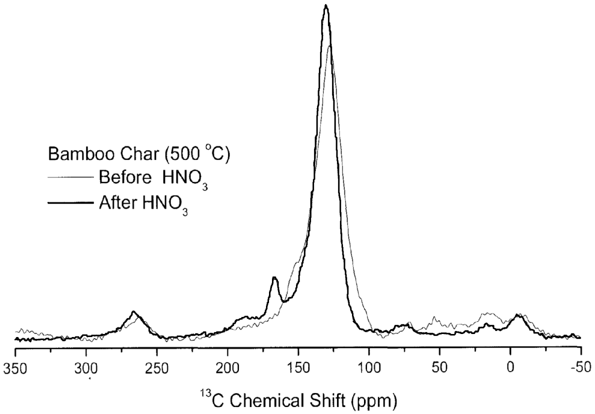

Figure 3. NMR Spectra Char 500°C before and after 4hr chemical oxidation by NHO_3.

The NMR spectra clearly indicate that the core fused aromatic structures survive the nitric acid oxidation process, and the oxidation was likely to involve only the peripheral aromatic structures. As slow oxidation of char takes place in soil carboxylic acids will form from the peripheral aromatic groups. These will confer cation-exchange properties characteristic of indigenous soil humic and fulvic acids. In addition the persistence of the char confers carbon sink properties to the char added to soil. Our ongoing studies are investigating the surface areas and porosities of chars. Porosity is considered to be an important feature of chars, and pore sizes may well be functions of the materials used in their genesis, and of the methods of preparation employed. The pores are considered to provide a refuge for microorganisms, such as Arbuscular mycorrhizal fungi. These form symbiotic relationships with plant roots and extend the abilities of roots to take up soil plant nutrients, and to improve moisture retention properties.

4. CONCLUSIONS

The research reported in this communication indicates that the essential features of char can be retained when char materials are chemically oxidised with nitric acid. Because N is incorporated in the char structures during the nitric acid treatment it may be possible to include the N in this way and provide a source of slow release N. The research programme will be extended to detailed investigations of the surface properties of chars made from different substrates and under different conditions, and the microbial colonisation and agricultural effectiveness of the products will be investigated in green house and field plot experiments. Because of the inevitable introduction of second generation biorefining technologies it will be important to study the properties of the chars formed during the gasification of biorefinery residuals. These materials are likely to provide valuable soil amenders. It will be important to investigate the agricultural effectiveness and the economic viability of biochar as a soil ameliorant in parallel with investigations of the bioenergy products from the pyrolysis of biomass and biorefinery residuals.

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Retention of Plutonium on Model Kaolin-Humic Complexes

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Keywords: kaolin-humic complexes, plutonium, adsorption, desorption

1. INTRODUCTION

In terrestrial environments a substantial fraction of humic substances (HS) exists as humic-clay colloids. Kaolinite is the most abundant clay mineral. Actinides transported through soil horizons and aquifers can sorb onto colloids containing humics. Evaluation of actinide retention onto humic-clay colloids is important for estimating actinide fate and transport in the subsurface environment, as the presence of HS in groundwater may alter the mobility sorbed actinides.

The goal of this work was to investigation the effect dissolved HS in groundwater on the plutonium retention to kaolin a humic complex.

2. MATERIALS AND METHOD

A kaolin-humic adsorption complex was used as a model for humic-clay colloids. Humic acid (HA) from brown coal were adsorbed to a suspension kaolinite colloid with particle sizes <1 µm. Kaolinite (Kaolin CF 70) was obtained from Caminauer Kaolinwerk GmbH, Germany. Particles <1 µm were obtained by ultrasonic treatment and separated by centrifugation. The kaolinite suspension was then washed and saturated with Ca^{2+} in 0.001 M $CaCl_2$ at pH 5.5. Commercially available potassium humate (Humintech Ltd, Germany) was used to isolate humic acids (HA) of leonardite. Humic-kaolinite complexes were created by adsorping HA onto kaolinite followed by subsequent sequential desorption of weakly bound HS in 0.001 M $CaCl_2$ at pH 5.5.

^{239}Pu(V) was used for these experiments. Sorption of Pu(V) onto the model colloids was studied using ultrafiltration through 30 kDa membranes. 0.001 M $CaCl_2$ solution was equilibrated with Pu(V) hydroxide during several weeks. Before ultrafiltration, colloids were added to solutions containing plutonium and then diluted giving a respective colloid and

Pu(V) concentrations of 50 mg/L and 1.55×10^{-9} M. The strength of plutonium sorption was estimated using fivefold washing of membranes with colloids using solutions of 0.001 M $CaCl_2$ and HA from brown coal in concentrations 0, 10, 25 and 50 ppm.

3. RESULTS AND DISCUSSION

Model colloids were saturated with Pu(V) and then washed with different HA -$CaCl_2$ solutions as shown on Figure 1.

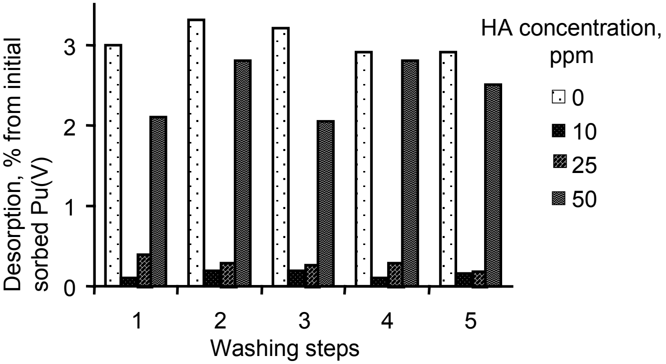

Figure 1. Desorption of Pu(V) from colloids wash in different HA-$CaCl_2$ solutions.

Each HA solution of a given concentration desorbed approximately the same quantity of Pu(V) for each washing step. This fact suggests colloid-Pu(V) systems were in equilibrium. Generally Pu(V) desorption was limited (0.1-3 % from quantity sorbed Pu(V)). The percent of Pu(V) desorped increased with increasing of HA concentration. Pu(V) complexation with HA facilitated Pu(V) desorption from colloids. However, in the absence of HA, 0.001 M $CaCl_2$ desorped the most Pu(V). Probably in absence of HA Pu(V) is partially displace by Ca^{2+} but in presence its Ca^{2+} is bounded of HA.

4. CONCLUSIONS

In this study, give evidence of Pu(V) desorption from kaolinite-humic colloids occurring via effect of HA or Ca^{2+}.

ACKNOWLEDGEMENTS

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Synthesis, Biological Activity and Detoxifying Properties of Carbonylated Humic Substances

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Keywords: humic substances, carbonylation, detoxification

1. INTRODUCTION

It is known that functionally enriched humic substances possess high binding ability in respect to heavy metals (1). The promising approach for enhancing detoxifying ability of humic derivatives (HD) with respect to heavy metals is incorporation of C=O-groups into the structure of humics. The objectives of this study were to synthesize and characterize structure carbonylated humic materials and to estimate biological and detoxifying ability of the obtained HD with respect to copper. It was expected that introduction of additional CO-groups in the structure of humics would enhance their complexing and/or redox properties and, hence, would lead to an increased detoxifying ability of humic derivatives with respect to heavy metals.

2. MATERIALS AND METHODS

Humic acids (HA) of oxidized brown coal of two Kyrgyz deposits (Kara-Keche and Kyzyl-Kiya) were used as parent material for subsequent modifications. Carbonylation was performed by the treatment of HA by formic acid (Hatterman-Koch reaction). Elemental analyses (C, H, N) were performed on a Carlo Erba Strumentazione elemental analyzer. Oxygen contents were calculated as a difference. The H/C and O/C atomic ratios were derived from the contents of the elements calculated on ash- and water-free basis. The data of chemical analysis and molecular-mass distribution are presented in Table 1.

Size-exclusion chromatography (SEC) analysis was performed according to a previous report (2). Redox capacity of humic materials was determined using ferricyanide as oxidizing agent as described in previous issue (3). Biological activity of the preparations was estimated by bioassay technique with seedlings. Seedlings of wheat *Triticum aestivum* L. were used as a target, and a root length was used as a response.

Table 1. Elemental and functional group composition of the carbonylated derivatives

Sample	Content of elements on ash-free basis, % (mass)						Content of functional groups, mmol/g			
	C	H	N	O	H/C	O/C	Total acidity	COOH	Ar-OH	Ar-CHO
Humate Kara - Keche	65.5	3.9	1.0	29.6	0.71	0.33	7,54	3,84	3,70	1,70
Humate Kyzyl-Kiya	64.3	4.0	1.1	30.4	0.73	0.35	7.63	4.21	3.42	1.61
Carbonylated Kara - Keche	63.0	4.2	0.8	32.0	0.40	0.91	7,69	4,27	3,42	2,19
Carbonylated Kyzyl-Kiya	65.2	2.5	0.9	31.2	0.46	0.34	7.62	4.18	3.44	2.17

3. RESULTS AND DISCUSSION

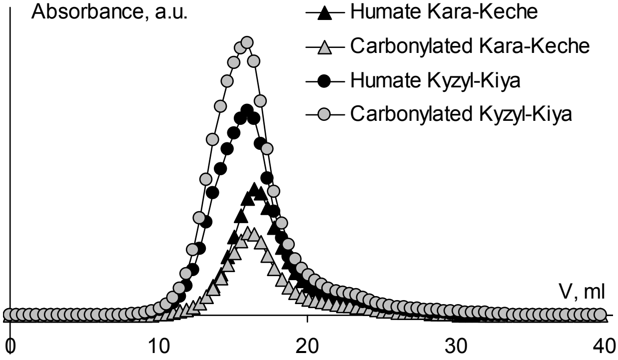

Figure 1. SEC-profiles of the parent humic material and carbonylated derivatives.

Chromatograms of all the parent humic materials and humic derivatives exhibited a sharp single symmetric peak. This finding confirms that the SEC-fractionation was conducted under conditions appropriate for the substances under study, as no artifacts such as ionic exclusion or specific adsorption were observed (2). That was also indicative for complete suppression of the surface negative charge of HA (ibid.).

Calculated values of molecular weight for all humic preparations are given in Table 2.

The HD were characterized with higher molecular weight Mw comparing with the parent humic material. This finding was evident for the partial polymerization occurred.

The values for redox capacity of the parent humic materials were 0.20, for Carbonylated Kara-Keche and Carbonylated Kyzyl-Kiya humics are 0.28 and 0.32 mmol/g respectively. All the HD possessed redox capacity considerably exceeding that of the parent humic materials. This finding was evident for increase in reducing capacity of humic derivatives comparing initial samples.

Table 2. Peak molecular weight Mp, number-average molecular weight Mn, weight-average molecular weight Mw, and polydispersity Mw/Mn of humic parent materials and obtained derivatives

Derivative Cipher	Mp, kD	Mn, kD	Mw, kD	Mw/Mn
Humate Kara-Keche	6.9	1.5	8.2	5.5
Humate Kyzyl-Kiya	8.5	1.9	12.9	7.0
Carbonylated Kara-Keche	8.1	0.8	9.5	11.9
Carbonylated Kyzyl-Kiya	9.2	0.9	13.7	14.3

The results on detoxifying ability activity of the humic derivatives are given in Figure 2.

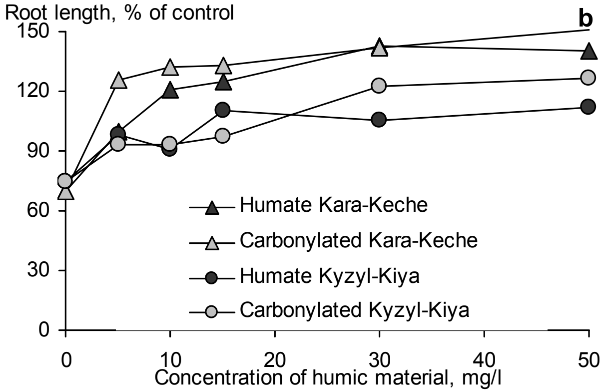

Figure 2. Dose-response relationships in the presence of 1 mg/L of copper(II) for the parent humic materials and carbonylated derivatives.

All the humic preparations studied significantly decreased toxicity of copper (Fig. 2). At their concentrations 30-50 mg/L no copper toxicity was registered. At low concentrations of HA (5-15 mg/L) the tendency was equal or slightly higher than that of the parent sample was observed.

The calculated values of the toxicological binding constants of the humic materials studied with copper(II) are given in Table 3.

It should be noted, however, that in all the cases of the carbonylated derivatives examined strongly pronounced toxicity was observed (Figure 3) what made incorrect calculations of toxicological binding constant K_{OC}^{tox}.

Table 3. The toxicological constants of copper binding to CHP-K$^+$ and its derivatives

Derivative Cipher	K_{OC}^{tox}, L/kg
Humate Kara-Keche	3.0×10^5
Humate Kyzyl-Kiya	8.7×10^5
Carbonylated Kara-Keche	4.0×10^5
Carbonylated Kyzyl-Kiya	5.0×10^5

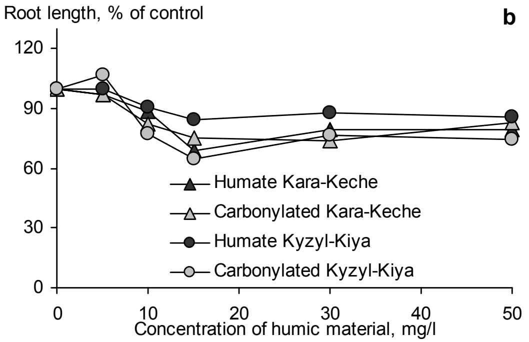

Figure 3. Dose-response relationships for the parent humic materials and HD.

4. CONCLUSIONS

The unknown phenomenon of humics biological activity was observed when carbonylated derivatives served as copper antagonist. Both copper and humic derivatives showed marked toxicity towards plant seedling when applied solely, while combined application of the above substances resulted in pronounced beneficial effect on plant growth.

ACKNOWLEDGEMENTS

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Extraction and Characterization of Organic Fractions isolated from a China Ball Clay Quarried in Devon South West England

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Keywords: China Ball Clay, organic fraction, humic, NMR

1. INTRODUCTION

China Ball clay has been used for centuries in the tiling and pottery-ware industry. Because it is much finer than the other components in the casting slip the ball clay dominates the particle-particle interactions. Therefore, the ball-clays give the cast its plasticity and cohesion. The clay under investigation is a dark slightly sandy china ball (DSSCB) clay with a high content of finely divided kaolinite and is mixed with other substances for casting. Because of its surface area properties and its associated organic matter it has an important influence on the cohesiveness and visco-elastic properties of the clay bodies used in manufacturing pottery ware. The fine particles in the ball clays have an important influence on the rheology of the wholebody, and stabilise the flow and casting properties of the slip.

A number of analytical procedures have been used in this study in order to gain a better understanding of the clays and their associated organic materials. Identification of the amount and nature of the active organic fraction and of its mode of action is important. The work described here is part of a larger project that investigates the chemical properties and compositions of the clay and lignite materials, and the nature of clay-humic complexes and of lignite materials in clay and lignite deposits.

2. MATERIALS AND METHODS

The ball clay was pre-washed with 1 M HCl acid followed by washing with distilled water until the conductivity approached 200 µS. The ball clay was first exhaustively extracted with 0.5 M NaOH from which one fulvic acid and two humic acid fractions were isolated (samples FA1, HA1 and HA2), then (exhaustively) with 0.1 M NaOH plus 6 M urea to give a ‘urea humic acid’ fraction (sample HAU). All extractions were in an atmosphere of

N_2. The soluble fractions were separated from the insoluble clay material by centrifugation (15,000 g). The soluble fractions were then adjusted to pH 9 using 6 M HCl. These were then pressure filtered through 0.2 µm cellulose acetate membrane filters. The soluble fractions were then adjusted to pH 2 using 6 M HCl. The FA and HA was separated due to precipitation of the HA under acidic conditions. The FA fraction was pumped on to XAD-8 column, desalted, back eluted with 0.1 M NaOH and was ion exchanged with IR120 resin, and was then freeze dried. The HA fractions were dialysed in Visking tubing over 3 to 4 days using 0.3 M HF plus 0.1 M HCl acid. The conductivity of the HA solution was reduced to 200 µS using distilled water before freeze drying.

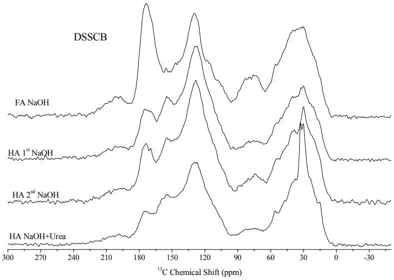
Characterisations of the series of organic fractions were carried out using Solid-state ^{13}C NMR spectroscopy using a VARIAN INOVA spectrometer at ^{13}C and ^{1}H frequencies of 100.5 and 400.0 MHz, respectively. Cross-polarization times of 1 ms, acquisition times of 13 ms, and recycle delays of 500 ms were used. For Variable Amplitude Cross-Polarization (VACP) experiments a Magic-Angle Spinning (MAS) frequencies of 13 kHz was used. Additional experiments using Chemical Shift Anisotropy (CSA) filter (C-sp^3 only spectra) and Dipolar Dephasing (DD) filter (non-protonated mobile C only spectra) were carried out. The spectral data along with a number of other ^{13}C NMR spectra along with thermogravimetry analyses (TGA) of the same samples were analysed using Partial Least Squares (PLS).

3. RESULTS AND DISCUSSION

The NMR spectra show high contributions from aliphatic functionalities. There are broad peaks in the aliphatic hydrocarbon region for both the FA and HA1 spectra (Figures 1a and 1b) corresponding to methylene carbons in alkyl chains, and CH_3 groups of acetyl groups attached to aliphatic structures. Evidence for CH_3 functionality is provided by the prominent resonance at 10-20 ppm in the DD spectrum. The broad resonance at about 30 ppm is characteristic of the significant contributions from methylene C. The peak centred around 32 ppm is characteristic of crystalline, or ordered methylene groups, and this feature is more pronounce for the HA2 and the HAU fractions. The HAU spectra show two distinct peaks at 30 and 34 ppm which is likely to indicate long chain amorphous and crystalline methylene structures, respectively.

The wide band at around 75 ppm for both the FA and HA samples (TOSS spectra) and the peaks at around 75 and 102 ppm (CSA filtered spectra) indicate the presence of sp^3-hybridized C, which are probably due to cellulose and other polymeric carbohydrates.

The broad peaks in the 125 ppm region are assigned to variety of aromatic structures, that may include C_1 quaternary carbons of guaiacyl and syringyl lignin units and the C_6 carbon of guaiacyl structures. The ratio of DD to TOSS in the aryl region (110-150 ppm) (Table 1) shows that there is a high degree of condensation in the aromatic structures. This may be attributable to lignite material.



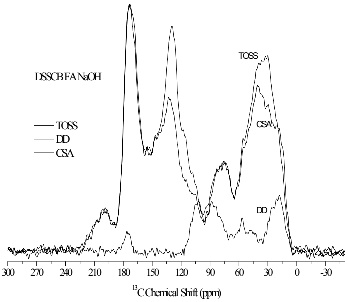


Figure 1a. ^{13}C NMR Spectra isolated from the DSSCB clay; from top to bottom: FA, HA1, HA2, and HAU fractions. Figure 1b. FA TOSS spectra, including the changes of the spectra due to DD and CSA experiments

Table 1. Relative values of the ^{13}C NMR spectra for TOSS, DD and CSA for the four organic fractions isolated from the DSSCB clay

Samples		Alkyl	Methoxyl	O-Alkyl	di-O-Alkyl	Aryl	Aryl-O	Carboxyl	Carbonyl
		0-45 ppm	45-65 ppm	65-90 ppm	90-110 ppm	110-150 ppm	150-160 ppm	160-185 ppm	185-225 ppm
FA	Abundance%[a]	23.5	9.5	8.4	4.6	26.1	4.4	18.6	4.9
	DD/TOSS[b]	21.7	21.4	41.3	85.2	75.6	103.0	102.5	92.7
	CSA/TOSS[c]	90.0	92.4	101.9	83.8	1.5	-0.0	4.4	7.0
HA1	Abundance %[a]	18.5	6.55	3.9	4.4	30.8	5.75	9.8	3.5
	DD/TOSS[b]	24.85	26.1	61.9	93.2	80.7	100.0	95.5	44.3
	CSA/TOSS[c]	83.6	79.65	101.8	38.1	0.2	-0.3	4.2	10.5
HA2	Abundance %[a]	22.5	7.7	5.45	5.1	33.0	6.0	10.7	3.9
	DD/TOSS[b]	22.7	21.3	38.2	67.1	69.9	90.8	98.1	99.1
	CSA/TOSS[c]	89.4	82.2	100.2	43.6	0.6	0.6	5.6	12.2
HAU	Relative %[a]	22.5	6.1	3.75	3.7	23.7	4.8	7.3	3.0
	DD/TOSS[b]	21.4	23.0	42.8	66.0	68.0	85.8	91.75	77.6

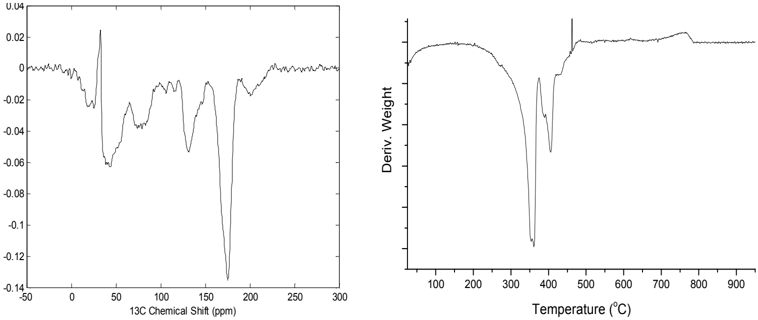
a; Abundance % is the percentage of the area of the different subsections of the spectra over the total area of the spectra

b; DD/TOSS is the ratio of DD (%) to the TOSS (%) for each subsection of the spectra

c; CSA/TOSS is the ratio of CSA (%) to the TOSS (%) for each subsection of the spectra

There are a number of similarities throughout, although some differences are apparent. The DD to TOSS ratio in the carbonyl group between the HA1 and HA2, 44.3 and 99.1 %, respectively, may indicate some protonated carbons in mobile macromolecules in the HA1 sample. The low CSA values in the aryl and O-aryl regions of the four spectra indicate that the peaks present in these regions are due to aromatic moieties.

The latent variable (60% of the variance from NMR data and 36% of TGA data) (Figures 2a and 2b) show the correlation between labile compounds and the thermal events at 352, 360, 386 and 407°C respectively. The latent variable from NMR have positive and negative signals, the positive signals are probably due mainly to crystalline polymethylene and carbohydrates which have a positive correlation with the exothermic thermal events and negative signals indicating a negative correlation with carboxyl and aryl groups present.



Figures 2 a and 2 b showing the latent variance from 60% of NMR data and 36% of TGA data

4. CONCLUSIONS

^{13}C NMR has indicated compositional characteristics of the organic matter associated with the clay matrix in DSSCB, and in combination with TGA in the PTS given insights into their thermal properties.

ACKNOWLEDGEMENTS

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Efficiency of Lignite as an Antioxidant for Polyolefines

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Keywords: lignite, antioxidants, thermal analysis, stability

1. INTRODUCTION

A perusal of literature shows many scattered applications of humic substances or humified substrates. Due to the high content of polyphenols and unsaturated fatty acids these materials may exhibit an antioxidant effect when added to various polymeric materials. Therefore it is reasonable to apply these materials as additives in polymers used in specific applications.

In this work, we used thermal analysis to determine the antioxidant efficiency of humic-rich South Moravian lignite applied as an additive at different concentrations in polypropylene (PP) and high and low density polyethylene (HDPE and LDPE, respectively).

2. MATERIALS AND METHODS

HDPE, LDPE and PP were melted and mixed (in an extruder) with milled lignite having particle size up to tens of micrometers to obtain concentrations 0.5; 1; 2; 5; 10; 20; 30 % (w/w). The various mixtures were analyzed at seven different heating rates under the oxygen flow by differential scanning calorimetry to assess their thermo-oxidative stability. To estimate stability, the onset temperatures of degradation were measured. Isoconversional methods allowing for induction period determination were used to determine the stability of investigated material at 100°C (1). For this purpose three different approaches were evaluated using Arrhenius and non-Arrhenius equations to describe temperature effects (2). Obtained results were compared with that for the pure polymer.

3. RESULTS AND DISCUSSIONS

Obtained results can be summarized in the following statements:

- Application of different temperature functions to fit the data gave slightly different results regarding to the stability of polymers under study. The most reliable data were obtained using the non-Arrhenius temperature function.

- Lignite added to HDPE, LDPE and PP showed a stabilizing effect at any concentration, with some concentrations of lignite being more efficient. The highest efficiency for PP was shown at a concentration around 5%, for HDPE at 10% and for LDPE at 30%.
- At the above-mentioned concentrations, the presence of lignite increased the durability of HDPE 3 times, for LDPE 15 times and for PP 46 times.
- The composition of lignite also affected the physical structure of investigated polymers. Application of antioxidants above a concentration around 5-10% is quite rare in polymer industry and changes were observed in polymer physical properties, mainly in their rigidity and elasticity.

4. CONCLUSIONS

Lignites represent raw materials with unique composition and high content of humic substances. Up to now, however, they are used almost exclusively as a source of energy in thermal power stations. Results of this work show one potential non-fuel application of lignite – as a polymer additive. Lignite affects thermo-oxidative stability of common large-scale polymers which can be attributed to its polyphenolic and unsaturated structures. In agricultural practice, lignite-filled polymers could be used for production of multifunctional mulching foils.

ACKNOWLEDGEMENTS

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Mitigating Activity of Humic Substances and their Si-enriched Derivatives in relation to Wheat Seedlings under Salt-Stress Condition

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Keywords: humic, bioactivity, silicium, stress

1. INTRODUCTION

Humic substances (HS) are known to possess bioactivating properties in relation to plants. Mitigating activity of HS is observed under various stress conditions including both biotic and abiotic ones, therefore non-specific action of HS towards plants can be hypothesized. The main non-specific damage induced by all the stresses is the plant cell injury. As silicon is responsible for cell wall stability, artificial enrichment of HS with silicon could results in increase in their mitigating activity.

This study was aimed to estimate bioactivating properties of coal humic acids and their derivatives enriched in silica under salt stress conditions induced by 0.15 M NaCl.

2. MATERIALS AND METHODS

Leonardite humic acids were obtained by desalting of the commercial potassium humate Powhumus – CHP-Pow (Humintech Ltd., Germany) and used for modification with 3-amino-propyltrimesthoxy-sylane (APTS) according to (1). Obtained derivatives were designated as CHP-APTS-5, CHP-APTS-20, CHP-APTS-50, CHP-APTS-100, CHP-APTS-200 and contained 2.58, 3.24, 6.44, 7.89, 11.27% of Si respectively.

To estimate mitigating activity of the CHP-Pow and produced derivatives, bioassay technique with seedlings was applied. Seedlings of wheat *Triticum aestivum* L. were used as a target object, and a length and weight of shoot was used as a response. Ten wheat seeds were placed in Petri dishes with solutions containing 10 mL of 5, 10, 25, 50 mg/L of parent humic material or humic derivatives. Distilled water was used for blank.

The stock solutions of humic materials were prepared by dissolution of a certain amount of the sample in a small volume of 1M NaOH. Values of pH of all the solutions were adjusted to 5.5-5.9 using 0.1M HCl. Seeds were grown for 72 hours at 25°C in the dark. Then 5 seedlings from every dish were transferred to plastic pots containing 0.5 l of

Knopp's nutrition solution for 96 hours. Salt stress was induced by sodium chloride (0.15 M) when required. Then wheat seedlings were harvested and shoots' and roots' length and weight were measured. Based on the data obtained, length increase as a difference between final and initial lengths was calculated. The experiment was performed in duplicate.

3. RESULTS AND DISCUSSION

The obtained data demonstrated that introduction of 0.15 M NaCl resulted in decrease in wheat seedlings growth. When seedlings were germinated in the presence of both parent and modified humic materials, they were more resistant to the salt stress as compared to those germinated in distilled water. At that, mitigating activity of used humic materials was more pronounced in relation to the shoots rather than roots.

The results of bioassay experiments are presented in Figures 1 and 2.

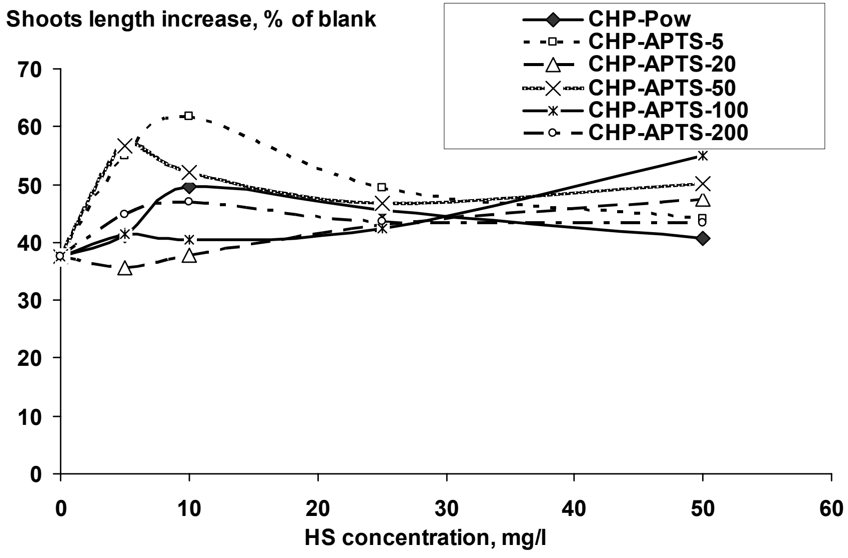

Figure 1. Concentration dependent wheat shoots' length increase in the presence of parent humic material CHP-Pow and its silicon derivatives under salt stress condition.

As it can be seen from the Figure 1, the most marked effect for CHP-Pow was registered at concentration 10 mg/l and was 49.6% of blank. That value exceeded significantly negative control (0.15 M NaCl), where increase of length was 37.6% of blank value. Derivatives CHP-APTS-5 and CHP-APTS-50 demonstrated maximal beneficial effect in relation to the shoots of wheat seedlings at concentrations of 5-10 mg/l (56.6-61.7% and 56.6-52% of blank respectively). Responses of wheat shoots to treatment with humic material CHP-Pow and its silica enriched derivatives at 25 mg/l gave no significant difference. The exception was CHP-APTS-100, where the length increase was 55%. When HS concentration was increased to 50 mg/l the maximum values were registered for CHP-APTS-50 (50% of blank) and CHP-APTS-100 (55% of blank).

To reveal relationship between mitigating activity of HS and contents of silicon, data on shoots' length increase and weight at HS concentration of 50 mg/l were compared (Figure 2). The highest values of both parameters were observed in case with CHP-APTS-100 containing 7.89% of silicon.

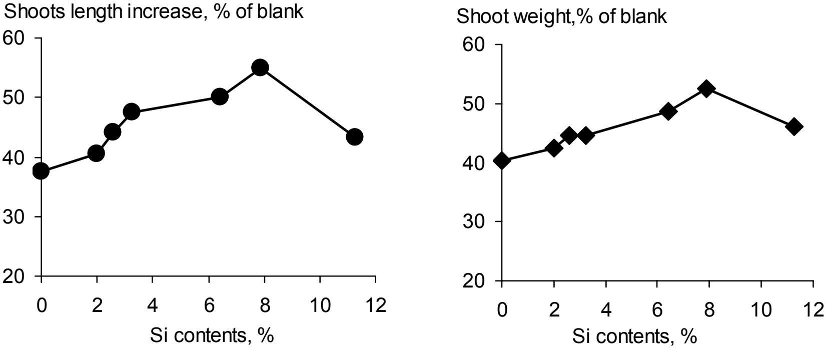

Figure 2. The influence of parent humic material CHP-Pow and its Si-enriched derivatives of different silica contents on wheat shoots' weight and length increase.

Therefore, Si-enriched humic derivatives were demonstrated to mitigate salt stress injury of wheat seedlings. At that himic derivative with silicon content of about 8 % was seemingly the most efficient.

4. CONCLUSIONS

The bioassay experiments with wheat seedlings aimed to estimate mitigating activity of coal humic acids and their derivatives enriched in silica under salt stress conditions induced by 0.15 M NaCl were performed.

The obtained results showed that both parents and Si-enriched humic materials mitigated salt stress damage of wheat seedlings. Among studied HS, derivative CHP-APTS100 with silicon content of about 8 % was seemingly the most efficient preparation.

Our findings indicated clearly that Si-enriched derivatives are the promising humic preparations for the further research in the field of development of agricultural humic-based materials.

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Evaluation of the Quality Assurance of Ultrafiltration Separation for Humic Substances by Chemical Probes

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Keywords: ultrafiltration, membrane, probe, characterization, polyethylene glycols, dextrans, polystyrene sulfonates, dyes

1. INTRODUCTION

The characterization of the molecular size of natural organic matter (NOM) is a valuable tool when assessing the effect of this material on the performance of water treatment systems as well as its geochemical origin (1). Size fractionation is usually accomplished by size exclusion chromatography (SEC) or Ultrafiltration (UF). The UF separation uses membranes with different cutoffs (e.g., 1, 5 and 10 kDa). Unfortunately, membrane manufacturing generates a range of pore sizes and many membrane manufacturers use molecular weight cutoff (MWCO) metrics based on a 90% retention of given solute after specified duration of filtration (2). Present calibration studies are incomplete and differ by manufacturer: usually a single brand of membranes and a limited number of shaped and charged molecular probes are used (3-6). Thus, MWCOs are subjective and depend upon the shape and charge properties of the solutes used.

The objective of this study was to characterize the Millipore™ ultrafiltration membranes YM 1 (1 kDa), PB 5 (5 kDa), YM 10 (10 kDa) with neutral and charged molecules of various sizes and shapes. Polyethylene glycols (PEGs), dextrans, polystyrene polysulfonates (PSSs) and highly charged dyes were chosen to be studied under natural water quality conditions of pH and ionic strength. The goal is to develop, a calibration curve to ascertain which fractions of NOM are retained by the membranes.

2. MATERIALS AND METHODS

In this study, Millipore YM 1000 and 10000 MWCO, and PB 5000 MWCO membranes were used. PEGs were purchased from J.T. Baker, dextrans from Fluka, polystyrene sulphonates from Polymer Standards Services and dyes from Fisher. Dyes used in the study include bromocresol green (BCG), congo red (CR), methyl red (MR) and methyl orange (MO). All solutions were prepared in 200 mM phosphate buffers at pH 7.2. Ultrafiltration was done on Millipore solvent-resistant stirred cell (XFUF 076 01). Two hundred ml of bulk solution was put in the cell and 100 ml was filtered through. Fractions were analyzed by Shimadzu TOC 5050 Total Organic Carbon Analyzer for polymers, and Shimadzu UV-1700 Pharmaspec UV-Vis spectrophotometer, for dyes. Maximum peak absorbance wavelength was used for each dye. Dyes were selected based on their shape, charges at pH 7.2, and similar molecular weights. Retention coefficient was calculated using $\% \text{ retained} = \left(1 - \frac{C_f}{C_b}\right) * 100$ where C_f is concentration in filtrate and C_b is concentration in the bulk solution prior to ultrafiltration (7).

3. RESULTS AND DISCUSSION

Figure 1 generally shows that the UF results do not agree with MWCOs indicated by the manufacturer. It appears that shape and charge of the molecule has a significant effect on the passage through membrane pores. Millipore specifies its MWCO as 90% retention of globular solute (8). For globular dextran molecules the MWCO definition applies well for 1K Da membranes. The definition does not hold for 5K and 10K Da membranes as dextrans of 5,000 and 12,000 g/mol are only 11 and 24% retained, respectively. For linear PEG compounds 1K Da membrane fits well with manufacturer's definition, but 5K and 10K Da membranes are much too loose as shown on Figure 1. The high variability of PEGs for the 10K Da membrane may be due to orientation of the molecules i.e. if the linear molecule enters the pore vertically it will pass, whereas if it enters the pore horizontally it will be rejected. From the data it appears that length of PEGs does not have an effect on the passage through the membrane as rejections for all 10K Da membrane probes are within the standard deviation.

Dyes were selected based on their weight, shape and charges at pH 7.2, creating 2 groups – heavier dyes (~ 700 g/mol) with –1 and –2 charges and lighter dyes (~300 g/mol) with the same charges. From the dyes with –2 charge, it is apparent that shape, as well as charge, plays a role in the sieving. It was hypothesized that highly charged molecules would be more rejected than less charged ones. Linear CR molecules were retained about 96% under both 5K and 10K Da membranes, while globular BCG molecules passed the 10K Da membrane, and were 96% retained by the tighter 5K Da membrane. Therefore, even though double-charged, the globular BCG was able to pass through the loose 10K Da membrane, while elongated CR was hindered. Similar trends are apparent with smaller, single-charged MR and MO dyes as both are able to pass through the loose 10K

Da membrane, while elongated MO is retained 94% and globular MR 41% on the tighter 5K Da membrane. Dyes were not tested at 1K Da membranes as the rejections were quite high for looser 5K and 10K Da membranes. Dyes of about 700 g/mol with –1 charges will be run to establish which parameter, shape or charge is more dominant during ultrafiltration.

To simulate elongated highly charged NOM molecules, PSSs with high –1 charge densities throughout the polymer were used. PSSs of similar sizes to the MWCOs were all 100% rejected by their respective membranes, as compared to neutral PEGs which were able to partially pass through if properly oriented with respect to the pore. This result is expected since dye molecules, which are at least an order of magnitude smaller than the shortest PSS polymers, and with only a single –1 charge were retained by the membrane.

Figure 1 shows the "calibration curves" for the probes. The calibration curves indicate that PEGs and dextrans cannot be used to define MWCOs whereas PSSs with their evenly distributed negative charges follow the stated MWCOs of the membranes. Although humics do not have an even charge distribution, they do contain charges and a variety of polar and non-polar functional groups that make humics more comparable to PSSs than to PEGs or dextrans. A variety of biomolecules with similar characteristics will be used in future studies to characterize the membranes. Currently, a polarity rapid assessment method (PRAM) is being developed which will be able to characterize NOM by polarity and would be utilized in conjunction with UF, since charge and polarity together play a significant role in the UF sieving process (9).

There is consistent reproducibility of the analysis especially for the 1 and 5 K Da. Therefore the primary value of UF is completing comparative analysis of temporal or spatial variation or between input and output of a water treatment process, and whether there is a difference between 2 locations in a reservoir. SEC with the same polymer probes is being studied to determine the relationship to MWCOs and if there is any relationship between the gel and UF methods for size of samples.

4. CONCLUSIONS

The study shows that UF is not only a mechanical sieving process, but that charges on the membrane and the constituent groups play a significant role in the rejection process. Uncharged molecules larger than specified MWCOs are able to pass through pores, while small charged molecule do not pass. Thus, fractionated NOM samples will contain a mix quite different than what is expected by simple mechanical sieving. PSSs were found to be representative of specified MWCOs while PEGs and dextrans were not.

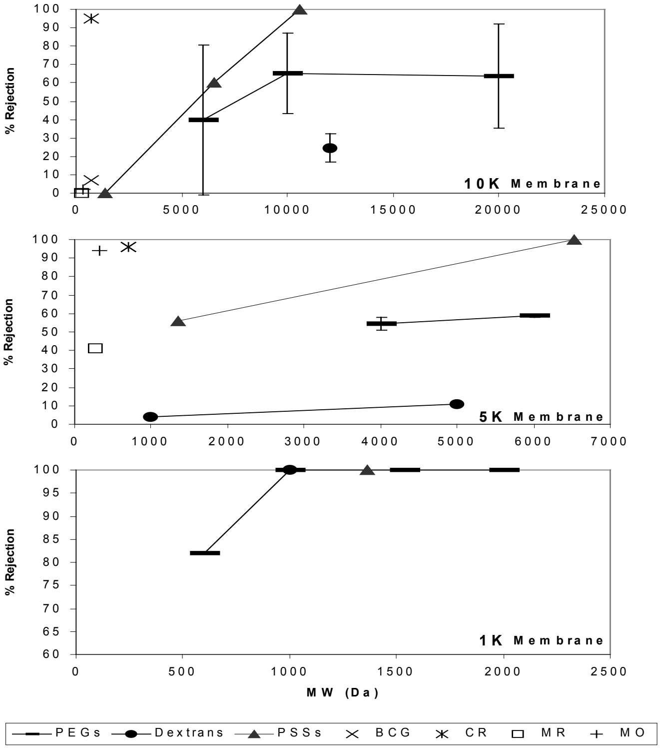

Figure 1. Rejection of probe solutes for 10, 5 and 1 kDa UF membranes.

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Results of a Study on Obtaining of the Humic Containing Plasticizers and Their Influence on the Physic-Mechanical Properties for the Cement and Concrete Mixtures

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Keywords: plasticizer, humic substances, cement, concrete

INTRODUCTION

In the modernization of the technology on concrete the important significance has physical chemistry and colloid chemical ideas about mechanism and to the kinetics of the processes of structure formation in the dispersal systems and questions on the influence of different chemical additives, regulated performance properties of concrete. The chemical additives of the organic and inorganic origin, which are introduced into the composition of concrete they contribute to a change in the kinetics of forming of micro-, the macrostructure concrete mixtures due to the adsorptive modification of hydrated phases, formed in the initial setting time and hardening of the binding materials, decrease water-requirements, increase mobility and fluidity of concrete mixture, increase frost resistance and strength indices of concrete.

In recent years, for purposes of the modernization of the technology of concrete the new chemical additives superplasticizers, which are actually surface-active synthetic polymeric substances, widely are used. The countries of superplasticizers, in the industrially developed countries of superplasticizers obtain, in essence, from the individual chemical substances, such, as naphthenic acid, sulfonated naphthalene, melamine-formaldehyde oligomers, polycarboxylates, in connection with which, the cost of these supers-plasticizer is sufficiently high, which limits their uses in practice on a wide scale.

For the Mongolia when, almost they are absent the production of chemical substances, the development of the method of obtaining the plasticizers of high efficiency on the basis of natural organic raw material has great practical value for the production of concretes of different designation. By the purpose of this work is the experimentally substantiation of the method of obtaining the strongly acting plasticizer from the oxidized brown coal high the content of humic substances.

2. MATERIALS AND METHODS

The highly oxidized brown coal of Mongolia served as the starting material of experiment. The proximate analysis of the all-level sample from Baga-Nuur coal deposit face layer as oxidized brown coal, used in this work showed that the output of volatile substances composed about 50,0%, ash content -15,0%, humidity 11,3%. They composed the element composition of all-level sample (to the combustible mass): C 65,34%, N 4,62%, N 1,27%, O 28,47% (by the weight difference).

The content of the humic acid, extracted by 1% solution of NaOH from this test composed approximately - 60% on the initial dry coal. With the well-known method of obtaining the sulfur-coal cationites on an industrial scale the sulfonation is achieved by an action of the concentrated sulfuric acid or by 20% solution of oleum. In this work for the sulfonation of the humus substances of brown coal was used, on an example of Kulgrena*, soft method sulfonation with the application of the sodium salts: $NaHSO_3$, Na_2SO_3.

Presence of different functional groups in (- SO_3^{2-}, COO^-, CH_3, CH; OH, SO^{2-}, $C=C^-$, > C=O and other) for the end product were studied by the method of IR spectroscopic studies.

A quantity of chemically bound SO_3 in the end product they were determined by the Eshka method and the average content SO_3 in it was equal to 4,5-4,8%. The influence of the end product of synthesis sulfohumic formaldehyde condensate, in abbreviated form as SHFC on the kinetics structure formation of cement test were studied with the aid of conical plastometer of the construction of MSU. The effects of dosage SHFC on the setting time and after spreading cement test, and also water requirements and the strength of the concrete mixtures were studied by standard methods.

3. RESULTS AND DISCUSSION

It is established that the most suitable time of the process of the sulfonation of the oxidized brown coal (OBC) should be considered 12-14 hours, at a temperature of 120-130°C. The most rational quantity of components, for reacting of sulfonation and polycondensation were as following weitgh percent: OBC 14,75; Sodium sulfite 3,68; Sodium hydrosulfite 7,37; Formaldehyde 0,48; H_2O the rest.

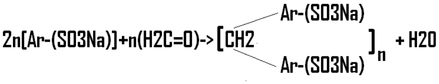
The proximate scheme of the reactions for obtaining sulfohumic-formaldehyde condensate it is possible to describe as follows:

1. Stage of the sulfonation:

n (Ar- H) + n (NaHSO3 + Na2SO3) of → n [Ar (SO3Na)],

where Ar - is the aromatic nucleus of humus substances in OBC.

2. Stage of polycondensation with formaldehyde:



$$2n[Ar\text{-}(SO3Na)]+n(H2C{=}O)\text{->}\left[CH2 \begin{array}{l} \diagup Ar\text{-}(SO3Na) \\ \diagdown Ar\text{-}(SO3Na) \end{array}\right]_n + H2O$$

Studies of surface activity $\delta = f\ (C_{SHFC})$ showed that the surface tension of aqueous solutions SHFC gradually is reduced and with C_{SHFC} ~ 09% the surface tension of water it decreases to ~ 68 erg/cm^2 (with the future with the increasing of the concentration of aqueous solution SHFC surface tension it barely changes).

The dependence of the kinetics of the increase of the plastic strength (rheological characteristic) of cement test on the dosage of additive SHFC (%) is represented in Fig.1

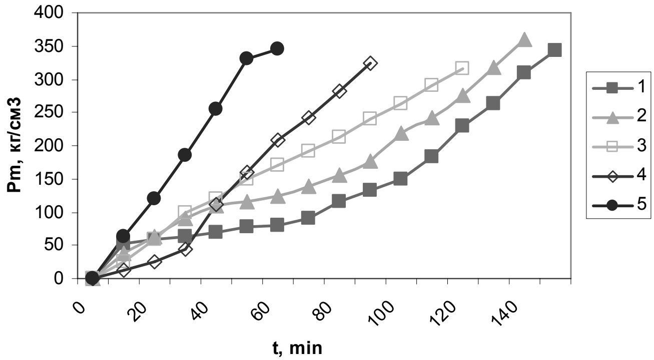


Figure 1. Dependence of the increase of the plastic strength of cement test on the dosage of additive SHFC (%). 1 without the additive it is; 2 0,1% of SHFC; 3 0,9% SHFC; 4 1,5% of SHFC; 5 0,5% of SHFC.

It is evident from Fig. 1 that during 10-15 minutes from the beginning of mixing with the concentrations of the additive SHFC=0.1, 0.9, 1.5 values of plastic strength are lower than in the system without the additive SHFC; beginning 30-40 minutes in the given systems it is observed the acceleration of an increase in the plastic strength in comparison with the system without the additive. When the concentration of addition SHFC =0,5%-Pm is noticeably more than in model without the additive SHFC.

Table 1, presents data on testing of the influence SHFC of lignosulfonate of sodium (obtained by us) and number of imported plasticizers on the strength indices of concrete mixtures.

Table 1. Results of the tests of influence SHFC, LST- SC and imported plasticizers for the concrete pressure strength*

Type of additives	Cement, kg	Sand, кg	Gravel, kg, 5-10 мм	Gravel, kg, 10-20 мм	Dosage of additive, g	Water, kg	w/c	Compression strength, 28 day, mPa
Control	3,6	4,6	4,7	4,7	0,0	1,16	0,30	32
SHFC	3,6	4,6	4,7	4,7	33	0,97	0,27	59
LST-SC	3,6	4,6	4,7	4,7	33	0,97	0,27	68
LS Russian	3,6	4,6	4,7	4,7	33	0,97	0,27	74
LS Chinese	3,6	4,6	4,7	4,7	33	0,97	0,27	74

*Study was conducted by cubes of 100x100x100 mm, aqueous storage with the standard conditions; SHFC ~0,91% of the weight of cement. It is evident from Tabl.1 that the chemical products obtained by us LST- SC, SHFC increase the strength of concrete 1,8-2,1 in comparison with the strength of concrete without the additive. The strength of concrete with the additives SHFC is approximately on 10 mPa lower than with the use of imported LST, but this fact apparently related with that circumstance that this dosage of additive for SHFC is not optimum, and also imported plasticizers have more complex composition in the plan of the content of additional chemical constituents.

4. CONCLUSIONS

1. Shown the possibility of obtaining sulfohumic - formaldehyde condensate, by being appeared as surfactant anion, from the oxidized brown coal with the high content humic containing of products by their sulfonation by the mixture of sulfites of sodium (Na_2SO_3+$NaHSO_3$) and subsequent polycondensation with formaldehyde with the relatively soft conditions.

2. The infrared-spectroscopic studies SHFC showed that in this end product are contained such functional groups, as - COO^-, SO^{2-}, -OH, -SO_3^{2-}, and structural elements as =CH-, -C=C-, -CH_2-, -CH_3.

3. SHFC, obtained by us they are characterized by high surface activity and adsorptivity on the surfaces of the finely dispersed particles of the basic cement minerals (C_3A, C_4AF).

4. Tests on the influence SHFC on the rheological, physic-mechanical properties of cement test and concrete mixtures showed that its application in the technology of concrete of the described

   a. to decrease the water-required in concrete 15-20%;

   b. increases the mobility of cement test and concrete to 10 times;

   c. to increase the strength of studied test and concrete 1,5-1,8 times.

5. It is established that [SGFK], obtained from the oxidized brown coal, which are the withdrawal of coal industry, satisfy basic technical requirements by that presented to the highly active plasticizers of group of chemical additives to the technology of concrete.

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Humic Substances as Stabilizing Agents for Superparamagnetic Nanoparticles

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Keywords: humic, biocompatible magnetic nanoparticles, γ-Fe_2O_3

1. INTRODUCTION

Magnetic fluids are stable colloidal suspensions usually containing ferrite-based (MFe_2O_4) magnetic nanoparticles dispersed in organic or inorganic liquid carriers. Magnetic fluids stable under physiological conditions are of great interest for potential biomedical applications as contrast agents for magnetic resonance imaging (MRI) or colloidal mediators for cancer magnetic hyperthermia. However, magnetic nanoparticles without additional coating often tend to aggregate in water or tissue fluid limiting the range of their applications (1). To prevent nanoparticles aggregation, modification of iron-oxide nanoparticles by humic acids has been studied in the proposed research for the first time.

2. MATERIALS AND METHODS

Humic acids (HA) of brown coal derived from commercially available potassium humate of leonardite were used for stabilization of oxide nanoparticles. The composite of γ-Fe_2O_3-NaCl was obtained using aerosol spray pyrolysis method (ASP) as described in (2). Sodium chloride was used to encapsulate nanoparticles. The aerosol stream was burned in furnace at 650°-700°C. The as-prepared samples were examined by XRD, TEM, Mössbauer spectrometry, magnetic measurements and dynamic light scattering (DLS). For modification with HA, 2% solution of composite was prepared in 100 ppm HA solution at pH 7 using ultrasonic bath. Stability of colloid obtained was monitored using DLS measurements. Iron content in precipitate and fractions of different particle size was determined using spectrophotometry analysis with o-phenantroline after oxidative digestion of organic matter.

3. RESULTS AND DISCUSSION

The composite particles of γ-Fe_2O_3-NaCl obtained by ASP represent hollow nanostructured microspheres consisting of ~50 nm nanoparticles. Mössbauer spectra measured in the range of 300-16K proved the presence of metastable phase of maghemite γ-Fe_2O_3. The isomer shift relative to α-Fe and quadruple splitting at 16K were 0.46 and 0.04 mm/s, respectively. The magnetic measurements illustrate the superparamagnetic behavior of the particles. According to DLS results, the obtained humic-composite colloid was composed of particles with a peak size at 145±60 nm preserved within ten days of observation. According to the chemical analysis iron content exceeds 4 mg per liter for the refined fraction of modified nanoparticles after ten days of ageing.

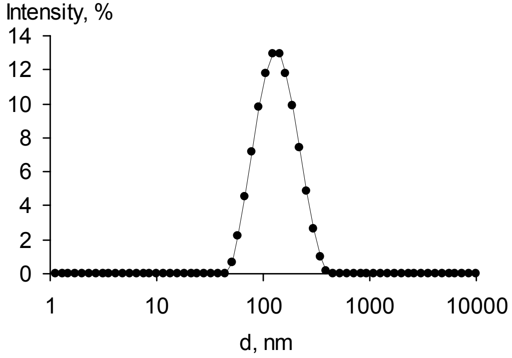

Figure.1 Particles size distribution for magnetic nanoparticles obtained at 650°C and stabilized by HA as measured by dynamic light scattering (DLS); (D=145±60 nm).

4. CONCLUSIONS

The prospects of novel applications of humic substances as stabilizing agents for γ-Fe_2O_3 superparamagnetic nanoparticles in aqueous solutions were demonstrated.

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Sorption of Np(V) onto Hybrid Clay-Based Materials: Montmorillonite-Melanoidin

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Keywords: melanoidin, neptunium, sorption, montmorillonite, composite, hybrid clay-based materials

1. INTRODUCTION

Humic substances may be found in the interlayers of swelling clays (1). There are many laboratory evidences of defined organic compounds absorbed into the interlayers of clay minerals, but little is known about the existence of those complexes in natural soils, due to the extraction methods, which are able to destroy the silicates. There is evidence for the interlayer sorption of fulvic acids at pH below 5.0 (2), but the higher weight humic acids may be too large to penetrate the interlamellar spaces (1). In order to simulate natural conditions, a different approach has to be followed, i.e. the preparation of hybrid clay-based materials (HCM) may throw new light on the problem as humic-clay-complexes are likely to be formed in statu nascendi of the humic substances (3).

Melanoidins are polymers obtained after the condensation and dehydration of amino acids and sugars; they were first described by Louis Camille Maillard (4) in 1912 and are the products of a chemical reaction between an amino acid and a reducing sugar, usually requiring the application of heat. The use of melanoidins as natural analogues has been reported for the last years, in order to understand the reactions of humic substances with the actinides. The most successful approach was the synthesis of the melanoidin M42, which has similar properties to Aldrich Humic Substances (5).

Neptunium will become a major contributor to the radiation inventory in nuclear waste due to the long half-life of its isotope ^{237}Np ($2.14 \cdot 10^6$ years). It can exist in the oxidation states III – VII, but the cation NpO_2^+ dominates the aqueous speciation under a wide range of environmental conditions.

The negative charge of smectites make this group of clay minerals extremely reactive in soils, they are responsible for most of the swelling properties of soils. They are widely distributed throughout the world, and montmorillonites and beidellites are the most commonly found. Due to the little amount of iron, we chose the Texas montmorillonite STx-1 for our investigations.

2. MATERIALS AND METHODS

The montmorillonite (STx-1) sample was supplied by the Clay Minerals Society; no treatment was done with the montmorillonite before the synthesis. L-Glutamic Acid, L-Tyrosine, and D(+)-Xylose (Merck, Darmstadt, Germany), were used for the synthesis of the melanoidins and hybrid clay-based materials. The hybrid clay-based materials were synthesized in a batch experiment starting from 16.5 g xylose, 13.5 g L-tyrosine or 11 g glutamic acid, 3.2 g montmorillonite STx-1 and 150 mL of water. After reflux boiling (100°C, 90 h) for L-tyrosine and (80°C, 90h) for glutamic acid (5). A brown solid was formed, after centrifugation the solid product was ground with ethanol and ether (Acros Organics), and again centrifuged. This solid was stirred with 150 mL 2 M NaOH (Merck) 8 hours under N_2. After centrifugation the solid was again stirred with 100 mL 2 M NaOH (Merck). After centrifugation the HCM were isolated, dialyzed using dialysis tubes (Spectrum Laboratories Inc., exclusion limit MWCO <1000) against purified water and then lyophilized.

The samples were pre-equilibrated at least 3 days; the equilibrium with CO_2 was accelerated by adding $NaHCO_3$ and Na_2CO_3 (Merck). The pH was daily controlled and if necessary readjusted with $HClO_4$, or NaOH (Merck). The contact time with Np was 3 days, after that, phases were separated using a Beckmann Coulter Avanti J-30I High Performance Centrifuge (108 800g, 1 hour). For the determination of the organic substances in the supernatant of the samples UV/vis-spectroscopy was used.

^{239}Np(V) stock solution was produced by irradiation of ^{238}U ($UO_2(NO_3)_2 \times 6\ H_2O$) at the TRIGA Mainz research reactor at 100 kW for 6 h, followed by separation from uranium and other fission products via anion-exchange chromatography (6). Higher neptunium concentrations can be achieved with ^{237}Np, which is also separated from fission products using anion-exchange chromatography (6). ^{239}Np can be determined by γ-spectroscopy (lines 106.12 keV and 103.76 keV), ^{237}Np is determined using Liquid Scintillation Counting (LSC).

3. RESULTS

The yields for the reaction, concerning the HCM, are very similar for the synthesis with L-tyrosine and with L-glutamic acid, indicating no preference in the reaction for any reactant, e.g., L-tyrosine or L-glutamic acid. Similar yields were obtained while forming a caramel-clay nanocomposite under MW irradiation (8). The composites were characterized using NMR, XPS, SEM, STXM and DTA (9).

Table 1. Yields and C, N content for the two composites isolated, and comparison with the contents in STx-1

Sample	Yield (g)	C %	N %
GHS	1.7 ± 0.2	1.7 ± 0.3	0.11 ± 0.02
THS	1.8 ± 1.2	1.3 ± 0.2	0.06 ± 0.03
STx-1	–	0.03 ± 0.01	<0.02

Sorption of neptunium on the hybrid materials was studied for the pH-range from 6 to 8.5 under ambient conditions (presence of CO_2), and at Np concentrations of 10^{-12} M. With increasing the pH, dissolution of organics was observed, which was confirmed by UV/Vis absorption spectroscopy. It is interesting to note that the fraction of Np sorbed to the dissolved organics is equal to the difference in percent sorption on pure STx-1 and the HCM solid.

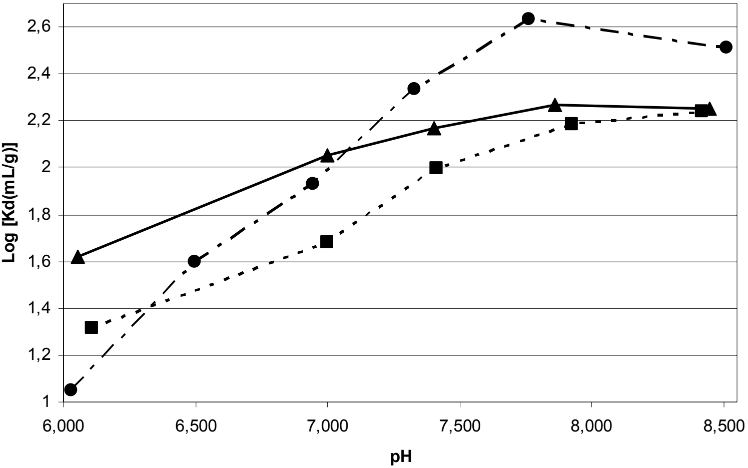

Figure 1. Sorption of ^{239}Np onto the two different hybrid materials (GHS ▲and THS ■) and onto pure STx-1 montmorillonite (●)(10).

Table 1. Summary of complexing properties of the >1 kDa organics dissolved from the hybrid materials

Sample	% Np sorbed at pH 8.4	% Np bound to organics > 1kDa	% Np free
GHS	41.6	16.1	42.4
THS	41	7.4	51.6

4. CONCLUSIONS

In situ polymerization of melanoidins in the presence of montmorillonite lead to hybrid materials. The sorption of neptunium on those materials was investigated. The presence of melanoidins in montmorillonite reduces the sorption of neptunium above pH 7 in comparison with pure montmorillonite, due to the release of organic material from the hybrid material into the aqueous phase. Below pH 7, the sorption on the HCM is increased. This behavior assembles that of the sorption of actinide metal ions on kaolinite in the presence of Aldrich humic acid (11).

The dissolution of organic material from the HCM samples points to the formation of aggregates of organic material on the surface of the montmorillonite.

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Section V

Industrial production, standardization and innovative application of humic materials

Primary Energy Production by Photoreduced Humic Materials

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Keywords: photoreduction, redox, bacteria

1. INTRODUCTION

Many bacteria are capable of generating energy for cell maintenance and growth by oxidizing electron donors such as Fe(II) or hydroquinones within reduced humic substances (HS) under nitrate-reducing conditions. Organisms capable of these respiratory forms are prevalent in the environment, existing at up to 10^6 cells/g soil or sediment in some environments (1, 2). However, the production of reduced humic material or Fe(II) in relatively high-redox, nitrate-rich environments is poorly understood. Data presented here indicates that exposure of HS to incident radiation can generate humic-born hydroquinones and/or reduced functional groups capable of reacting with Fe(III) to Fe(II). These results suggest that photoreduction of HS may act as a form of primary energy production via two mechanisms. Firstly, generation of hydroquinones by the photoreduction of HS represents a conversion of light energy into reducing equivalents directly suitable for supporting respiratory processes such as nitrate reduction. Secondly, indirect production of Fe(II) by the reaction of reduced HS functionalities such as hydroquinones, semiquinones, or semiquinone radicals with Fe(III) offers an alternative route for light energy to be converted to a bioavailable electron donor.

Quinones represent a major class of redox-active functional groups within HS (1). A wide variety of model quinone compounds can be reduced to hydroquinones, semiquinones, or semiquinone radicals in the presence of light (3, 4). Suitable electron donors for these reactions include organic compounds, as well as inorganic compounds such as H_2O (3, 4). In this work, photoreduction of humic acids, as well as model quinones, by full spectrum, UV, and gamma photons derived from isotopic decay is observed. Photoreduced quinones are found to be directly bioavailable as electron donor sources for model nitrate-reducing microorganisms. Further, photoreduced HS and quinones were capable of chemically reacting with Fe(III) upon its introduction to the solution in the absence of light, generating Fe(II). Although HS are known to photosensitize the reduction of Fe(III), this work demonstrates that photoreduction of humic material can lead to Fe(III) reduction in the absence of co-illumination.

2. MATERIALS AND METHODS

Anoxic solutions of Aldrich humic acids and/or 2,6-anthraquinone disulphonate (AQDS) were prepared by dissolving the solute into 10mM phosphate-buffered nanopure water containing 0.1g/L KCl, which was boiled and cooled under N_2, anoxically dispensed into serum bottles, and then autoclaved. Filtered (11μm pore size filters) creek water was substituted for nanopure water where noted. Phosphate-buffered (10 mM) artificial groundwater medium lacking bicarbonate (1) was also used where noted. For photoreduction reactions, solutions were anoxically transferred to glass or fused quartz tubing, gassed out with N_2, and capped with thick butyl-rubber stoppers. Photoreductions were carried out in light chambers equipped with full-spectrum or UV fluorescent lights (ambient temperature varied between 35 and 45 degrees C). Photoreduction was also achieved by exposing humic solutions dispensed in anoxic glass vials to gamma radiation derived from decay of a Cs-137 source (total exposure of approximately 4.8 kGy). Reducing equivalents from photoreduced HS or AQDS were quantified by iron back-titration as previously described using ferric citrate, $FeCl_3$, or synthetic amorphous ferric (hydr)oxides as a titrant (1). Production of Fe(II) was monitored via the ferrozine assay. Microbial experiments with *Azospira suillum* were performed under growth conditions as previously described (1).

3. RESULTS AND DISCUSSION

Initial photoreduction experiments were conducted in anoxic glass tubes. Artificial groundwater medium amended with 5mM AQDS underwent a gradual color change from yellow to red over an 8-hour period when exposed to full-spectrum light. A back-titration of the photoexposed AQDS solution with Fe(III)-citrate led to the generation of Fe(II) (Figure 1). Fe(II) production was not observed in controls foregoing light treatment, indicating that light catalyzed development of reduced-redox active species. Likewise, exposure of artificial groundwater medium lacking AQDS to light did not lead to reduced species capable of reducing Fe(III)-citrate. After the photoreduction reaction was halted. *Azospira suillum* strain PS was added to photoreduced medium in the presence of nitrate as an electron acceptor. Microbial oxidation of photoproduced reduced quinoid species was apparent, as observed by back-titration analysis. This result indicated that strain PS was able to directly access and oxidize the reduced product generated from the exposure of AQDS to light.

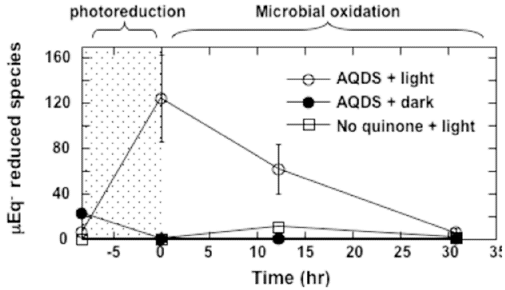

Figure 1. Photoreduction and subsequent re-oxidation by strain PS of artificial groundwater medium containing AQDS exposed to full-spectrum light.

Photoreduction of quinone solutions was much more efficient when glass tubing was replaced with fused quartz. Photoreduction was also accelerated by replacing the full-spectrum light source with a UV source bulb (maximum output = 254nm). Although photoreduction of AQDS to reduced species was achieved in the absence of any other solute other than AQDS and buffering components, photoreduction was further enhanced in the presence of buffered 11micron-filtered creek water (data not shown) or when AQDS solutions were mixed with humic acid suspensions (1.25 mM AQDS, 0.1 g/L HA) (Fig 2a). The AQDS/humic acid mixture was able to rapidly reduce synthetic Fe(III) hydr(oxides). After 4 days, the Fe(II) generated in this reaction remained unaltered within the standard deviation of the initial reading, indicating the Fe(II) was relatively stable as a function of time (data not shown). Similarly, when phosphate-buffered 0.1 g/L humic acids solutions alone were exposed to UV light in quartz tubing, reduced species were formed which readily reacted with Fe(III) to generate Fe(II) (Fig 2b).

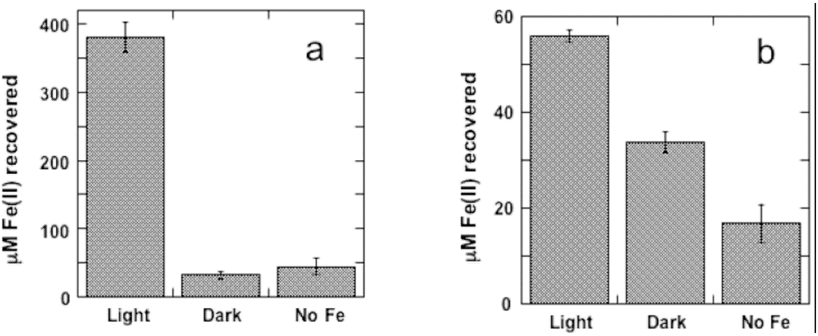

Figure 2. Photoreduction of AQDS/HA mixtures by exposure to UV for 2.3 h (a) as indicated by reduction of introduced Fe(III). Photoreduction of HA alone under the same conditions was achieved with an 18 h illumination period (b).

Glass bottles containing anoxic, phosphate buffered solutions of humic acid (1g/L) were placed in a gammator fitted with a Cs-137 gamma source. Exposure of humic acid solutions to radioactive decay products resulted in production of redox-active species capable of reducing Fe(III)-citrate to Fe(II) (Fig 3). Controls not exposed to radiation did not exhibit an increase in the reducing capacity of the humic acid solution.

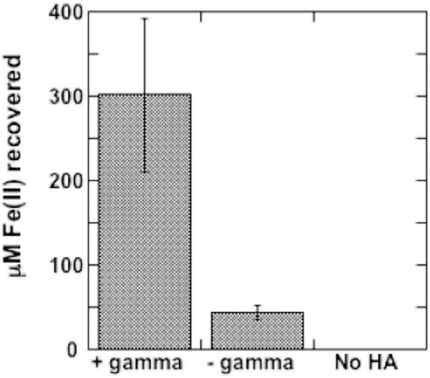

Figure 3. Recovery of Fe(II) after titrating gamma-irradiated HA solutions with Fe(III)-citrate.

4. CONCLUSIONS

Photoreduction of humic substances and quinones yields two potential electron donating compounds capable of supporting microbial respiration: reduced quinone species and Fe(II). Photoreduction of humic substances therefore represents a potential mode of primary energy production in some environments by converting incident light to a bioavailable format.

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Characterization of Organic and Organomineral Liquid Fertilizers

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Keywords: carbon, methodology, humic substances

1. INTRODUCTION

Use of organic and organomineral fertilizers has increased very much in Brazil and the world in the last two decades. In Brazil, this rapid increase in the demand for these products leads the Ministry of Agriculture, Livestock and Food Supply to search for new methodologies for the verification of their quality. This work aims to obtain information about the nature of these fertilizers.

2. MATERIALS AND METHODS

Six samples of liquid organic fertilizers commercialized in Brazil were selected, being made of different raw material. Density, pH, electric conductivity, and total solids in the commercial samples (Table 1) were determined. Additionally, aliquots of commercial products were lyophilized for elementary, thermogravimetric, and infrared analysis.

Table 1. Characteristics of liquid organic and organo-mineral fertilizers

Samples	Classification	Origin material	pH*	Density (g cm^{-3})	Total solids (g L^{-1})	Electric Conductivity**,(dS m^{-1})
L1	organomineral	Leonardyte	9.96	1.15	412.06	0.01
L2	organic	Leonardyte	10.68	1.11	267.20	0.01
P1	organic	Peat	9.30	1.17	514.22	0.03
P2	organic	Peat	10.38	1.03	95.60	0.00
H1	organomineral	HOR***	4.84	1.17	531.32	0.02
H2	organic	HOR***	3.49	0.88	253.16	0.01

*dilution 1:10; **dilution 1:100; *** Hydrolizated organic residues.

The humic acid fraction of each product was extracted according to the methodology of the International Humic Substances Society. Humic acids extracted and the lyophilized solid portions of the commercial products were analyzed by using the elementary analyzer (CHN), Perkin-Elmer CHNS/O 2400, employing 5 mg ± 0.100 mg of sample.

Samples of humic acid were also analyzed by thermogravimetry, with aliquots of 3-4mg in a DP Union TGA Q-500 thermo-analyser, under synthetic air atmosphere at a flow of 60 mL min^{-1}. The FTIR spectra in the region of the infrared of humic acids were obtained in a Perkin-Elmer Spectrum One infrared spectrometer in pellets of KBr (mass proportion of 1:200).

Speciation of the most reactive functional groups present in the sample of humic acid was made with potentiometric titration. The experimental data were analyzed by using the Best7 program (4). The distribution of species was determined with the SPE and SPELOT programs (2).

3. RESULTS AND DISCUSSION

The determined pKas values (Table 2) could be attributed to carboxylic groups (phthalic and acetic acids), phenolic acids (phenol and cathecol) and salicylic acid (1). After speciation of the organic molecules present in the samples, carboxylic acidity was observed to be predominant. This characteristic is important, since carboxylic groups are more active in tropical soils (3).

Table 2. Potentiometric titration data of the samples of organic and organomineral fractions of fertilizers

Sample	Sites	pKa	%	Acid	mmol/g	Total acidity (mmol g^{-1})	Predominant Acidity
L1	S1	4.77	46	Phtalic Cathecol Phenol	0.532	1.152	carboxylic
	S2	7.21	23		0.262		
	S3	9.48	18		0.205		
	S4	10.34	13		0.153		
L2	S1	4.75	44	Phtalic Phenol	0.516	1.181	carboxylic
	S2	6.52	21		0.248		
	S3	10.09	35		0.417		
P1	S1	5.10	43	Phtalic Phenol	0.314	0.734	carboxylic
	S2	7.69	30		0.217		
	S3	9.55	28		0.203		
P2	S1	4.90	62	Phtalic cathecol	0.419	0.671	carboxylic
	S2	6.88	17		0.117		
	S3	9.28	20		0.135		
H1	S1	4.44	31	Acetic	0.615	1.981	Phenolic
	S2	9.07	69	cathecol	1.366		
H2	S1	4.18	48	Acetic cathecol	1.408	2.932	carboxylic
	S2	6.44	27		0.777		
	S3	9.09	25		0.747		

Despite being materials of different origin, the results of the elementary analysis (Table 3) have showed level of carbon with narrow range among the samples. This difference is even smaller in purified humic acid. However the level of nitrogen presented a wide range among the samples. The samples P1, P2, H1 presented significant differences between the amount of nitrogen in the commercial lyophilized product and purified humic acid. This fact could be is explained by the addition of nitrogen to the fertilizer in its manufacturing process. Through the analysis of the H:C atomic relation, it was possible to separate the samples with contrasting characteristics. Purified humic acids of the same raw material present a similar atomic ratio. Leonardyte and Peat-based fertilizers presented low H:C relation while hydrolysed-based fertilizers showed a high H:C relation. This higher H:C ratio is related with aliphatic chains and this is usually related to a higher biodegradability of these substances. It was also observed a high C:N ratio in the purified humic acids of the samples of fertilizers with leonardyte as the origin material.

Table 3. Amount of carbon, hidrogen and oxigen of the commercial lyophilized product and in the corresponding humic acid determined by the elementary analysis

Sample	Lyophilized commercial Product					Purified Humic Acid				
	C%	H%	N%	H:C	C:N	C%	H%	N%	H:C	C:N
L1	33.79	4.52	0.93	1.61	36.33	54.19	4.64	0.69	1.03	91.63
L2	42.74	5.39	0.75	1.51	66.48	64.43	5.24	1.05	0.98	71.59
P1	23.74	5.43	24.44	2.74	1.13	54.19	4.75	2.37	1.05	26.68
P2	29.07	4.56	13.81	1.88	2.46	57.66	3.94	1.75	0.82	38.44
H1	32.51	6.83	23.25	2.52	1.63	47.96	6.28	8.02	1.57	6.98
H2	29.41	5.89	4.54	2.40	7.56	49.30	8.90	6.15	2.17	9.35

Hydrolysed-based fertilizers had a predominance of organic composts thermodegradable below 200°C. On the other hand, the samples of leonardyte and peat-based fertilizers had thermal decomposition of their composts at temperatures between 400 and 500°C, hence explaining a higher recalcitrance of these compounds (Figure 1).

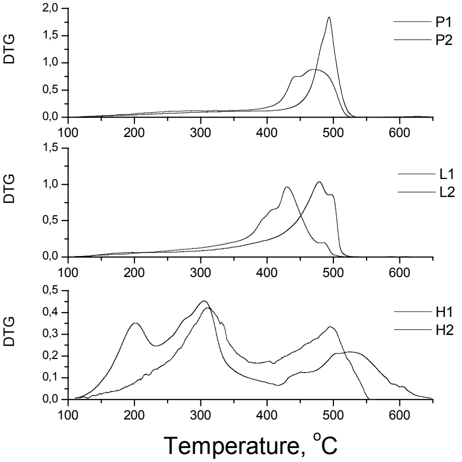

Figure 1. Thermogravimetric curves of the first derivative of HA extracted from fertilizers based of leonardyte (L1 e L2), peat (P1 e P2) and hydrolysed composts (H1 e H2).

The infrared spectra of these samples of fertilizers of the same origin material presented similar characteristics. Peat-based samples presented peaks characteristic of carboxylic and aromatic groups; and predominance of hydroxyl. The hydrolysed-based samples presented characteristic peaks of aliphatic compounds (2940; 2840; 1470 cm^{-1}), amide (1540-1500 cm^{-1}; 1080-1034 cm^{-1}) and carbohydrates (2918; 2850; 1544; 1508; 1452 cm^{-1}). Leonardyte samples have displayed spectra similar to those of peat. Peaks characteristic of aromatic groups and C-C bond in aromatic ring are predominant (1548-1510 cm^{-1}) (Figure 2).

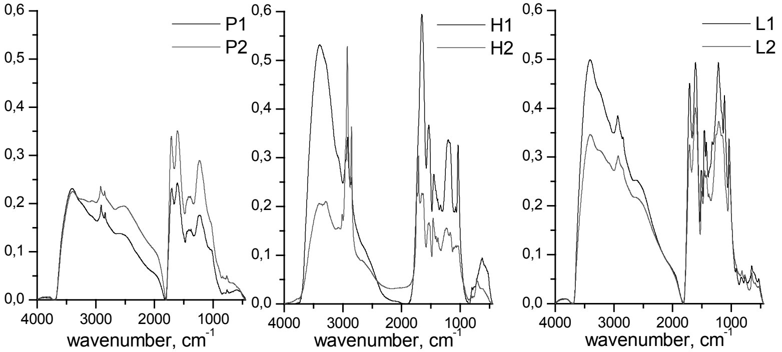

Figure 2. Infrared spectra of humic acid samples extracted from fertilizers based of peat (P1 e P2), hydrolysed composts (H1 e H2) and leonardyte (L1 e L2).

4. CONCLUSIONS

By the techniques employed for the characterization, fertilizers have presented distinct features. It was possible to identify the different compounds through them and to observe resemblances to samples of the same origin material. We have concluded that the information obtained trough those techniques can support the Ministry of Agriculture, Livestock and Food Supply, and contribute to a better standardization of organic and organomineral liquids fertilizers.

ACKNOWLEDGEMENTS

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Standardization and Legislative Regulations of Commercial Humic and Humic-Based Products

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Keywords: legislative regulations, humic substances, organic carbon

1. INTRODUCTION

Humic substances (HS) are complex, heterogeneous mixtures of polydisperse components formed by biochemical and chemical reactions during the humification processes. The HS are major components of natural organic matter in soil and water as well as in organic rich geological deposits such as lake sediments, peats, brown coals and shales. The HS are involved in many processes in soils and natural waters, e.g. soil weathering, plant nutrition, pH buffering, trace metal mobility and toxicity, bioavailability, degradation and transport of hydrophobic organic chemicals, formation of disinfection by-products during water treatment, and heterotrophic production in blackwater ecosystems (web-site http://www.ihss.gatech.edu/ of the International Humic Substances Society).

In agriculture HS and HS-based products (solid and liquid) are generally used in plant nutrition: their role in increasing bioavailability of nutrients and biological activities is well supported in scientific literature (1, 2). For a proper use of HS it’s fundamental to know their elemental composition as well as some basic features. This information is basically included and well described in scientific papers.

In commercial products such data are often lacking. The main reason is that at a national and, especially, international level (3) the specific rules neglected HS. In fact, it must be kept in mind that even the recent European Regulation 2003/2003 (3) has completely neglected organic and organic-based fertilizers and amendments. At the best the existing rules require the declaration of total content of organic and humic carbon (4). Among others, the Italian Legislative decree 217/06 (5) is one of the most advanced in Europe from this point of view.

The need of standardization and regulations of commercial humic and humic-based products is imperative in a modern agriculture and for a market globalization.

Table 1. Some parameters to be declared in organic amendments containing humic substances according to the Italian Law of fertilizers (5)

Product	Compulsory statements	Requested content
Humified peat	Organic matter (OM)	40% min. (dry weight)
	Humified/extractable OM(a)	60% min.
	Total organic carbon (TOC)	≥ 20% (d.w.)
	Humification rate HR(b) = (HA+FA)/TOC•100	≥ 50%
Leonardite	Total organic carbon (TOC)	≥ 30% (d.w.)
	Organic matter (OM)	not stated
	Humified/extractable OM(a)	not stated
	Humification rate HR(b) = (HA+FA)/TOC•100	≥ 60%
Lignite	Total organic carbon (TOC)	≥ 30% (d.w.)
	Humified carbon (HA+FA)	≥ 15% (d.w.)
	Degree of humification DH(b) = (HA+FA)/TEC•100	≥ 50%
Humic extracts(c)	Total organic carbon (TOC)	≥ 30% (d.w.)
	Humification rate HR(b) = (HA+FA)/TOC•100	≥ 60%
Soluble humates(c)	*Solids*	
	Water soluble organic carbon (WSOC)	≥ 19.5%
	Humification rate HR(b) = (HA+FA)/TOC•100	≥ 90%
	Fluids	
	Water soluble organic carbon (WSOC)	≥ 2.8%
	Humification rate HR(b) = (HA+FA)/TOC•100	≥ 90%

(a)OM extracted with 0.1M NaOH + 0.1M $Na_4P_2O_7$. (b)See Ciavatta et al. (6). (c)Isoelectric focusing technique is recommended to recognise the matrix (peat, leonardite, lignite, etc.).

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A Perspective on Over a Decade of On-Farm Research on the Influence of Humates Products on Crop Production

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Keywords: humates, crop production

1. INTRODUCTION

Since 1900, soil organic matter has declined drastically in America's farmlands and around the world as a result of carbon turnover and cropping systems. Soil is a living system: When organic matter enters the community of soil organisms known as "the soil food web," it is degraded through chemical, physical, and biological pathways. Humic substances, major constituents of soil organic matter, have long been known to have properties such as chelation, mineralization, buffer effect, clay mineral-organic interaction, and cation exchange. Since 1990, we evaluated commercial humic acid products derived from lignite and leonardite in different cropping systems. The results of these evaluations differed as a result of the source, concentration, processing, quality, types of soils and cropping systems.

2. MATERIALS AND METHODS

Research trials were established between the years 1990 and 2007, to evaluate the efficacy of different humate products in potatoes, sugar beets and alfalfa in western Idaho, USA. For each research trial, humic acid treatments were arranged in a randomized complete block design with four replications. The products were applied as a foliar, side-dressed, or top-dressed. For the top-dressed, the hills were opened above the potato seeds prior to application of the products for pre emergence treatments. Liquid humic products were applied with solo pack sprayers to the opened furrow. Furrows were closed immediately after application of different treatments. In different trials, granular humates were weighed (40.48 kg ha^{-1}) and spread as evenly as possible to treated rows, according to block design randomizations. Liquid humic products were applied according to manufacturers' recommendations. At the end of crop season, potato, sugar beet and alfalfa plots were harvested, graded, weighed, and quality parameters were evaluated.

3. RESULTS AND DISCUSSION

Data from humic acids trials conducted over a seventeen-year period showed that different products responded differently in relation to yield and quality. The important qualifying factors were: a) source; b) concentration; c) processing; d) chelating or complexing capacity of the humic acid products; e) functional groups (Carboxyl -CO2H; Phenol -Ohp; Hydroxyl -Oha; Ketone -C=O; Ester O=C-O-R; Ether –C-O-C-; Amine –NH_2,-NH,-N); f) rotation and soil quality factors; i) consistency of the product in enhancing yield and quality of crops; j) mineralization effect; and k) influence on fertilizer use efficiency.

A comparison of statistical analysis of field data to determine the efficacy of twenty different humic acids products has shown that, in general, good-quality products consistently showed significant yield results. Under our research, crop yield increased from a minimum 9.4 percent to a maximum 35.8 percent. Also, the use of good-quality humates with complexing macro- and micronutrients in the solution enhanced fertilizer use efficiency and potato quality.

Overall, we did more research on potato fields than on sugar beet fields. Collectively, the consistent use of good quality products in our replicated research plots in different years resulted in a yield increase from 11.4 percent to the maximum of 22.3 percent. Humic acid products enhanced nitrogen mineralization in early season by an average of 9.6 percent. I must note that caution should be taken to evaluate the C:N ratio early in the season, to prevent higher mineralization after mid-June. This will help to maintain low conductivity and high sugar level by the end of the crop season. Alfalfa research plots had yield increases from a minimum of 8.2 percent to the maximum of 20.9 percent.

4. CONCLUSIONS

In the past decade, there has been a drastic increase in the quality of research and development of organic and humic acids by some well-established manufacturers. Our experimentations with these products showed an increase in the yield and quality of crops. We have emphasized IHSS findings and quality factors in hundreds of presentations, workshops, seminars and applied research field days that we have given. As a result, humic acid companies have been establishing sound applied research summaries for the use of their different products on different crops. We highly recommend that the growers and other users review each manufacturer’s crop research profiles for results and economic outcomes.

Organogenic Waste Materials as Promising Metal Sorbents

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Keywords: humics-rich waste, metal binding, sorption efficiency

1. INTRODUCTION

Studies on metal binding onto NOM (peat) from strongly acidic electroplating waste in batch and flow-through systems showed high potential of organic matter for metal removal from polymetallic solutions (1). The increasing need for efficient metal sorbents for pollution prevention and control in a variety of bulk applications, as well as a limited availability of peat in many regions and requirements of wetland protection, directs increasing attention to abundant and easy-to-use organogenic wastes such as sewage sludge as potential sorbents of metal cations.

2. MATERIALS AND METHODS

In these studies, a representative sample of air-dried stabilized sewage sludge (SS) from the Zabrze-Downtown Sewage Treatment Plant (Poland) was used. Its physicochemical characteristics and selected parameters pertinent to sorption, such as pH, content and composition of mineral fraction, organic fraction, HA/FA, HS/insoluble organic ratio, CEC_0 and its composition, specific surface and initial metal content were determined. Complex sorption studies comprised laboratory, bench and pilot-scale batch and flow-through experiments with use of mono-, binary and polymetallic solutions at pH 1.5-4.0. Here, the efficiency of SS as a metal sorbent is exemplified in selected results, and major general conclusions are presented.

3. RESULTS AND DISCUSSION

Studied SS appeared to be environmentally problematic due to the high initial content of PTE – Potentially Toxic Elements (Table 1) enriched in mobile and mobilizable fractions. This material though showed a sorption capacity for metal cations, which in monometallic batch systems was comparable with NOM (peat) and was similarly influenced by the kind of dominant anion (2). The sorption capacity of SS for metals (in mass units) represented ascending sequences Ni<Cd<Zn<Cu<Cr<Pb for Me-Cl and Ni<Zn<Cd<Cu<Cr<Pb for Me-SO_4 systems and was several orders of magnitude higher than the initial content of these ions in SS (Table 1). In equivalent units, the sequences for both systems were the same: Cd<Ni<Zn<Cu<Pb<Cr.

Table 1. Initial content (C_0) and sorption capacity of SS for PTE (S_t) (mg/kg, d.w.)

Metal ion	Ni	Pb	Cr	Cu	Cd	Zn
C_0	25	132	53	123	23	2162
S_t	24600	181500	48900	40600	39000	28700

SS demonstrated a high sorption capacity for metals also under flow-through conditions specific for reactive permeable barriers (Fig. 1 – example).

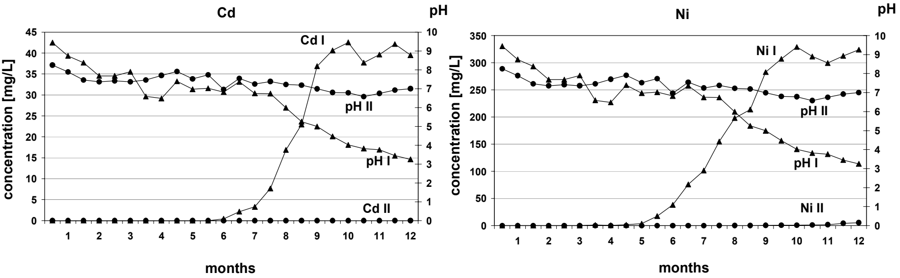

Figure 1. Concentrations of Cd and Ni in leachate from extractive waste
(I – reference column; II – column with a reactive barrier of SS). Input pH 1.5.

4. CONCLUSION

SS as a complex humics-rich waste material exhibited high efficiency as a sorbent of metal cations from acidic polymetallic solutions. Due to abundance and low cost, this material could thus be widely used in various applications as a substitute for natural sorbents.

ACKNOWLEDGEMENTS

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Commercial Humates: The Origin of Organic Matter and Its Environmental Role

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Keywords: humate

1. INTRODUCTION

One of challenges in the transition to sustainable agriculture is the development of environmentally friendly, and cost-effective natural fertilizes, plant growth stimulators, and soil conditioners. One of the most promising natural tools is the use of humic substances (HS). Since the beneficial role of HS in environment is well known, today many companies all over the world produce commercial humates (HUM) from organic raw materials and promote them as soil conditioners, plant growth stimulators, for the remediation of degraded soils, and the sequestration of toxicants (1).

The biochemical mechanism behind the effects of humates on plants growth can be linked to specific features of HS molecules, which in turn depend on origin of organic matter. In this study a possible connection between the origin of organic matter, and the ability of HUM to stimulate plant growth was investigated.

2. MATERIALS AND METHODS

A detailed chemical analysis was conducted on available humate-based products (50 samples) for commercial humates produced from variety of organic deposits in U.S., Canada and Europe. *In vitro* experiments were conducted with plants treated with and without HUM. Reddish seeds (*Raphanus sativis* L.) have been soaked in solutions or water suspensions of HUM at concentrations giving 5-500 mg water-soluble C per liter, and germinated at dark at 27°C for 72 h. The physiological activity of HUM was estimated as a change in primary root length of seedlings.

3. RESULTS AND DISCUSSION

Since HS are complex, stochastic molecules, chemical analyses of HUM were performed at 3 different levels of details, starting from simple basic parameters and continuing to advanced investigations of molecular structure. Results are discussed in presentation. In brief, molecular structure for the majority of HUM tested was enriched in aromatic moieties and close to HS of Mollisols, whereas other soil humic acids together with lignin and plant residues defined other groups (Fig. 1).

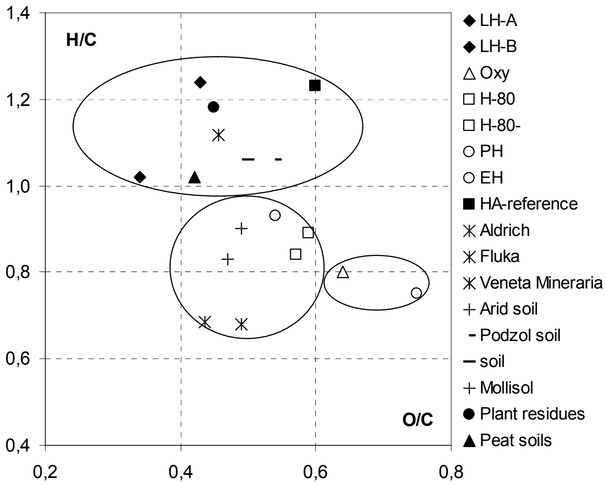

Figure 1. Atomic ratios diagram for HUM and natural HS of different origin.

All of the HUM tested demonstrated auxin-like activity at concentrations 100-5000 mg l^{-1}. However, the level of growth-stimulating activity varied greatly for different HUM-brands, depending on their chemical structure and organic matter origin.

4. CONCLUSIONS

Commercial humates vary greatly in their properties (solubility, elemental content, amount of humic acids, and molecular structure) depending on source material. Each individual commercial humate product must be tested first for their activity and potential risk to the environmental in an authorized laboratory.

ACKNOWLEDGEMENTS

The support of Fulbright Program and RFBR (grant 07-04-01510) is deeply appreciated.

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Greenhouse Experiment on the Effect of Humic Substances on Solubilization of Ogun Phosphate Rock

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Keywords: Ogun phosphate rock, humic substances, phosphorus

1. INTRODUCTION

Sustainability of human life on earth depends on sustainable agriculture (1). Phosphorus (P) is one of the major essential nutrients needed to ensure this sustainability. Presently in Nigeria, high cost and scarcity of mineral P fertilizers (single superphosphate and triple superphosphate is shifting attention to direct use of indigenous phosphate rock as viable alternative to mineral fertilizers. The aim of this experiment is to determine influence of humic substances on solubilization of Ogun phosphate rock.

2. MATERIALS AND METHODS

Greenhouse experiment was conducted at International Institute of Tropical Agriculture, Ibadan, to monitor the effect of humic substances from decomposition of legume biomass on solubilization of Ogun Phosphate Rock (OPR). Soil samples taken to the depth of 20 cm was collected from three experimental sites (International institute of Tropical agriculture, Institute of Agricultural Research and Training (IAR&T) and University of Agriculture Abeokuta) in South Western Nigeria (Table 1).

Four rates of Ogun phosphate rock (0,30, 60 and 90 kg P ha^{-1}) along with 40 kg P ha^{-1} as Triple superphosphate (TSP) was mixed with 1 kg soil in a 2 kg plastic pots arranged in complete randomized block design in three replicates with and without legume biomass. Mucuna and cowpea were used as green manure while newly released rice (*Oryza sativa*) variety NERICA 1 was used as test crop.

Table 1. Chemical properties of soil samples taken at soil depth 0-20 cm from the three experimental sites

Parameters	Ibadan (IITA)	Ikenne (IAR&T)	Abeokuta (UNAAB)
pH (H_2O) 1:1	5.8	6.6	6.2
C (g kg^{-1})	1.21	1.23	0.96
N (g kg^{-1})	0.11	0.11	0.08
P (mg kg^{-1})	54.36	7.34	10.18
Exchangeable cations (cmol kg^{-1})			
Ca	2.77	2.91	2.96
Mg	0.70	1.18	0.68
K	0.77	0.17	0.23
Na	0.10	0.06	0.05
Micronutrients (kg ha^{-1})			
Fe	138.9	98.8	95.9
Mn	236.44	202.88	246.43
Zn	2.39	1.08	1.83
Cu	1.41	1.65	1.35
Particle size (%)			
%Sand	74	68	80
%Clay	12	10	8
%Silt	14	22	12

3. RESULTS AND DISCUSSION

Highly significant treatment effect was observed on dry matter yield and P uptake in the plant tissue (2) with soil samples from all the three sites according to Fisher's LSD at 0.05 probability level (Table 2). Highest value (2.58 g kg^{-1}) in dry matter yield was with pots treated with mucuna and TSP. Treatment combination of cowpea and OPR at 90 kg P ha^{-1} biomass significantly increased rice dry matter yield (2.19 g kg^{-1}) by 16% over pots treated with cowpea biomass alone and 42% over control pot (no legume biomass &no OPR). Soil available P increased significantly as rate of OPR increases with pots treated with green manure (Table 2). Reactivity of phosphate rock on soils that are slightly acidic to slightly alkaline (Table 1) could be effectively enhanced through the action of humic substances (3).

Table 2. Effects of treatment on dry matter yield of Rice in the greenhouse at 9 weeks after planting using soil samples from the three sites

Treatment		Dry matter yield (g kg^{-1})		
Legume	P rates, kg ha^{-1}	IITA	IAR&T	UNAAB
No Legume	0	1.54	0.80	1.01
	30	1.68	0.92	1.1
	60	1.72	1.07	1.27
	90	1.38	1.14	1.52
	TSP	2.30	1.38	1.40
Mucuna	0	1.89	0.98	1.11
	30	1.94	1.02	1.27
	60	2.02	1.23	1.36
	90	1.69	1.55	1.71
	TSP	2.58	1.56	2.10
Cowpea	0	1.89	1.14	1.27
	30	2.19	1.27	1.36
	60	2.02	1.16	1.60
	90	2.06	1.34	1.71
	TSP	2.49	1.38	2.15
LSD($p \leq 0.05$)	L	***	***	***
	P	***	***	***
	L x P	***	***	***

4. CONCLUSIONS

Results from the greenhouse experiment showed that humic substances produced as a result of decomposition of legume biomass enhanced rate of release of phosphorus from phosphate rock to rice plant on slightly acidic soils to slightly alkaline soils.

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Structural Components and Biological Activity among Humic Substances of Low-mineralized Silt Sulphide Muds

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Keywords: fulvic acids, hymatomelanic acids, humic acids, humin acids, humic substances, biological activity, pelotherapy

1. INTRODUCTION

Health resort treatment by native muds faces the number of challenges among which there is exhaustion of conditioned muds resources, muds dirtyness by products of anthropogenic origin (1). In spite of broad popularity of traditional mud therapy, it is unacceptable because of medical indications for some categories of patients (2, 4).

Scientists make attempts to extract biologically active fraction from peloids and to build peloid-specimen on basis of peloids, which without yielding in therapeutic activity allow to reduce the amount of contraindications, make therapy dosated, enhance the possibility of combined application both with medications and with physical agents to lighten the medication transportation and storage, improve work conditions of medical staff, make therapy more available to solve above-named problems. It is the opinion of some authors that muds application in the form of medications is more adequate stimulus in comparison with traditional pelotherapy (3).

Listed factors are the foundations to search biologically active peloids preparations and their introduction into health-care service practice.

The aim of research. To study structure functional properties, to disclose biologically significant effects and to ascertain the most active fractions of humic substances of low-mineralized peloids.

2. MATERIALS AND METHODS

Low-mineralized silt sulphide muds being the object of our research are compound multicomponent combination of substances. Unlike the soil and peats, biochemical processes which inherent to silt sulphide muds formation, take place in them at high values of biological activity period, in the presence of easy-to-assimilate organic substances, soluble sulphides and appropriate microflora by microorganisms. Humin acids of peloids form and exist under anaerobic conditions, which is reflected in their structure and chemical activity.

All-round description of humin acids function-group mixture, biogeotransformation degree, time history of group mixture according the seasons of the year are described on the basis of research methods combination both classical and modern. Element structure of macromolecules and properties describing their structure are determined at submolecular level. Functional groups contents are quantitatively described which are responsible for acid-base properties.

The influence of fulvic acids, himatomelanic acids, humic acids and summary medication on a chronic inflammation dynamics was studied by experimental model of adjuvant-induced arthritis. Activity of pro- and antioxidative systems was estimated according the activity of catalase and superoxide scavenger, the intensity of diene conjugation of unsaturated higher fatty acids and concentration of malonic dialdehyde.

3. RESULTS AND DISCUSSION

On the whole humin acids are presented by more or less coloured high-molecular, polycoupled, polydisperse, polycondensat organic compounds of acid nature having electron-donor and electron-acceptor properties. They have community of structure but differ from each other according the degree of listed properties manifestation as well as molecular weights, particles sizes, amphiphile, configuration, conformation, adsorption property, particle mobility in solution. Such properties lead to appearance different biological activity of humic acids.

Toxicity absence of humic substances is experimentally proved. Negative manifestations were not discovered after enteral introduction of drug dose at 15 g/kg mass of experimental animals. The comparative effect analysis of physiological solution and humic acids have showed activation of monocytic-macrophage system and descent of reaction evidence under emotional-painful stress of healthy experimental animals during the influence of peloid medication.

The research of biological activities of fulvic acids, hymatomelanic acids, humic acids and summary medication in wide concentration range on the model of acute inflammation discovered polymodal dependence of all the medications actions from doses. Comparison antiinflammatory activity with influence of 2,5% diclofenac sodium solution showed more apparent action of humic peloid preparation.

Behavior of immunological blood values during medical treatment of acute inflammation by humin acid allows to make a conclusion that antiphlogistic effect is determined by influence on functional-metabolic activity of neutrophilic granulocytes and monocytes. Such activity is described by humin acids capability to reversible inhibit the epactal interleukin by 1β overactivity macrophages, to level strengthen issue of neutrophilic granulocytes from marrowy depot into blood, to reduce oxygen demand by activated phagocytes with posterior reduction of oxygenous radical generation, which leads to reduction of inflammatory reactions intensity as a result. The most biological activity is discovered in humic acids.

The results were received during the system multifactorial analysis on 19 quantity indicators describing change in cellular blood structure, functional activity of monocytic-macrophage system. They have shown that introduction of test substances at the time of inflammation development considerably changes the degree of integrated parameters deviation of blood system functional condition from specified values.

The received results are evidence of complex polymodal influence of all the peloids preparations from the humuc series on various stages of inflammatory process. Organism reaction under separate components of humic acids in investigated dozes is ambiguous but all the studied connections forward normalization of general balance of redox processes in an organism. Change dynamics of hematologic and immunologic parameters under animal introduction of humic acids with developed adjuvant-induced polyarthritis shows the intensity decrease of inflammatory process with autoimmune component here the therapeutic effect intensified with course dose increasing. The research of humic acids influence on activity of inflammatory process and animals immunologic status with experimental polyarthritis caused by Freund's adjuvant introduction has shown apparent immunocorrected effect of present peloids preparation.

Anti-inflammatory and immunotropic action of humic acids was expressed in blood leukogram normalization, functional-metabolic properties of monocytic- phagocytal system, disbalance levelling of immunoregulatory T-lymphocyte subpopulations, that, apparently, was an evidence of activity decrease of autoimmune reactions under the condition of experimental joints affect of animals.

The histologic feature is an evidence of developing apparent pathologic changes such as parenchyma and stroma edema, sclerosis, vessels hyalinosis and others during rats rheumatoid joint inflammation in peripheral immunogenesis organs (spleen and regional lymph nodes) simulation. Peloid preparations in terms of hymatomelanic, humic, humin acids develop obvious protective effect according to the morphological feature. We research preparations on the basis of fulvic acids according to the morphological characteristics which do not have influence on rats immune inflammation model. The more coupling system is shown in macromolecules of humic peloid preparations the more their biological activity is expressed.

High reparative activity of argentum humate in the form of ointment on hydrophilic basis is experimentally determined. The therapeutic effect of humic acids is proved when hemolytic anemia of laboratory animals are treated. Electrophoretic introduction of humic and humin acids were effective during rheumatoid joint inflammation treatment at the stage of remission. Suppositories on the basis of humic acids have shown high efficiency during chronic prostatitis treatment. The complex dynamical system of humin acids sustains an internal homeostasis of biosystems at organismal, cellular and subcellular levels, forwarding restoration of physiological functions at pathological states and in extreme cases.

4. CONCLUSIONS

Highly effective peloid preparations obtaining has pantobiological significance in studying biotransformation laws of organic substances as well as it will forward the efficiency increasing of pelotherapy, the economy of mud resource depletion and the preservation of ecological biogeocenosis equilibrium from natural zones.

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Beneficial Effects of the Complex Humic Microfertilizer Applied to the Ornamental Plum *Hessei*

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Keywords: complex microfertilizer, humic, plum Hessei, beneficial effects

1. INTRODUCTION

There is a whole variety of industrial humic-based products on the market, which includes humates produced from peat, lignites, sapropel, and organic wastes. Many humate producers incorporate nutritional elements into the commercial products. These elements are usually introduced as water soluble salts, e.g., iron(II) sulphate, or a broad spectrum of salts is mixed with the humate as in case of Humate+7 product (Irkutski Gumat Ltd., Russia). The objective of this work was to test the efficiency of the new complex humic microfertilizer containing Fe(III), Zn(II), Mn(II), Cu(II), and B in the field conditions. The major difference of the tested humic microfertilizer from the industrial humic preparations currently available on the market is a high total content of the nutritional elements (up to 9 % mass) which makes it comparable to the artificial chelates (e.g., DTPA, EDTA, EDDHA).

2. MATERIALS AND METHODS

The plants of the ornamental plum Hessei were used. The experiments included: 1) blank (no microfertilizers added), 2) soil application of the commercial iron(II) humate (Humiron) – Humintech Ltd. (Germany), 3) soil application of artificial Fe(III) chelate in the form of EDDHA complex, 4) soil application of the synthesized complex humate enriched with Fe(III), Mn(II), Zn(II), Cu(II), and B, 5) soil application of the sodium humate (Sakhalin Humate, Russia), 6) foliar application of microelements without humates (Fe, Mn, Zn, Cu, B); 7) foliar application of the synthesized complex humate (Fe(III), Mn(II), Zn(II), Cu(II), and B).

Two years old plants of plum Hessei grown on the common carbonate chernozem were used in these experiments. The amount of plants used for each experiment was from 30 to 34. The plants from the blank experiment did not receive additional microelements. The plants from the humate experiments were treated three times per season with 0.5% humate solution. At the same time the foliar application was conducted for the appropriate experiments using microelement mixtures and complex humate solution at concentration of 0.05%.

Efficiency of the microfertilizers used was estimated by measuring stem diameter, size of the plant, and increase in size of the plant. The latter was calculated by multiplying the length of new branches with their amount.

3. RESULTS AND DISCUSSION

All experiments were conducted during three months. The obtained results are described below: the “stem diameter” has increased up to 205% for blank and sodium humate experiments and it has reached 216% for the complex humate experiment. Foliar application of the complex humate also did not influence this parameter – it accounted for 207%. An increase in the plant size characterizing the growth intensity of the plant foliage, was 335 % for the blank experiment, and 408% for the experiment with the complex humate. Of importance is that the complex humate not only improved the growth of the foliage, but its quality as well. The trees looked much prettier with a thick, uniformly developed foliage. The pure microelements did not provide the difference in the foliage quality observed for the complex humate.

4. CONCLUSIONS

The conducted research has revealed beneficial effects of the application of the complex humate tested on the conditions of the young plants of the ornamental Hessei plum. The observed beneficial effects were the most distinct for the quality of the plants foliage. The conclusion can be made of the promising potential of the application of the humic-based microfertilizers in the organic agriculture.

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Use of Natural and Modified Materials to Minimize Influence of Sulfide Tailings

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Keywords: mine waste treatment, peat-humic agent, organic-mineral complex

1. INTRODUCTION

The huge amount of waste has been collected in the world during the last century as a result of industrial activity. Most hazardous wastes were produced by the ore mining-processing industry as they contain high concentration of heavy metals. Various reagents (carbonaceous rocks, activated carbon, zeolites, iron hydroxides, cellulose and others), different protective screens (6, 12, 14, etc.), microbial populations (1, 7, 9, etc.) are used for area decontamination of the mining industry. In the given work effective treatment ways of liquid and solid waste have been developed on the basis of natural (clay, peat, limestone, etc.) and modified materials (peat-humic agent (PHA), organic-mineral complex, etc.).

2. MATERIALS AND METHODS

The following materials were used in experiments: 1) kaolinite clay ("Dorogino" deposit, Novosibirsk region); 2) peat ("Krugloe" deposit, Kochenevski area, Novosibirsk region); 3) peat-humic agent which has been produced on the basis of peat from "Krugloe" deposit by Planet-RA Ltd.; 4) limestone ("Chernorechensk" deposit, Iskitim area, Novosibirsk region). Laboratory researches were carried out in analytical center of the Institute of geology and mineralogy SB RAS.

3. RESULTS AND DISCUSSION

Exploration of sorption conditions of chosen elements (Zn, Cd, Pb, Cu) on the natural and modified materials (clay and peat) has been carried out in the first series of experiments. Adsorption isotherms are constructed from experiment results. It was shown, that the clay modified by PHA (organic-mineral complex) has increased sorption capacity in 1.5-2.5 times comparing to natural clay and expanded pH interval approximately to 5-8 (4). Binding of potentially toxic elements with humic acids usually goes via the exchange mechanism (interaction of a carboxyl group with metal ions is prevailed) with formation of poorly soluble humates. Formation of organic-mineral complex mainly happens due to the formation of hydrogen bond (-OH…H), COO-…HO-Al bonds, and in a sample with sorbed elements also with R_1-COO-Me-OOC-R_2 bonding. During this process solid particles are getting bigger, easily settling out from a solute, and also sorb metals more completely. Virtually all studied elements are badly desorbing into a solution (the degree of element desorption is less than 3%). It is significant, that metals are most strongly bonded to the organic-mineral complex.

Some ways of waste treatment have been devised basing on the data obtained. Sulfide tailings are oxidized under the action of atmospheric oxygen and as a result acid mine drainages (AMD) with high concentration of SO_4^{2-}, Fe, Zn, Cu, Cd, Pb and other elements are generated. According to static experiments it is possible to use quite simple methods for neutralization of technogenic waters. For waters with the pH 4-5, small additives of limestone (5-10 g/l) and peat-humic preparation (1-2 ml PHA/l) are enough to alkalize medium up to subacidic and about neutral pH (3). For strong-acid drainage waters, the positive effect increases with small additives of sodium bicarbonate or simultaneous use of limestone and peat-humic agent. All used compounds (clay, peat, limestone, PHA, sodium bicarbonate) and their combinations are applicable for treatment of AMD with high contents of potentially toxic elements. The greatest purification efficiency achieved by modified clay (with the addition of sodium bicarbonate (1%) in case of strong-acid technogenic waters) and peat with limestone in the ratio 10:1-2:1, accordingly.

The method of drainage water treatment with the use of peat-humic agent is based on the properties of humic acids (8, 10, 11, 15, etc.). The peat-humic agent is a good metal sorbent and can alkalize acid and especially subacid drainage waters. Required addition of the peat-humic agent depends on water composition. For example, the good results were obtained using A2 ratio (see Table 1) for mine drainage of Belovo hydraulic-mine dump (Kemerovo region, Russia). Subsequently formed flaky sediment can be removed by filtration through activated coal and haydite sand. Activated coal is better to use for afterpurification of strong-acid mine drainage. From the financial point of view it is better to use haydite sand especially for subacid mine drainage as it is relatively cheap material. Acid mine drainage of old sulfide tailings is often spread without any control into environment and pollute rives, reservoirs, and soils. In that case it is possible to use the following method of drainage water treatment. Firstly, it is required to dig out a trench around sulfide tailings for AMD collection. The peat-humic agent is added to a trench with certain concentration. Then it is necessary to put bags with haydite sand in drainage streams or special deepening for filtration of acid mine drainage mixed with the peat-humic agent. Suggested method will help to decrease harmful effect of mine wastes onto environment.

Table 1. The pH and element concentration in drainage water and treated drainage waters, mg/l

Sample	pH	Fe	Zn	Cd	Cu	Ni	Co
Б1	4.8	0.22	112	1.2	270	0.68	0.6
A1	6.4	<0.005	24	1.1	3.2	0.53	0.47
A2	7.1	<0.005	4.3	0.29	1.9	0.27	0.26
A3	8.2	<0.005	<0.005	<0.005	1.8	0.08	0.037
MPC (13)	–	0.3	5.0	0.001	1.0	0.1	0.1

A1 – PHA:AMD ratio = 1:1000;
A2 – PHA:AMD ratio = 1:500;
A3 – PHA:AMD ratio = 1:100;
B1 – Belovo hydraulic-mine dump;
MPS – maximum permissible concentration.

The next experiments leaded to a decrease of technogenic influence of sulphide wastes which occupy huge territories in the form of tailing piles. The eolian transportation of tailings particles is a potentially hazardous process. Particles of thin fraction are transferred on adherent territories: woods, reservoirs, arable lands, fields, and courtyards of local residents. Some solutions of this problem existed before, e.g. the tailings could be covered by break stone or clay. We propose alternative solution in this article: the covering of sulphide tailings by peat-humic agent. In the carried out experiments we separated shallow fractions of substance (particle size is 0.16-0.25 mm and less 0.16 mm) which gave the greatest contribution to particle size distribution of investigated wastes (approximately 50-60%), and also to process of waste dusting. It is recommended to use a solution of peat-humic agent in the ratio PHA:water = 1:500 for prevention of tailings dusting. It is shown, that particle-size distribution of tailings material was changed (2). Fraction of large particles was increased to approximately 50-80%. On the basis of experiment series it is possible to offer following methods of conservation of tailings with the use of peat-humic agent. Acidity of medium was decreased to subacidic and perineural values by using peat-humic agent with small additives of sodium bicarbonate (0.2-1.2 mass. %) or limestone (4-6 mass. %). The purification efficiency was sharply increased along with pH due to loading of soda and limestone. There are quite complex processes going on in the given system which influence on retention of elements. The complex geochemical barrier is formed. Poorly soluble humates of heavy metals, iron oxyhydroxides and poorly soluble carbonates of some elements were formed onto barrier (5).

4. CONCLUSIONS

The proposed cost-effective method of area decontamination will allow quickly improving ecological situation nearby industrial facilities. A wide distribution of raw materials required for sorbents (clay minerals, peat, etc.) in various regions of Russia and usage of modified materials will allow applying the given methods at many industrial enterprises.

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Humic Acids of Brown Coals of the Russia South Far East: Extraction and Complexing Properties toward Gold, Platinum, and Palladium

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Keywords: humic, brown coal, precious metals, gold, chromatography

1. INTRODUCTION

In the last decades brown coals attract high interest as promising unconventional raw materials for industrial production of rare and precious metals. According to literature data, 85-88% of germanium world reserves are concentrated in coals; besides, the content values of rare and precious metals in coals is often higher than in the ores of non-ferrous metals. Brown coals of Primorsky Territory (South Far East of Russia) are characterized as low-calorie fuel, which is not economically suitable for power plants but can rather be used with higher efficacy as raw materials for extraction of gold and metals of platinum group. Here we discuss preliminary data on the possibility to extract precious metals with brown coal humic substances and selectively separate metal-rich and metal-depleted humic fractions, the latter of which can be further applied as agricultural products.

2. MATERIALS AND METHODS

The brown coals studied were collected in different parts of the Khanka, Partizansk and Bikin-Ussuriysk coal basins and South Primorye coal district. Alkaline extracts of coal humic acids were obtained at solid/liquid ratio 1:50 under constant stirring at 60°C during 4h and then filtered through blue ribbon filter paper. 0.1 M and 1 M NaOH, and 0.1 M NaOH / 0.4% H_2O_2 were used as extractants. The extraction degree of organic matter varied from 30 to 68% (taking into account the ash content).

Humic acids of brown coals were obtained from 0.1 M NaOH extracts by precipitation at pH 1, centrifugation at 10000 rpm for 10 minutes. Triplicate re-precipitation was used to purify samples. Ash content of the studied humic acids was less than 5%.

The atomic absorption spectroscopy was applied for the analysis of gold, platinum and palladium content in brown coal alkaline extracts after decomposition of organic matter with nitrohydrochloric acid.

Alkaline extracts of humic acids containing ions of gold, platinum and palladium were fractionated using a chromatographic column packed with Toyopearl 65 with distilled water as an eluent. HLPC was performed on a column Shodex Asahipak GS-520 HQ (Showa Denko, Japan) by means of Shimadzu LC-20A Prominence (Japan) with refractometric detector RID-10A and diode matrix detector SPD-M20A. Elemental compositions of humic acids were determined using the CHNS-O analyzer EuroEA3000 (Eurovector, Italy). Potentiometric titrations of humic acids were performed in a PC-controlled system under constant argon flow. 6-10 mg of air-dried sample was dissolved in 10 ml of 0.1 M KCl adjusted to pH 10 by addition of 0.1 M KOH to allow complete solubilization. Prior to titration soltions were readjusted to pH 2.5 with 0.1 M HCl to attain complete protonation of functional groups. The results of potentiometric titration were processed using the density function method (1) which yields pK-spectra of humic acids.

3. RESULTS AND DISCUSSION

Characterization of extracts by means of HPLC revealed that increase of alkaline concentration or addition of oxidizing agent promoted the extraction of humic fractions with higher molecular weights and facilitated the recovery of precious metals. However, the effect of increasing NaOH concentration from 0.1 M to 1 M was comparable with addition of H_2O_2 (0.4%) that allows reduction of alkaline consumption in the presence of oxidizing agent.

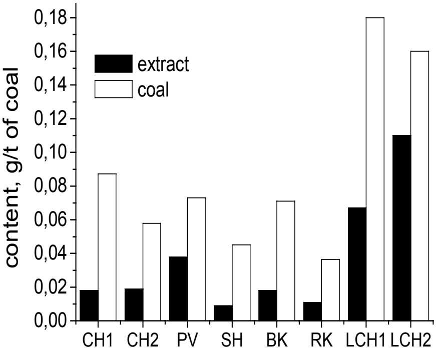

Figure 1. Efficacy of gold recovery with extracts of humic substances from brown coals of Pavlovsky (PV), Shkotovsky (SH), Bikinsky (BK), Cherny-shevsky (CH), Luchegorsky (LCH) and Rakovsky (RK) coal fields.

Analysis of precious metal bulk content in brown coal and in related humic extracts showed that gold can be extracted with efficiency of 20-69% (Fig. 1), the reliable evaluation of extraction degree for palladium and platinum was complicated by low bulk content of these metals in most studied samples of brown coals. Humic extracts of brown coals of Pavlovsky and Luchegorsky coal fields, which showed the highest degrees of gold recovery, were used for further fractionation by gel-filtration and fraction separation by centrifugation at different pH and rotation speeds. Considering relatively low natural content of gold, platinum and palladium, to raise reproducibility of analysis ions of these metals were added to humic extracts in quantities corresponding to metal content in coals up to 10 g/t.

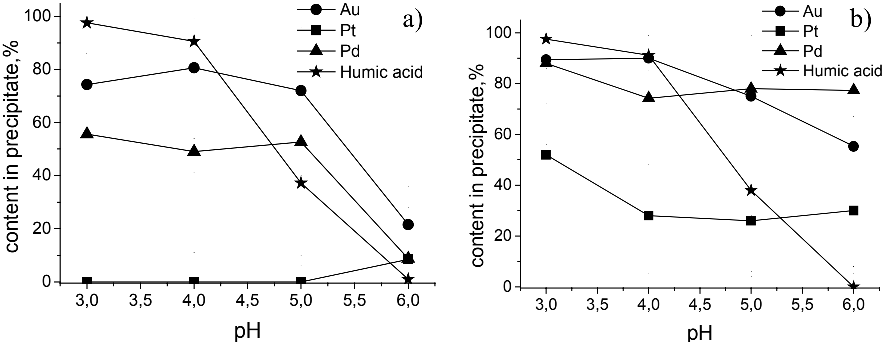

Figure 2. Precipitation of metal-rich fraction of humic acids from LCH2 extract (Luchegorsky coal field) at 5000 rpm (a) and 10000 rpm (b).

Fig. 2 shows that precious metal-rich fractions of humic acids can be selectively precipitated by centrifugation under conditions when the main fraction of organic matter remains stable – pH=5-6. Most efficient recover was observed for gold-rich fraction, while the recovery of platinum-rich fraction was less efficient. Similar trends were obtained for humic extracts of brown coals from Pavlovsky coal field (not shown).

Table 1. Elemental composition of brown coal humic acids

Coal field	Content, $\frac{\text{mass}\,\%}{\text{at}\,\%}$				Atomic ratios	
	C	H	O	N	H/C	O/C
Pavlovsky	$\frac{54.76}{40.85}$	$\frac{4.01}{35.92}$	$\frac{37.42}{22.30}$	$\frac{1.371}{0.93}$	0.88	0.55
Luchegorsky	$\frac{52.44}{40.05}$	$\frac{3.98}{36.50}$	$\frac{37.38}{21.4}$	$\frac{3.066}{2.04}$	0.91	0.53

It should be noted that although elemental compositions of humic acids from these coal field were rather close (Table 1), their acid-base properties differed significantly. Fig.3 illustrates higher content of strong carboxylic groups in humic acids extracted from

Pavovsky brown coal that can explain higher overall colloid stability of humic acid fractions and lowering pH of efficient separation of metal-rich fraction.

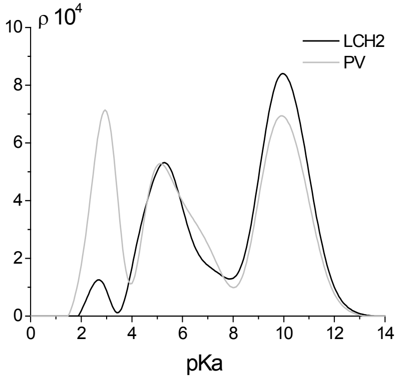

Figure 3. pK-spectra of humic acids from brown coals of Pavlovsky (PV) and Luchegorsky (LCH2) coal fields.

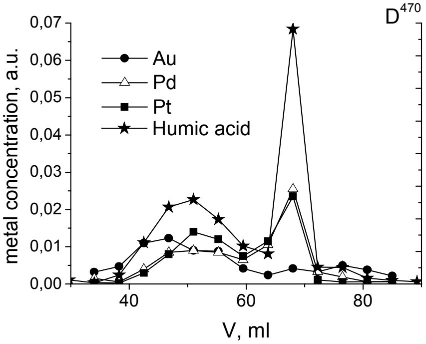

Figure 4. Distribution of gold, platinum and palladium between humic fractions (gel-filtration chromatography on Toyopearl 65).

The distinguished efficiency of the gold-rich humic fraction separation by centrifugation suggests the lowest colloidal stability of gold-rich fraction, possibly, due to reduction of Au(III) to Au(0) and formation of nanoparticles. Indeed, Fig. 4 shows that gold was predominantly eluted with the high molecular weight fraction, while significant amount of platinum and palladium were eluted with the low molecular weight fraction or remained unbound. The lowest binding degree to humic acids observed in sorption experiments for platinum explains very poor efficiency of platinum recovery by centrifugation.

4. CONCLUSIONS

Here we have shown that gold and palladium can be recovered from brown coals with extracts of humic substances. Due to predominant binding of gold to less colloid-stable high molecular weight fraction and possible formation of Au(0) nanoparticles selective recovery of gold-rich fractions with efficacy up to 80% can be reached by centrifugation at pH 5-6.

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Influence of Humics Containing Materials on Poinsettia Hydroponics

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Keywords: humic, nutrition, poinsettia

1. INTRODUCTION

The poinsettia nutrition by subirigation with hydroponical solution creates danger of nitrate tactility (1). Some producers use urea-rich fertilizers in combination with humic substances.

The possibility of biological humification of lignites for production of leonardite-type humus, suitable for bioremediation of polluted sites and for application as anti stress agent in the reclamation of problematic anthropogenic substrate was observed in our previous study

The aim of this study is observation and determination of the influence of humic acid from biologicaly humified lignite on poinsettia (Euphorbia pulcherima) cultivation.

2. MATERIALS AND METHODS

The test was carried out with poinsettia variety Already Red in 4 variants and 4 plants per variant. The plants were potted in 11 cm standard pot in neutralized sphagnum peat with supplemental 5% clay. Irrigation was performed with commercial hydroponical solution products of ADVANCED NUTRIENTS Canada with high urea content. Supplemental humic acid was 12 ml/L per treated variant. The final EC of solution was 2300 μS/cm with humic acids and 2100 μS/cm in standard solution for the first 5 weeks and 3200 and 2800 μS/cm on 5th-10th weeks. At the end of cultivation all plants were irrigated with standard nutrient solution as during the first week.

Ornamental quality of the plant was observed – number of leaves, bract, and height in cm, stem diameter, total leaf area, and number of red leaves.

The standard analyses of plant tissue were conducted on well developed leaves in the initial phase of color development.

Experimental scheme was as follows: 1. Control; 2. Treatment with 5% solution of humic substances isolated from Biotransfotmed lignite; 3. Treatment with 5% solution of humic substances isolated from Biotransfotmed lignite+ poliacrilamid gel (0,5 g/dm^3 coal); 4. Treatment with biotransfotmed lignite – 1g/pot. The method for humic substances preparation was reported in our previous work (2).

Agrochemical characteristics of peat clay media is as follows (mg/100g):

pH	EC µS/cm	P_2O_5	K_2O	N-NH_4	N-NO_3	P_2O_5	K_2O	CaO	MgO
Water extraction 5:1				Extraction with KCL		Morgan extraction buffered at pH 4,8; 10:1			
5,96	2142	41	11	48	35	590	136	1215	65

3. RESULTS AND DISCUSSION

The data presented in Table 1 show characteristic parameters of humic substances. The highest content of C h was in humic substances isolated from Biotransfotmed lignite.

The data presented in Table 2 show the effect of humic substances on poinsettia quality. Humic acids isolated from Biotransfotmed lignite increased number of bracts and leaves, especially area of red leaves. Biotransfotmed lignite strongly influenced coloring of leaves. The stems were wider in humic treated plants.

Table 1. Characteristics of humic substances

Constituents ^{a} % of the sample ^{a} % of the total OC	Biotransfotmed lignite	Humic substances isolated from Biotransfotmed lignite	Humic substances isolated from Biotransfotmed lignite gel
Dry matter,%	59,55	91,90	92,38
Total org. C, %	43,13	33,11	36,27
C,% in pyrophosphate extract	24,59^a 57,01^b	26,42 79,79	25,79 71,16
C h,%	19,87 46,07	20,01 60,43	20,13 55,55
C f, %	4,72 10,94	6,41 14,86	5,66 15,62
N,%	1,46	1,22	1,67
P_2O_5, %	0,086	1,46	1,54
K_2O, %	0,44	18,03	7,36
Na_2O, %	0,20	0,54	0,71
CaO, %	5,82	4,58	1,83
MgO, %	0,78	0,56	0,56
Fe_2O_3, %	1,42	1,01	0,71
Al_2O_3, %	2,24	1,43	1,37
S, %	1,82	1,43	1,29
Cu, mg/kg	29,80	37,40	33,80
Zn, mg/kg	53,00	66,50	412
Mn, mg/kg	99,30	84,80	256
Ash, % in 600°C	20,91	40,24	31,71
Organic mater,%	79,09	59,76	68,29

Development of the poinsettia culture was improved due to increase of N/K, ratio which is a function of better uptake of nitrogen from urea by plants. However, treatment with Biotransfotmed lignite balanced nutrition. The coloring of poinsettia started earlier.

Diameter = 1/ (0, 0616 + 0, 0559 / (N /K));

Diameter = exp (1, 603 + 0, 180*N)

Red leaves = 1/ (0, 047 - 0, 0225/ (N /K))

Table 2a. Biometrical data of treated poinsettia plants

Variant	Bract	Bract, % to control	Leaves	Leaves, % to control	Red leaves	Red leaves, % to control	H, cm	H, % to control
1	6,33	100	62	100	30	100	24	100
2	7,17	113	78	127	30	99	29	119
3	6,00	95	67	108	30	101	25	104
4	5,67	89	62	101	37	122	27,	112

Table 2b. Biometrical data of treated poinsettia plants

Variant	Red leaves in plant, %	Leaves area, cm^2	cm^2/pl, % to control	Red leaves area, cm^2/pl	Red leaves, % to control	Stem D, mm	Stem D, % to control
1	50,20	2410	100	809	100	9,26	100
2	38,11	2507	104	979	121	11,66	1256
3	45,99	2333	97	1087	134	11,55	125
4	62,69	2788	115	1197	148	9,36	101

Table 3. Macroelements in leaf tissue, % of dry mater

Variant	N	P	K	Ca	Mg	N / P	N / K
1	3,56	0,35	2,75	1,04	0,29	10,26	1,29
2	4,20	0,31	2,73	1,23	0,51	13,68	1,54
3	3,94	0,32	2,29	0,98	0,55	11,35	1,72
4	4,00	0,35	2,84	1,22	0,50	11,30	1,41

The photo presented in Figure 1 shows ornamental quality of treated plants.

Figure 1. The treated Poinsettia plants view: from left to right var. 1, 2, 3 and 4.

4. CONCLUSIONS

Treatment of poinsettia with humic substances from Biotransfotmed lignite improved nitrogen nutrition with urea. The ornamental quality of the culture became better.

ACKNOWLEDGEMENTS

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Thermal Analysis of Standard Humic Acids

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Keywords: IHSS standards, humic acids, TG-DTA, DRIFT

1. INTRODUCTION

Humic substances (HS) play an important role in many environmental processes in soils, sediments and water (1). Their chemical structure has been discussed from different viewpoints including molecular conformation, molecular aggregation, supramolecular characteristics and many others (2). The International Humic Substances Society (IHSS, http://www.ihss.gatech.edu/) has collected a number of reference HS originated from specified locations, and all samples have been extracted using recommended and supervised procedures. Thus, these materials can be considered a representative pool of overall HS.

Among analytical techniques, thermal analysis is considered an important tool to understand how functional group composition and different origin can influence the thermal behaviour of HS (3-6).

In the present paper we have compared the thermal behavior of soil, peat, and leonardite standard IHSS humic acids (HA) with those HA extracted from the respective IHSS bulk solid sources and purified using dialysis procedure.

2. MATERIALS AND METHODS

The following standard IHSS samples were used: Elliott soil standard HA 1S102H (E-HA), Pahokee peat standard HA 1S103H (P-HA), Leonardite standard HA 1S104H (L-HA); and the respective bulk solids from Elliot silt Loam Soil (1BS102M), Pahokee Peat Soil (2BS103P) and Gascoyne Leonardite (1BS104L).

Humic acids from bulk solids were extracted according to IHSS procedure until precipitation of HA to pH <1 and then the HA were dialyzed against distilled water (cut-off 14,000 Da). The soil (S-HA-D), peat (P-HA-D) and leonardite (L-HA-D) HA obtained were freeze-dried before analysis.

TG-DTA analyses were carried out simultaneously using a TG-DTA92 instrument (SETARAM, France) as described in a previous paper (5).

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) (5).

3. RESULTS AND DISCUSSION

In general, the thermograms of standard HA were characterized by an unique strong exothermic reaction at around 500°C as showed by TG curves (Figure 1, a). This decomposition reaction was mainly produced by combustion of aromatic structures and cleavage of the C–C bond (7). DTA curves showed also a slight shoulder at lower temperature suggesting the presence of functional groups and aliphatic chain as confirmed by DRIFT data (7, 9). Despite of the different origin of HA the thermal changes seem to be due to the slight temperature shifts of the same exothermic reaction. On the contrast the thermal profiles of S-HA-D, P-HA-D and L-HA-D (Figure 1, b) were significantly different than those of respective standard-HA. In particular, two well defined exothermic reactions characterize the DTA curves.

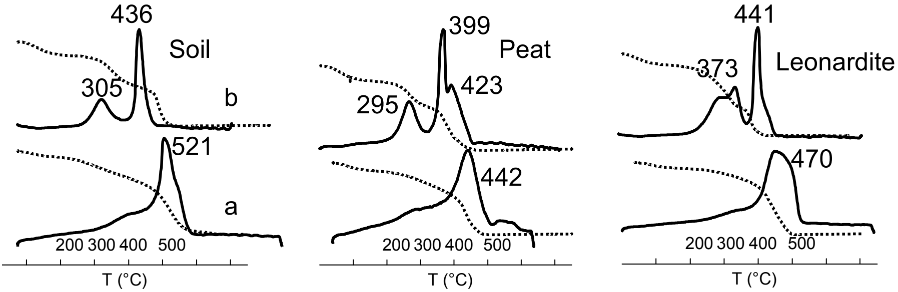

Figure 1. TG-DTA curves of IHSS standard HA (a) and
HA extracted from IHSS bulk solid sources (b).

The first exothermic peak was considered to be the result of thermal combustion of polysaccharides, decarboxylation of acidic groups and dehydration of hydroxylate aliphatic structures (8). The mass losses related to the presence of these compounds were 37%, 40.1% and 51.8% (ash free) for S-HA-D, P-HA-D and L-HA-D, respectively. The intense combustion reaction of the second peak might be due to the cracking of higher molecular weight polynuclear systems (10). The temperature of combustion reactions related to first and second exothermic peaks were lower in S-HA-D and P-HA-D than in L-HA-D. The observed differences are unequivocally related to their humification rank. However the noteworthy changes of thermal profiles between these and standard HA samples depend on purification processes applied.

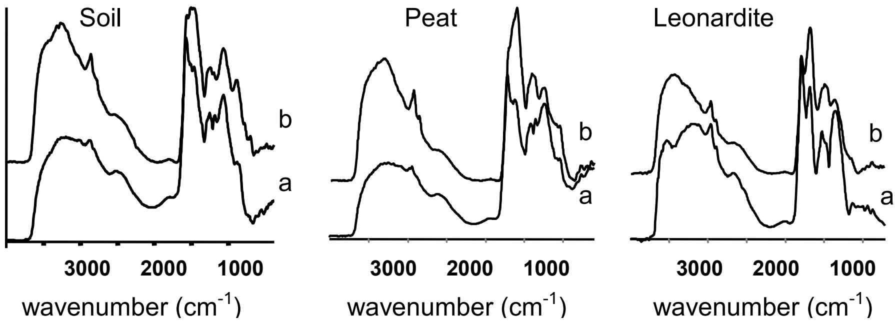

Figure 2. DRIFT spectra of IHSS standard HA (a)
and HA extracted from IHSS bulk solid sources (b).

The DRIFT spectra of standards HA were similar (Figure 2, a) in accordance with their thermal behavior. They were characterized by two strong bands at around 1710 and 1230 cm^{-1} that are characteristic of C=O stretching (9) and C–O stretching motions in carboxyl acids, respectively. In addition, the broad band at 2629 cm^{-1} suggested the formation of intermolecular hydrogen bonding between OH groups of COOH groups in acid dimers (9). The presence of bands at around 1600 cm^{-1} and 1400 cm^{-1} indicated that the carboxylic groups were not too extensively protonated.

The DRIFT spectra of HA extracted from bulk solid sources (Figure 2, b) were not much different from those of HA standards. The main modifications can be ascribed to a shiftment of the frequencies of carboxylic groups because of highest deprotonation.

4. CONCLUSIONS

The purification procedures has influenced the thermal behavior of HA analyzed. Samples purified by dialysis procedure showed very well resolved TG-DTA curves allowing to distinguish between polysaccharides, carboxylic groups and aliphatic structures, and aromatic compounds in HA.

ACKNOWLEDGEMENTS

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Photoremediation Properties of Dissolved Organic Matter Extracted from Compost

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Keywords: humic-like acids, water extractable organic matter, photoinduction

1. INTRODUCTION

There are two main stages in the composting procedure. The first stage of aerobic biodegradation by thermophilic bacteria occurs during the first month in order to eliminate pathogens. Next follows the curing stage where organic matter is stabilized. It has been shown that during this stage lasting from 2 months to 6 months, organic matter evolutes. When composting time increases, the part of hydrophobic fraction increases while that of water extractable organic matter decreases (1).

In agriculture, compost is mostly used as an organic amendment or as a mulch. These two applications lead respectively to soil fertilization or to improving of soil structure and texture. Other compost applications are also possible. Organic matter from compost can absorb sunlight and photosensitize organic pollutant degradation. It was recently shown that humic- and fulvic-like substances and water soluble fractions from compost can promote pollutants degradation under light excitation (2, 3).

The aim of this study is to compare photoinductive properties of various extracts: humic-like acids (HLAs) and water extractable organic matter (WEOMs).

2. MATERIALS AND METHODS

Two different composts were chosen for this study : Green Manure (GM) and Urban Biowaste (UB). They are made of green manure, trimming and crop residues for GM and of a 60:40 (w:w) blend of green manure and municipal wastes for UB. For each compost, three different times of composting were collected. Each sample was sieved at 5 mm and air-dried for two weeks. The dried sample was milled and sieved at 0.5 mm. GM060, GM120, GM180 correspond to composts obtained after 60, 120 and 180 days, respectively. UB030, UB045 and UB090 correspond to composts obtained after 30, 45 and 90 days, respectively. WEOMs were obtained by extracting 50.0 g (oven dry equivalent) of air-dried sample in a 500 mL centrifuge tube with 250 mL of ultrapure water for 30 minutes at room temperature (25°C) in an end-over-end shaker under N_2 atmosphere. After extraction centrifugation of the suspension at 7500 g for 30 minutes at 20°C was made. Then the supernatant solution was separated and filtered through a 0.4 μm filter (polycarbonate). Finally the filtrate was freeze-dried and stored in an amber glass vial. HLAs were extracted as previously described (4).

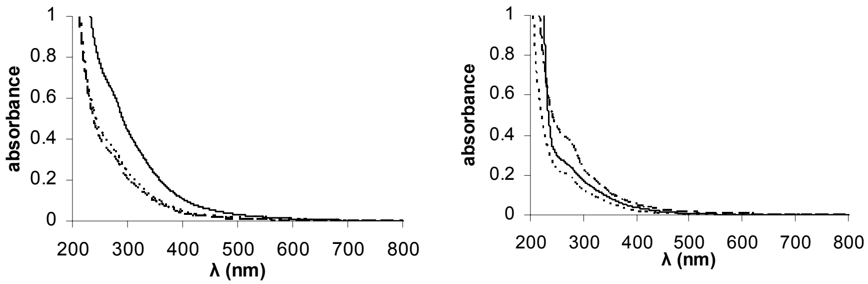
Excitation Emission Matrix (EEM) spectra were recorded on a Perkin-Elmer LS-55 luminescence spectrometer that was equipped with a xenon excitation source. Excitation and emission slits were set to 10 and 5 nm band pass, respectively. Correction for instrumental configuration was made using the calibration data for excitation and emission factors provided by the manufacturer. In order to minimize the excitation inner filter effect, an absorbance of 0.100 ± 0.005 at 300 nm was chosen for all the samples solutions.

Irradiation experiments were conducted in a device equipped with polychromatic lamps simulating solar light, in a pyrex reactor. WEOMs and HLAs were irradiated with furfurylic alcohol (FFA) at an initial concentration of 10^{-4} M. Photochemical reactions were investigated at a constant initial absorbance of 0.1 at 300 nm. FFA loss was monitored by HPLC.

3. RESULTS AND DISCUSSION

3.1 Samples characterization

WEOMs and HLAs were first characterized by UV-visible and fluorescence spectroscopy and DOC measurements. DOC contents of WEOMs and HLAs were around 20% and 50%, respectively. Figure 1 shows UV-visible spectra of WEOMs.



a) Green manure

b) Urban Biowaste

Figure 1. UV-visible spectra of normalized 100 mg L^{-1} WEOMs of GM (a) and UB (b) composts for different composting times:

. . . . GM060 and UB030, - - - - GM120 and UB045, and ____ GM180 and UB090.

Using fluorescence spectroscopy, we determined the humification index HIX as described in reference (5). Table 1 shows HIX values. It is generally admitted that he higher HIX value is, the more humified the dissolved organic matter compost is. We observed that (i) HIX generally increased with increasing composting time and (ii) WEOMs showed higher HIX values than corresponding HLAs.

Table 1. HIX values for WEOMs and HLAs of GM and UB at different composting times

	UB030	UB045	GM060	UB090	GM120	GM180
WEOM	3.2	5.7	5.6	9.2	3.6	10.0
HLA	1.8	3.0	3.3	3.0	4.2	4.6

3.2 Photodegradation experiments

All these samples were irradiated in the presence of a singlet oxygen scavenger, FFA (6). Table 2 gives the rate coefficient, k* normalized at 1 mg L^{-1} of organic carbon for WEOMs and HLAs. We observed that

- In WEOMs and HLAs series, k* generally increased as composting time increased.
- WEOMs were generally better photosensitizer than corresponding HLAs.
- The higher HIX value is, the higher k* is.

Table 2. k* ($/10^{-6}$ s^{-1}) values for WEOMs and HLAs of GM and UB
at different composting times

	UB030	UB045	GM060	UB090	GM120	GM180
WEOM	1.38 ± 0.07	1.70 ± 0.07	2.08 ± 0.08	3.83 ± 0.12	2.26 ± 0.06	4,20 ± 0.14
HLA	1.60 ± 0.34	1.31 ± 0.32	1.65 ± 0.09	2.15 ± 0.60	1.06 ± 0.15	2.90 ± 0.87

4. CONCLUSIONS AND PERSPECTIVES

Organic matter from compost was characterized by UV-visible and fluorescence spectroscopy and DOC analysis. This allows us to have a qualitative view of WEOMs and HLAs from two composts with three different composting times.

We show that organic matter obtained from compost are able to sensitize the production of singlet oxygen which is a very oxydant species. The way organic matter is extracted conditions photosensitizing and fluorescence properties. Interestingly, the simplest procedure (extraction using water) yields the organic matter showing the best photosensitizing and fluorescence properties.

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Potentials of Technical Lignins for Application as Soil Amendments: Free Radical Scavengers and Biological Activity

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Keywords: lignin, antioxidant activity, biological activity

1. INTRODUCTION

The role of antioxidants in human and animal physiology and healthcare has been good recognized owing to studies carried out for at least 50 years. In recent years, research into antioxidant properties of naturally occurring polyphenols has drawn increasing attention. Polyphenols have shown many favorable effects on human health (1-4) and are well-known to be effective antioxidants for food lipids (5). In plants and soil biology impact of reactive free radicals, in particular reactive oxygen species, and the antioxidants that control them, is the subject of much recent researches aimed at understanding of mechanisms of the processes involved in soil organic matters (SOM) dynamics and of the chemical stabilization of SOM in particular (6).

A natural phenolic polymer lignin in plants serves to affect water transport, protect plant tissue against chemical and biological attack, and provide structural integrity. In its turn, lignins isolated from plant tissue, so called technical lignins – co-products of wood and agricultural plants chemical processing are intensively investigated as antioxidants for various materials. It was reported that kraft lignin was as effective as vitamin E as an antioxidant of corn oil (7). Lignin functions as an effective radical scavenger to prevent autoxidation and depolymerization of cellulose in pulps and papers (8). Incorporation of lignin into synthetic polymer systems can stabilize the material against photo- and thermal oxidation (9,10). As a major component in dietary fibres, lignin can inhibit the activity of enzymes related to the generation of superoxide anion radicals and obstruct the growth and viability of cancer cells (11).

The main objective of this study was to investigate the radical scavenging activity of the 12 lignin preparations obtained by alkaline delignification of deciduous and coniferous wood species, agricultural plants and waste from their processing, identifying the structural features of the lignin most important for its antiradical efficiency and to search relationship between lignin antioxidant and biological activities.

2. MATERIALS AND METHODS

In the present work, lignin preparations obtained by alkaline deignification (pulping) of spruce (*Picea abies*), aspen (*Populus tremula)*, birch (*Betula pendula)*, grey alder (*Alnus incana)*, ash-tree (*Fraxinus excelsior*) wood, flax (*Linum usitatissimum)*, hemp (*Cannabis sativa)*, abaca (*Musa textiles)*, sisal (*Agave sisalana)*, jute (*Corchorus capsularis*), wheat (*Triticium Vulgarae*) straw and bagasse were characterized as potential antioxidants and their biological activity was assessed. Rutin, epicatechin and ethanol extract from bee propolis were tested in order to assess the efficacy of lignin preparations as antioxidants.

The key structural features governing antioxidant activity of lignin molecules, in particular functional groups and polyconjugated sub-structures (12, 13), were determined by relevant chemical and physico-chemical analytical methods described in (14, 15).

The procedures widely used to test the antioxidant properties of polyphenolic molecules, namely assays with stable free radical DPPH (12), radical cation ABTS (16) and superoxide radical-anion generated in the system hypoxantine/xanthine oxidase (17), were applied. The kinetics and mechanisms of the interaction between lignins and the DPPH radical was investigated by EPR spectroscopy.

Antimicrobial effects of lignin preparations were tested using 24 isolates of soil Gram negative bacteria and 1 yeast culture (four types of agarized medium, i.e. modified M8* medium with two different amendments, Tryptone Glucose Yeast Extract Milk Agar Plate Count Agar with Skimmed Milk (TGA, SIFIN), and Eosyne Methylene Blue Agar (EMB, SIFIN) as well as pure culture of bacterium *E. coli* (yeast-peptone medium).

3. RESULTS AND DISCUSSION

The results of the present work (Table 1) indicated that all lignin preparations under investigation possess antioxidant activity in all test systems used.

For some lignins the radical scavenging efficacy is close to that for natural low-molecular antioxidants – bee propolis and flavonoids, applied in medicine and veterinary. The most promising data were obtained for aspen and flax lignins. These samples could be considered as prospective polymeric antioxidants of natural origin, able to compete with low-molecular commercialized antioxidants.

The same preparations (aspen and flax lignins) also revealed good results in the test on supression of growth of pathogenic microorganisms exemplified by pure culture of *E. coli* (Fig. 1).

Table 1. Characterization of antioxidant activity of lignin preparations

Sample	DPPH test, EC_{50}[a]	Superoxide radical anion test, IC_{50}[b]	ABTS test, IC_{50}[c]
Spruce alkaline lignin	40.2±2.1	35.2±1.9	44.4±2.9
Birch alkaline lignin	17.6±0.5	7.6±0.8	7.8±1.1
Aspen alkaline lignin	14.1±0.3	1.8±0.2	5.8±0.1
Alder lignin	22.6±1.1	8.6±1.0	5.7±0.2
Jute alkaline lignin	15.0±0.5	52.0±2.1	15.2±1.0
Sisal alkaline lignin	14.5±0.5	38.8±3.3	n.d.
Flax alkaline lignin	16.0±0.5	1.9±0.2	6.1±0.1
Abaca alkaline lignin	16.2±0.3	20.2±2.1	n.d.
Bagasse alkaline lignin	44.2±3.1	38.2±1.1	69.2±5.1
Hemp alkaline lignin	58.2±4.3	44.6±1.1	n.d.
Wheat straw alkaline lignin	88.2±2.3	34.5±3.7	n.d.
Bee propoliss	41.7±1.7	1.7±0.2	7.1±0.2
Epicatechin	7.5±0.7	3.8±0.2	3.1±0.1
Rutin	41.8±2.0	12.4±1.5	12.6±0.1

[a] the concentrations of the sample, mg/L, needed to decrease the initial $DPPH^{\bullet}$ concentration by 50%

[b] the concentration, mg/L, of the sample, responsible for the 50% decrease in $O_2^{\bullet-}$ generation

[c] the concentration of the sample, mg/L, needed to decrease the $ABTS^{\bullet)}$ concentration by 50%

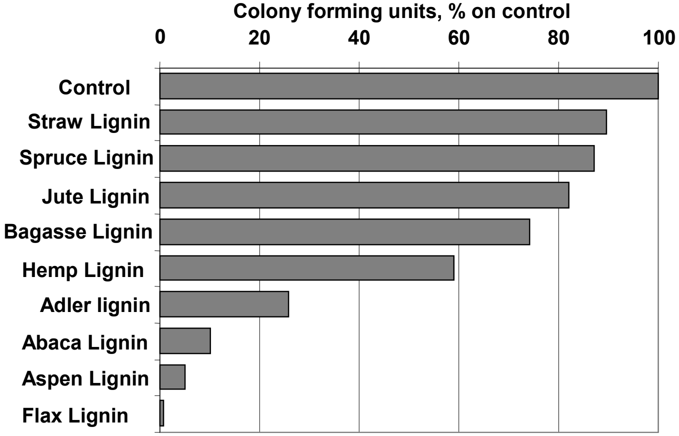

Figure 1. Lignin preparations influence on bacteria *E. coli* development.

The antimicrobial effect in the tests with soil isolates strongly depended on environmental conditions. Some bacteria species and yeast demonstrated resistance to all tested lignin preparations. The significant nonlinear Pearson correlation at the 0.05 level was found between development of polyconjugation system in lignin macromolecule in terms of mean conjugation length, lignin superoxide anion radical scavenging activity and inhibition effect towards *E. coli*.

4. CONCLUSIONS

Among the technical lignins studied the highest antioxidant efficacy close to that for some plant flavonoids, applied as antioxidants in medicine and veterinary, was detected for the lignins obtained from aspen and flax. The antioxidant properties of technical lignins highlight their good potentials for application in the „soil-plant” system.

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Influence of Selected Conditions on Chemical Properties of Humic Substances Formed during Composting of Municipal Solid Wastes (MSW)

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Keywords: composting of MSW, IR spectra of HA, elementary composition of HA

1. INTRODUCTION

Biochemical processes occur during composting of Municipal Solid Wastes (MSW) lead to total decomposition of organic matter (mineralization) or its disintegration and resynthesis (humification) and formation of humic substances, like humic acids (HA). Problems connected with genesis, structure, chemical and physicochemical properties of HA are still a subjects of many researches and experiments. Despite of the development in analytical methods, the chemical structure and composition of HA is still unknown (1-2). However, in opinion of many authors the structure and composition of HA depends mainly on: properties of fresh material, composting conditions and time of composting (4-6). Observation of structural and chemical changes of HA during composting is considered as one of the best way to estimate direction of biotransformation and to determine compost maturity stage. The aim of experiment was determine the properties and elementary composition of HA extracted from MSW, composted according KKO – 100 technology in Zielona Gora (Poland) in period of five months.

2. MATERIALS AND METHODS

Material for research was collected from ten points, directly from composting piles and averaged to a representative samples. In sampling material, the following determinations were performed: temperature, humidity, TOC (total organic carbon), fractional composition of humic substances. Additionally, for humic acids extracted from every representative sample (method recommended by IHSS) elementary composition (Perkin – Elmer 2000) and FTIR spectra of HA (Bruker FTIR 113v) were measured.

3. RESULTS AND DISCUSSION

Intensity and direction of biotransformation processes during composting are limited by: an amount and character of organic substances, time of composting, water content and the oxygen access. Figure 1 shows changes of value of composting parameters in time.

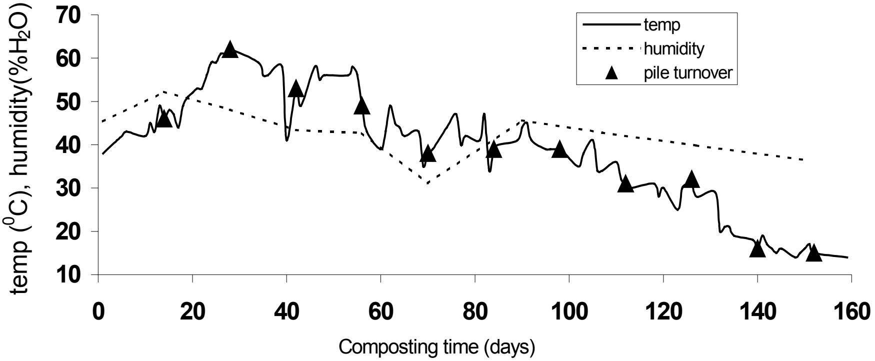

Fig. 1. Changes of temperature and humidity during composting of MSW in Zielona Gora.

The temperature and humidity of composting material was changed during composting time and had very significant influence on direction and intensity of organic matter transformation (Tab. 1). The contents of TOC, non humic fraction (CNHF) and residual carbon (CR) decreased during composting time, but amount of CFA$_1$, CHA$_1$, and CHA$_1$/CFA$_{1+2}$ ratio increased in more maturity compost.

Table 1. The contents of organic carbon in humic substances extracted from compost

Composting days	TOC	CAC extracted (0.1 M HCl)		CALC extracted (0.1 M NaOH)		CR residual	CHA$_1$/CFA$_{1+2}$
		CFA$_1$	CNHF	CHA$_1$	CFA$_2$		
	g·kg^{-1}						
1	205.90	1,94	8.70	8.68	10.19	176.39	0.72
28	155.50	3.46	10.20	8.74	12.66	120.44	0 54
56	126.10	3.37	13.20	13.24	14.80	81.49	0.73
90	106.80	3.60	6.40	13.14	11. 90	71.76	0.85
149	87.80	3.26	3.57	12.89	9.82	58.26	0.99

Changes of organic carbon of humic substances (Tab. 1) proofs, that biotransformation of organic matter in investigated material was caused mainly by microbiological activity of composted MSW in mesophilic and termophilic phases. The observation of presented parameters changes can be used as indicators of compost maturity.

The results of elementary composition of extracted humic acids shows, that content of C, N and O increases during composting time, while amount of H decrease (Tab. 2). That results in H/C, C/N and O/C ratio changes which indicate aromatisation and condensation of HA molecules during maturity of composted material.

Table 2. Changes of elementary composition of HA during composting of MSW

Composting days	C	H	O	N	H/C	C/N	O/C
	atomic %						
1	33.27	47.88	15.67	3.18	1.44	10.47	0.47
28	34.20	44.06	18.09	3.65	1.29	9.38	0.53
56	33.71	43.92	18.53	3.83	1.26	8.80	0.55
90	36.73	38.36	20.87	4.03	1.04	9.11	0.57
149	40.18	29.77	25.60	4.44	0.74	9.05	0.64

Although FTIR of extracted humic substances are very typical and similar to each other some changes in HA structures are noticeable (Fig. 2). Bands at 2920-2850 cm^{-1} attributed to aliphatic C-H stretch decrease in time of composting and confirm increase of aromaticity of humic acids and consequently maturation of the compost.

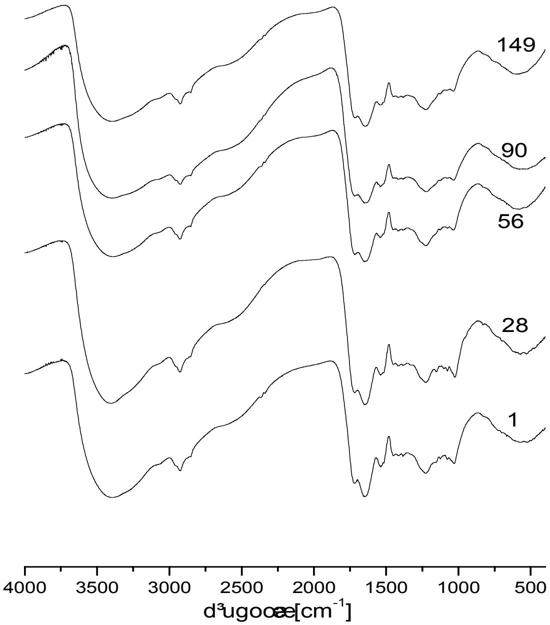

Figure 2. Changes of IR spectra of HA during composting of MSW in Zielona Gora.

The common features are bands at 1720 and 1650 cm^{-1} due to the C=O of carboxyl and amide groups and 1540 cm^{-1} band due to the C-O stretch of polysaccharides. Additionally, decrease of peak at 1720 cm^{-1} shows dissociation of carboxylic groups. The 1360, 1160 and 580 cm^{-1} bands were assigned as originating from sulfonyl units (7). Although these spectra were not well resolved, they did provide structural information that indicated the presence of aliphatic and aromatic components as well as degradation products originating from proteins and polysaccharides.

4. CONCLUSIONS

All presented studies prove that composting processes leads to extend of aromatic part of humic acids (C – HA) and directions and intensity of these changes are correlated with composting conditions. Obtained results shows that during composting of MSW changed significant properties synthesized humic substances.

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Effect of Potassium Humate on Advanced Potato Cultivars for Water Deficit Tolerance in Ardabil Region, Iran

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Keywords: potassium humate, potato, water deficit

1. INTRODUCTION

Water deficit is a common stress in potato cultivation. Because of potato’s high susceptibility to water stress (4), preparing sufficient water is very important for increasing potato quality and quantity. Climatic changes were occurred at the in recent years in the Ardabil region. These changes caused differences in precipitation dispersion, river flowing, and wells water. Therefore, we have to identify agronomic characters and water need of new potato varieties and extra improvement of their quality and quantity. Potassium humate is an active hormone with natural origin that extracts from plants and animal remains exist in the bottom of marshes. This material is formed from N, P, K and microelements namely Mo, Cu, Zn, B, Co, Mg (5). Potassium Humate increases accumulation of chlorophyll, sugar, amino acids and more improves the efficiency of nitrogen utilization, allowing for reduced fertilizer rates, the plant’s ability to withstand the stresses of heat, drought, cold, disease, insect and other types of environmental or cultural pressures and also increases general plant productivity, in terms of yield, as well as plant stem strength (2). Using of Potassium Humate increased root system, tuber yield, tuber number per plant (1), root number (3) and pea numbers and weight per plant and decreased nitrate amounts in leaves and roots (5) and decreased nitrate accumulation in potato tubers (6).

2. MATERIALS AND METHODS

This experiment was done on three potato varieties namely Agria (susceptible), Satina (semi-tolerant) and Ceaser (tolerant to water deficit) in 2007 and three irrigation treatments (after 30 mm evaporation from basin class A, after 60 mm evaporation +

spraying by Potassium Humate, and after 60 mm evaporation) used. Experimental design was split plot with three replications. Potassium Humate spraying with 250 ml in volume of 300 lit water per ha were done in the three stages of emergence, before tuberization and after tuberization. Plants were measured for main stem number, plant height, tuber number and weight in plant, total tuber yield and marketable tuber yield and dry matter percent, marketable tuber number and weight per plant in the growth period and after harvesting. Analysis of variances were done and comparison of means made by LSD (Least significant difference) test.

3. RESULTS AND DISCUSSIONS

Results showed that there were significant differences in irrigation treatments for tuber yield, marketable tuber yield, dry matter percent, tuber number and weight per plant, and marketable tuber weight per plant. There were significant differences between cultivars for dry matter percent, plant height and main stem number. Interaction of cultivars × irrigation treatments was significant for tuber yield, marketable tuber yield and tuber weight in plant.

Comparison of means for irrigation treatments showed that spraying by Potassium Humate in stress condition induced increase of tuber yield, marketable tuber yield, dry matter percent, tuber number and weight in plant, and marketable tuber number and weight per plant and main stem number (Table 1). The highest tuber yield, marketable tuber yield, dry matter percent, tuber number and weight per plant and marketable tuber number and weight per plant and main stem number were made at 30 mm evaporation and after 60 mm evaporation + spraying by Potassium Humate (Table 1). Spraying by Potassium Humate in water deficit condition increased tuber yield 15.87 ton/ha, marketable tuber yield 17.06 ton/ha, dry matter 2.69 %, tuber number per plant 2.72 number, tuber weight per plant 270.76g, marketable tuber number 2.58 numbers and marketable tuber weight per plant 287.95g (Table 1).

Caesar (tolerant to water deficit) had the highest tuber yield, marketable tuber yield, marketable tuber number, weight per plant and plant height (Table 2).

Correlation between tuber yield with marketable tuber yield and marketable tuber number and weight per plant was positively significant.

Table1. Mean of different traits on different irrigation treatments

Irrigation treatments	Total tuber yield (t/ha)	Marketable tuber yield (t/ha)	Tuber number per plant	Tuber weight per plant (g)	Main stem number	Plant height (cm)	Dry matter percent	Marketable tuber number per plant	Marketable tuber weight per plant (g)
after 30 mm evaporation	48.85 a	46.10 a	8.54 a	870.04 a	4.25 a	53.53 a	19.92 b	6.78 a	847.57 a
after 60 mm evaporation + spraying by Potassium Humate	45.79 a	44.80 a	8.68 a	858.64 a	4.56 a	52.62 a	21.56 a	7.07 a	839.97 a
after 60 mm evaporation	29.92 b	27.74 b	5.95 b	567.88 b	3.99 a	54.90 a	18.87 b	4.45 b	552.02 b

Table2. Mean of different traits on different cultivars

Cultivars	Total tuber yield (t/ha)	Marketable tuber yield (t/ha)	Tuber number per plant	Tuber weight per plant (g)	Main stem number	Plant height (cm)	Dry matter percent	Marketable tuber number per plant	Marketable tuber weight per plant (g)
Ceaser	42.42 a	40.24 a	7.45 a	779.43 a	2.71 b	59.87 a	20.51 a	6.04 a	763.50 a
Agria	40.89 a	39.02 a	8.08 a	767.45 a	4.95 a	55.63 a	20.85 a	6.42 a	746.82 a
Satina	41.24 a	39.38 a	7.64 a	749.67 a	5.14 a	45.56 b	18.99 b	5.88 a	729.24 a

4. CONCLUSIONS

Spraying by Potassium Humate in water deficit condition increased tuber yield 15.87 ton/ha, marketable tuber yield 17.06 ton/ha, dry matter 2.69 %, tuber number per plant 2.72 number, tuber weight per plant 270.76g, marketable tuber number 2.58 numbers and marketable tuber weight per plant 287.95g.

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Effect of Potassium Humate and Kadostim on Plantlets of Advanced Potato CVs

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Keywords: potassium humate, kadostim, potato, plantlet

1. INTRODUCTION

Kadostim is an organic cell pathway synthesized bio-stimulator that enhancing potassium leaf nutrient complex characteristics such as liquid formula with free amino acids and biologically active oligo-peptides developed for use in the post flowering stage and ripening of fruits. Products like as bio-stimulators and bio-fertilizers have effects and benefits for plants growth and development such as enhancing the process of seed germination, activating the formation of root system, stimulating the assimilation of micronutrients, accelerating the formation and growth of leaves, impacts over leaf stoma to control and regulate moisture, light, temperature, salinity and gas, activating the photosynthesis process of nutritional and substances and reserves, favors the formation of flowers, their capacity of fecundates, reducing the effects of stress and shock by transplantation, adverse weather (frost, drought and hailstorm) and chemical agents, facilitating the regeneration of damaged tissues, improving the absorption of all agrochemical products, stimulating the acquired immunology, increasing the resistance of the plants and yield and enhancing size, sorting and quality of the fruit, increasing the yield and fruit shelf life after harvest (1) (Table 3).

Potassium humate is an active hormone with natural origin that extracts from plants and animal remains exist in the bottom of marshes (Table 3). This material is formed from N, P, K and microelements namely Mo, Cu, Zn, B, Co, Mg (5). Potassium Humate increases accumulation of chlorophyll, sugar, amino acids and more improves the efficiency of nitrogen utilization, allowing for reduced fertilizer rates, the plant's ability to withstand the stresses of heat, drought, cold, disease, insect and other types of environmental or cultural pressures and also increases general plant productivity, in terms of yield, as well as plant stem strength (3). Using of Potassium Humate increased root system, tuber yield, tuber number per plant (2), root number (4) and pea numbers and weight per plant and decreased nitrate amounts in leaves and roots (5) and decreased nitrate accumulation in potato tubers (6).

This experiment was conducted for evaluation of effect of two biological complexes namely Kadostim and Potassium Humate on meristem culture of potatoes.

2. MATERIALS AND METHODS

Experiment was done in biotechnology laboratory and greenhouse of Villkej Company in Ardabil Province, Iran in 2007. In this experiment, plantlets produced from meristem culture of Agria, Advanced Clone 397007-9, Marfona, Sante, Satina and Ceaser propagated by single node cuttings. Experimental design was factorial on the basis of complete randomized block design in ten replications. Factor A was plantlets produced from meristem culture of six advanced cultivars (Agria, Marfona, Sante, Satina, Ceaser and Advanced Clone 397007-9) and factor B was seven treatments (four concentrations of Potassium Humate as 0.5, 1, 1.5 and 2 ml, one concentrations of Kadostim as 1 ml, compound concentration of Potassium Humate and Kadostim as 1 ml/1MS medium (7) and without them as Control). After planting, plantlets reached to enough growth after about 30 days in 18-22 centigrade temperature and 16/8 photoperiod with 5000 lux intensity light. The days to transplantation, stem strength and rhizo-genesis measured. Analysis of variance was done and means compared by LSD (Least significant difference) test. Then, plantlets cultured in planting beds of Pitmass (Biolan) with punce (1:1 v/v) in a greenhouse. Experimental design was factorial on the basis of complete randomized block design in three replications. All of plantlets irrigated after planting by normal water. Macro and micro nutrients were used to provide for nutrition of plantlets. All of practices such as irrigation and control of weeds, pests and diseases were done regularly during growth period. Control of pests and fungus diseases were done respectively by use of 250^{cc} per ha Confidor and 400 g/ha Equation-Pro. Mini-tubers harvested after about two months. Then some of traits measured such as average weight and number of mini-tuber per plant. Analysis of variance was done and means compared by LSD test.

3. RESULTS AND DISCUSSION

Results of analysis of variance showed significant differences between effect of Kadostim and Potassium Humate and advanced cultivars for transplantation to greenhouse, stem solidity and rhizo-genesis. Transplantation days of plantlets from *in vitro* to the greenhouse decreased from 30 days (control) to 13 days in MS media culture with compound of Potassium Humate + Kadostim by concentration of 1 ml/l MS culture, to 15 days in MS media culture with Kadostim by concentration of 1 ml/l MS culture and to 22 days in MS culture with Potassium Humate by concentration of 1 and 1.5 ml/l MS media culture and plantlets had the highest stem solidity and better rhizo-genesis in all of treatments. Agria, Sante and Marfona transplanted to the greenhouse earlier and Ceaser later than others.

Results of Analysis of variance in greenhouse showed significant differences between effect of Kadostim and Potassium Humate and advanced cultivars, for average of mini-tuber weight per plant and number and weight of mini-tuber per plant. Compound concentration of Potassium Humate and Kadostim with 1 ml/1 MS media culture had the highest number of mini-tubers per plant (2.56 tubers). Potassium Humate 0.5 ml/1 MS and Kadostim 1 ml/1 MS had the highest mini-tuber weight per plant (3.09 and 2.89 g) and average of mini-tuber weight per plant (1.74 and 1.62 g) (Table 1). Agria had the highest number (2.84 tubers) and average weight of mini-tubers per plant (4.87 g) (Table 2). Potassium Humate 0.5 ml/1 MS on Satina, Kadostim 1 ml/1 MS on Marfona and Potassium Humate + Kadostim 1 ml/1 MS on Agria produced the highest number of mini-tubers per plant.

The price of one mini-tuber is about 0.25$ in Iran. In our study Potassium Humate and Kadostim increased the number of mini-tubers per plant (with mean of 1). Therefore about 25$ increased for production of mini-tubers per square meter. Thus Potassium Humate and Kadostim caused the economic effects pay attention to results of this research.

4. CONCLUSIONS

The Potassium Humate and Kadostim decreased the days to transplantation of plantlets from *in vitro* to the greenhouse from 30 days to 13-22 days and increased number and average of mini-tuber weight per plant.

Table 1. Mean of traits on different levels of Potassium humate and Kadostim in the greenhouse

Treatments	Number of mini-tubers	Weight of mini-tubers (g)	Average weight of mini-tubers (g)
Kadostim 1 ml /l MS (KA=0.001)	1.78 b	2.89 ab	1.62 a
Kadostim 1 ml+ Potassium Humate 1ml /l MS	2.56 a	2.59 ab	1.01 b
Potassium Humate 2 ml /l MS (PH=0.002)	1.50 b	2.69 ab	1.79 a
Potassium Humate 1.5 ml /l MS (PH=0.0015)	1.67 b	2.16 b	1.29 b
Potassium Humate 1 ml /l MS (PH=0.001)	2.00 ab	2.61 ab	1.30 b
Potassium Humate 0.5 ml /l MS (PH=0.0005)	1.78 b	3.09 a	1.74 a
Control (C=0.0)	1.56 b	2.20 b	1.41 b

MS= Murashige and Skoog media culture; l= liter.

Table 2. Mean of traits for different cultivars in the greenhouse

Cultivars	Number of mini-tubers	Weight of mini-tubers (g)	Average weight of mini-tubers
Agria	2.84 a	13.81 a	4.87 a
Satina	2.70 a	9.381 b	3.47 b
Marfona	2.54 ab	10.10 b	3.98 b
Ceaser	2.25 b	7.143 c	3.17 b
Advanced clone	2.67 a	10.03 b	3.75 b
Sante	2.63 a	9.524 b	3.62 b

Table 3. Potassium Humate and Kadostim compounds

Kadostim Aminogram Distribution %				Potassium Humate	
Glycine	1.80	Total nitrogen(N)	5.00 % w/w	Nitrogen	2.8 %
Valine	5.10			Humid	5.0 %
Proline	8.40	Ammoniac nitrogen	1.60 % w/w	Phosphorus	0.4 %
Alanine	13.21			Potassium	10 %
Aspartic Acid	4.50	Nitric nitrogen	3.10 % w/w		pH=8.2
Arginine	8.40			N-NH4	460 mg/l
Glutamic Acid	0.90	Organic nitrogen	0.30 % w/w	N-NO3	890 mg/l
Lysine	5.10			P2O5	890 mg/l
Leucine	16.51	Organic matter	2.00 % w/w	K2O	8600 mg/l
Isoleucine	4.50			Mgo	620 mg/l
Phenilalanine	5.10	K_2O	6.00 % w/w	Cu	4.5 mg/l
Methionine	4.20			Zn	11.5 mg/l
Serine	3.90	Free amino acids	3750 mg/l	S	400 mg/l
Threonine	3.00			Fe	15 mg/l
Histidine	3.00			Mn	10 mg/l
Glycocoll	9.60			B	2 mg/l
Tyrosine	1.50			Mo	2 mg/l
Glutamine	0.90			Co	1 mg/l
Cystine	0.30				
Other	0.08				

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Effect of Potassium Humate on Production of Advanced Potato Mini-tuber CVs

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Keywords: potassium humate, minituber, potato, plantlet

1. INTRODUCTION

Potassium humate is an active hormone with natural origin that extracts from plants and animal remains exist in the bottom of marshes. This material is formed from N, P, K and microelements namely Mo, Cu, Zn, B, Co, Mg (4). Potassium Humate increases accumulation of chlorophyll, sugar, amino acids and more improves the efficiency of nitrogen utilization, allowing for reduced fertilizer rates, the plant's ability to withstand the stresses of heat, drought, cold, disease, insect and other types of environmental or cultural pressures and also increases general plant productivity, in terms of yield, as well as plant stem strength (2). Using of Potassium Humate increased root system, tuber yield, tuber number per plant (1), root number (3) and pea numbers and weight per plant and decreased nitrate amounts in leaves and roots (4) and decreased nitrate accumulation in potato tubers (5).

2. MATERIALS AND METHODS

In this experiment, plantlets produced from meristem culture of Agria, Advanced Clone 397007-9, Marfona, Sante, Satina and Ceaser propagated by single node cuttings. Then, plantlets were cultured in planting beds of Pitmass (Biolan) with punce in a volumetric form (1:1). Experimental design was factorial on the basis of completely randomized block design in three replications. Potassium humate spraying was done with two levels (0 and 250 ml in volume of 300 lit water per ha) and in the three growth periods (plantlet with 5, 10 and 20 cm length). This experiment was done in biotechnology laboratory and green house belonged to Villkej Company of Ardabil Province, Iran in 2007 year. All of plantlets irrigated after planting by normal water. Macro and micro nutrients were used to provide for nutrition of plantlets. Plantlets reached to enough growth after about 30 days in condition of 22-25 degrees centigrade temperature and 16 hours photoperiod with 5000 lux intensity light. Spraying with potassium humate was done in three growth stages (5, 10 and 20 cm in length of plantlets). All of practices such as irrigation, weed out, pest and diseases control were done regularly during growth stages. Control of pests and fungus diseases were done respectively by use of 250^{cc} per ha Confidor and 400 g/ha Equation-pro. Chlorophyll content was measured by CCM-200 made by OPTI-SCIENCES Company during of growth period and after spraying with potassium humate. Minitubers were harvested after about two months. Then the number and weight of mini-tubers per plant and average mini-tubers weight measured. Analysis of variance was done for noted traits and means compared on the basis of LSD (Least significant difference) test.

3. RESULTS AND DISCUSSION

Results showed that there were significant differences at probability level of 1 % between cultivars, potassium humate levels and their interaction for Chlorophyll Content Index, the number and weight of minituber per plant.

The highest Chlorophyll Content Index, the number and weight of mini-tuber per plant, and average weight of mini-tuber per plant was made by 250 ml/ha potassium humate treatment. Potassium humate caused to increase 4.76 Chlorophyll Content Index, 1.84 number of mini-tuber per plant, 17.15g weight of mini-tuber per plant, 2.04g average mini-tuber weight per plant (Table 1).

The highest Chlorophyll Content Index and number of mini-tuber per plant was belonged to Agria; weight of mini-tuber per plant to Sante and average mini-tuber weight per plant to Satina and Sante, when treated by 250 ml/ha potassium humate (Table 2).

The most uniformity of produced mini-tubers was observed in 250 ml/ha Potassium humate treatment. Potassium humate were caused increase of yield and crop quality in all of the plants (1, 2 and 6). Use of potassium humate in potato causes them to produce more than 70 % tubers with 35–55 mm seed size (2).

The price of one mini-tuber is about 0.25$ in Iran. In our study Potassium Humate increased the number of mini-tubers per plant (with mean of 1.86 tubers). Therefore about 47$ increased for production of mini-tubers per square meter. Thus Potassium Humate caused the economic effects pay attention to results of this research. On the other hand we would like to apply results of this research for increase of production of mini-tuber per plant, so decrease in production cost until farmers could buy cheaper mini-tubers.

4. CONCLUSIONS

Potassium humate caused to increase Chlorophyll Content Index, number of mini-tuber per plant and average mini-tuber weight per plant. The most uniformity of produced mini-tubers observed in 250 ml/ha Potassium humate treatment.

Table 1. Mean of different traits on different levels of Potassium humate

Potassium Humate	Chlorophyll Content Index	Mini-tuber number per plant	Mini-tuber weight per plant (g)	Average mini-tuber weight (g)
250 mlit/ha	32.10 a	4.92 a	36.12 a	7.48 a
0 mlit/ha	27.34 b	3.08 b	18.97 b	5.44 b
d	4.76	1.84	17.15	2.04

Table 2. Mean of different traits on potato CVs. and different levels of Potassium humate

Potassium Humate	Cultivars	Chlorophyll Content Index	Mini-tuber number per plant	Mini-tuber weight per plant (g)	Average mini-tuber weight (g)
K-Humate 250 ml/ha	Sante	34.06 b	4.50 bcd	40.68 a	9.03 a
	Ceaser	30.90 d	5.33 ab	36.40 c	6.82 cd
	Advancd Clone	24.36 h	4.66 bc	30.17 e	6.44 de
	Marfona	32.63 c	5.00 b	39.31 b	7.85 b
	Agria	36.66 a	6.00 a	33.32 d	5.52 f
	Satina	34.00 b	4.00 cde	36.86 c	9.21 a
K-Humate 0 ml/ha	Sante	27.63 ef	3.00 f	22.17 g	7.36 bc
	Ceaser	25.43 g	3.67 def	26.73 f	6.31 def
	Advancd Clone	25.33 g	3.00 f	19.36 h	3.00 g
	Marfona	28.33 e	2.00 g	13.78 i	6.75 cd
	Agria	30.33 d	3.50 ef	13.03 i	3.64 g
	Satina	27.00 f	3.33 ef	18.73 h	5.58 ef

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The Stimulation of Microorganisms Activity of Petropolluted Soils by Humic Preparations

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Keywords: humic acids, hydrocarbon-oxidizing microorganisms, petropolluted soil

1. INTRODUCTION

Due to development of nature-protecting biotechnologies the interest to the study of processes with participation of aerobic hydrocarbon-oxidizing microorganisms (HOM) has increased. First of all it is concerned with the pollution of soil, surface and underground waters by oil and oil products. The intensity and character of decomposition of oil hydrocarbons in soil basically is defined by a functional activity of HOM, capable to assimilate of oil as a unique source of carbon (1). The use of next ways for the bioremediation of soils and waters from oil pollutions is possible: the entering into soil of biological products on the basis of culture of active HOM and the stimulation of activity of own native petroassimilating microflora of soil. One of such ways is the stimulation of growth and activity of soil microflora by means of the entering of humic acids (HA) into the polluted soil.

The purpose of the present work was to investigate the influence of features of structure HA of different sources on dynamics of number and destructive activity of HOM of petropolluted soil.

2. MATERIAL AND METHODS

The number and activity of soil microflora studied on the example of next physiological groups: heterotrophs and actinomicetes. HA of low-moor wood peat of «Klyukvennoe» field of the Tomsk Oblast (Russia) with a degree of decomposition of 25% isolated by extraction of 0.1 N NaOH and used as a stimulating nutritious substratum. The preparation HA1 from initial peat was the control. Preparations HA2 and HA3 are extracted from a mechanoactivated peat (2). The mechanoactivation was carried out in the flow vibrocentrifugal mill VCM-10 without additives (HA2) and in the presence of solid alkali (3% NaOH) (HA3). HA4 – a commercial preparation of HA from brown coal (Aldrich).

For researches the oil in the concentration of 15 g/kg added in the soil to simulate soil pollution. Solutions of HA added in the soil on 3 and 5-th days of the cultivation. The doze of entering of the nutritious substratum on 100 g of the soil was 2 ml of a solution of HA in the concentration 5 g/l.

Fractions of paraffin-naphthene hydrocarbons have been analysed by means of gas chromatography with mass spectrometry (GC/MSD).

3. RESULTS AND DISCUSSIONS

Results of researches have shown that the addition of HA isolated from a mechanoactivated peat under the cultivation of microorganisms in petropolluted soils has the stimulating effect on the development of soil microflora, including HOM. The maximal growth of number of heterotrophs and actinomicetes – the basic destructors of oil, has noted after the addition of HA3 received from the mechanoactivated peat with alkali in the petropolluted soil (fig. 1).

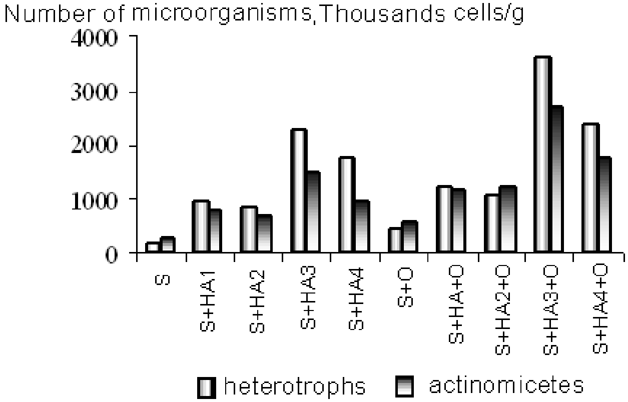

Figure 1. Number of heterotrophs and actinomicetes in the pure and petropolluted soil after the addition of HA on 30 days of growth.

S - soil; S+HA1 - soil+HA from initial peat; S+HA2 - soil+HA from mechanoactivated peat without additives; S+HA3 - soil+HA from mechanoactivated peat in the presence of alkali 3 % NaOH; S+HA4 - soil+HA from brown coal (Aldrich); S+O - soil+oil; S+HA1+O - soil+HA from initial peat+oil; S+HA2+O - soil+HA from mechanoactivated peat without additives+oil; S+HA3+O - soil+HA from additives peat in the presence of alkali 3 % NaOH+oil; S+HA4+O - soil+HA from brown coal (Aldrich)+oil.

The mechanoactivation of peat can cause the increase of the availability of separate components and parts of HA molecules, including micro-and macroelements, which are used as food by soil microorganisms. It was shown that the soil microflora, stimulated by HA, possesses an increased destructive oxidizing activity in relation to oil – additives of HA in the soil reduce the quantity of extracted bitumen-like compounds (residual oil) to a significant degree (fig. 2).

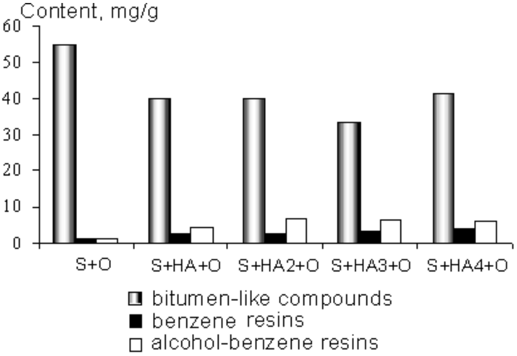

Figure 2. Changes in the content of bitumen-like compounds, benzene and alcohol-benzene resins in the petropolluted soil with additives of humic preparations.

The change of structure n-alkanes during a microbiological degradation was shown. It was noted that first of all low-molecular hydrocarbons destruct in a greater degree in the soil with additives of HA from mechanoactivated peat with alkali and HA of brown coal with a raised share of carbohydrate fragments and oxygen-containing groups in their structure (fig. 3).

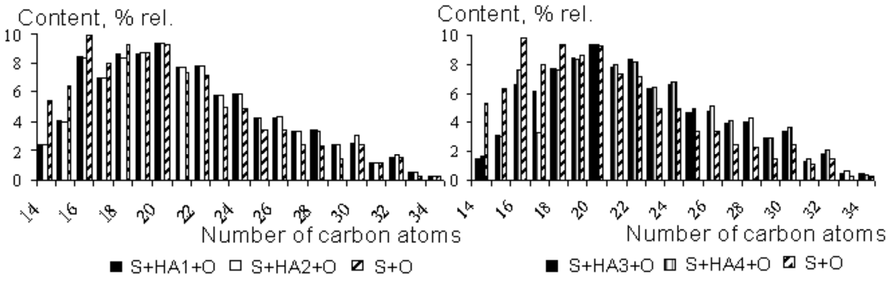

Figure 3. The molecular- mass distribution of n-alkanes in bitumen-like compounds extracted from petropolluted soils with additives of humic preparations.

Indexes of hydrocarbons biodegradation characterize the orientation, depth and character of the process of oxidizing destruction of oil hydrocarbons and testify about a microbiological decomposition of oil (tab. 1).

A ratio of n-C_{17}/n-C_{27} characterizes an essential reduction of hydrocarbons share with an average molecular weight and it decreases in two times in bitumen-like compounds from samples with additives of HA3 and HA4. An index and-C_{19}/н-C_{17} characterizes an increase of branching and testifies about deeper processes of oil biodegradation in variants with the application of preparations of HA3 and HA4. A coefficient of oddness defined from the formula of Philippi Ko = $2C_{29}/C_{28}+C_{30}$ are a third index of oil biodestruction (3). The value of Ko is close 1 for oil and slightly decreases in samples with additives of HA. It means that high-molecular n-alkanes are subjected to destruction to a lesser degree for the present level of microbiological oxidation.

Table 1. Indexes of hydrocarbons biodegradation in bitumen-like extracts of the soil and oil

Objects	Indexes of hydrocarbons biodegradation		
	n-C_{17}/n-C_{27}	i-C_{19}/i-C_{17}	$2C_{29}/C_{28}+C_{30}$
Soil+oil	3.3	0.13	1.1
Soil+oil +HA1	2.1	0.19	0.8
Soil+oil + HA2	2.0	0.19	0.8
Soil+oil + HA3	1.6	0.25	0.8
Soil+oil + HA4	1.5	0.24	0.8

4. CONCLUSIONS

Thus, results of researches have shown, that the addition of HA isolated from mechanoactivated peat in petropolluted soils has a stimulating effect on the development of soil microflora, including hydrocarbon-oxidizing microorganisms with an increased destructive oxidizing activity in relation to oil.

ACKNOWLEDGEMENTS

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Coal Biodegradation by Basidiomycetes for Production of Biofertilizers and Soil Conditioners

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Keywords: coal degradation, *Coriolus hirsutus*, biofertilisers, humic-like substances

1. INTRODUCTION

Coal is a traditional energy source for many countries and its use will not be reduced in the nearest future in spite of environmental damage by this energy source. Therefore great attention is paid for development of technological approaches to convert hard coal or lignite to a clean, cost effective energy source or to product value added products (1). The technological approaches focusing on conversion of coal slime and sludge as wastes of coal industry is also of great importance For Russian Federation.

The most perspective for conversion of coal slime considered to be biotechnological approaches based on microbial and/or enzymatic treatment (1, 2). These approaches are environmentally safe, cost efficient (carrying out at moderate pressure and temperature) and resulted in production of bioactive compounds.

The aim of the present study was to develop an approach for conversion of coal slime by Basidiomycetes and characterize the products formed during biotransformation.

2. MATERIALS AND METHODS

Pure culture of Basidiomycete *Coriolus hirsutus* 075 (Wulf. Ex. Fr) Quel. was used in this study. The samples of brown coal from Sonzevskoy deposit (Sakhalin, Russia) were used. For the experiments, the coal sample was air-dried, grinded and sieved through 1 mm sieve.

The strain was surface-cultivated in 250-ml flasks containing 50 ml of full or poor (without a favored carbon source glucose) nutrition medium, porcelain beads and inoculum. Full nutrition medium was prepared using following compounds (g/l): glucose (10.0), peptone (3.0), KH_2PO_4 (0.6), $ZnSO_4{\cdot}2H_2O$ (0.001), K_2HPO_4 (0.4), $FeSO_4{\cdot}7H_2O$ (0.0005), $MnSO_4{\cdot}7H_2O$ (0.05), $MgSO_4{\cdot}7H_2O$ (0.5) at pH 6.0. Inoculum was prepared according to (3). When required, 5 g of coal was added to the flask. Coal samples were sterilized by heating to 120°C for 1 h. Surface cultivation lasted for 30 days.

During the cultivation biomass of *Coriolus hirsutus* mycelium was monitored. At the end of cultivation coal was separated from the nutrition media by filtration, dried at 50°C, and weighted.

To estimate biological activity of the residual nutrition medium after fungi cultivation, bioassay experiments were conducted using seedlings of wheat *Triticum aestivum* L. as target object. Seeds were germinated in the residual nutrition medium for 72 h at 24°C. Then length of shoots and roots were measured and used as response.

3. RESULTS AND DISCUSSION

Coal biotransformation can be considered as complex process including enzymatic, non-enzymatic and enzyme mediated stages. The strain *Coriolus hirsutus* grew rather well both on poor and rich nutrition media under static conditions. The introduction of coal in the media resulted in significant increasing of fungal biomass. The most profound effect has been observed during the growth of fungus on rich medium with coal sample: the amount of fungal biomass has increased in 60 folds compared with blank (rich media without coal). During the growth on poor media containing coal, a 14,5 fold increase in fungal biomass has been determined. Analysis of the obtained data allowed concluding the ability of basidiomycetes namely *C. hirsutus* to utilize coal as nutrient source.

The cultivation of *C. hirsutus* on both media with introduced coal resulted in decrease in coal weight. Obtained results are presented in Table 1. The most drastic decrease (up to 83% of initial weight of coal) was observed when poor medium was used. The latter indicated for the coal utilization Basidiomycetes as a carbon source confirming our assumption.

Table 1. Losses of coal during liquid surface cultivation of *Coriolus hirsutus* 075 (Wulf. Ex. Fr) Quel. under full and poor nutrition media conditions.

Variant	Weight of coal, % of initial
Blank, poor medium	100
Poor medium	83
Blank, full medium	100
Full medium	90

The comparative study of biological activities of residual cultural liquids after fungus cultivation has been carried out. The data are presented in Fig. 1. Obtain results demonstrated that among the variants studied, the full nutrition medium after fungi cultivation in the presence of coal possessed pronounced stimulating effect in relation to both wheat shoots and roots growth. That finding indicated that basidiomycetes *Coriolus hirsutus* 075 produced some bio-solubilized compounds from coal that possessed physiological activity towards seedlings. Therefore, it can be concluded that residuary full nutrition media after basidiomycetes *Coriolus hirsutus* cultivation in the presence of coal could be recommended for further study for its biological activity.

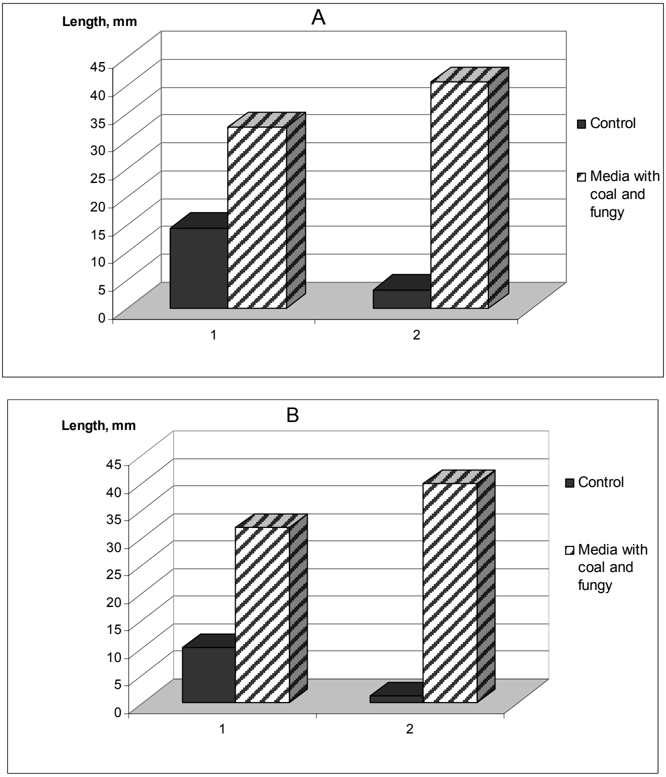

Figure 1. Influence of residuary nutrition media (A - poor media and B – rich media) on growth of shoots (1) and roots (2) of wheat Triticum aestivum L.

Analyzing the data it can be seen that limitation of carbon source (poor media) induced coal biotransformation with production of compounds utilized by fungi for biomass production. However growth on rich media resulted in enhance production of bioactive compounds. The further study will be focused on characterization of these compounds and their biological activity.

4. CONCLUSIONS

The performed experiments on *Corioulus hirsutus* cultivation demonstrated the ability of basidiomycetes to utilize coal as nutrient source under the surface cultivation. Among the variants studied (liquid surface cultivation conditions on full and poor media) the most pronounced decrease of coal weight was registered for the variant of liquid surface cultivation using poor nutrition medium. The most significant increase in fungal biomass production was observed during cultivation using rich nutrition medium.

Our findings indicated that basidiomycetes *Coriolus hirsutus* 075 produced some bio-solubilized compounds from coal that possessed physiological activity towards wheat seedlings. Residuary poor nutrition media after basidiomycetes *Coriolus hirsutus* 075 cultivation was seemingly not so effective as compared to full medium. Residuary full medium after basidiomycetes *Coriolus hirsutus* 075 cultivation possessed the greatest stimulating activity in terms of growth of both shoots and roots. This by-product of coal bio-solubilization could be therefore recommended for further study for its biological activity.

ACKNOWLEDGEMENTS

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Influence of Humic Substances on the Foam Formation and Cytotoxicity of Detergents

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Keywords: detergents, humic substances, foam formation, cytotoxicity

1. INTRODUCTION

Humic substances (HS) are known to interact with a variety of organic and inorganic substances which makes them an important regulatory factor in the environment (1). Along with the use of particular peat layers for the production of humic-rich bath additives the question of possible physicochemical and toxicological changes of the bath medium due to HS became relevant. The objective of the present study is therefore to examine interactions between HS and detergents intended to be used for manufacturing of body care products. In particular, foam formation, foam stability, changes in cytotoxicity as well as other parameters of interest were analysed.

2. MATERIALS AND METHODS

Detergents: The anionic surfactant sodium dodecyl sulfate (SDS, Ferak, Berlin), the non-ionic surfactants Plantacare® 2000 (PC-2000, Henkel KGaA, Düsseldorf) and Triton X-100 (TX-100, Ferak, Berlin), and the amphoteric surfactant cocamidopropylbetain (TEGO® Betain CKD, Th. Goldschmidt AG, Essen) were used.

Humic substances: Water-soluble parts of the Original Altteich® Bath Concentrate (BC) (BNS GmbH, D-02956 Rietschen, Germany) and the sodium salt of humic acids (sodium humate), extracted with 0.2 mol/l NaOH from the Altteich peat deposit at pH 9 were used for the experiments.

Foam height determination: The determination of foaming properties of detergents was carried out according to the ASTM (American Society for Testing and Materials) Standard D 1173 – 53 (re-approved 1997). The equipment consists of a calibrated 200 ml pipette and a 100 cm long glass tubing as the receiver. Detergents with and without HS were used as 0.1% (w/v) solutions in distilled water preheated at 49°C. The foam height formed during running out the detergent solution from the pipette into the receiver is measured every five minutes during a total period of 45 minutes.

Cells: Human promonocytic U937 cells (ATCC CRL 1593) were cultivated in RPMI 1640 medium containing 10% foetal bovine serum and 5% CO_2. They were split every two days at a ratio of 1:3 and used for the experiments two days after passaging.

Cytotoxicity: The surfactants were tested for cytotoxicity singly and in combination with humic substances 1h after addition to the cells. The cytotoxicity was measured by means of the tetrazolium reduction assay WST-1 (Roche Diagnostics GmbH, Mannheim, Germany).

3. RESULTS AND DISCUSSIONS

The foam height after 0.1% SDS dropped continuously from 15 cm in the beginning to 2 cm within 45 min. A mixture of 0.1% SDS and peat included bath concentrate (1:60) Promote the foam formation within 10 min after the start (about 5 cm higher than SDS alone). The foam formation curve crosses the SDS curve at 15 min, and a 2 cm deeper foam height course was registered (Fig. 1).

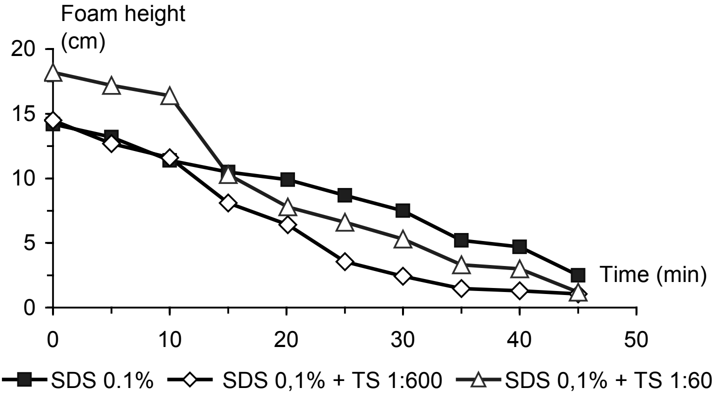

Figure 1. Influence of peat bath concentrate (BC) on foam formation and foam stability of the detergent sodium dodecyl sulfate (SDS).

Triton X-100 proved to be a weak foam producer developing very short-living foam. Already after 20 min the foam height dropped down from 13 cm at the beginning to <2 cm at the endpoint (45 min). BC 1:60 and BC 1:600, respectively, had no influence on the foam formation of triton X-100 (data not shown).

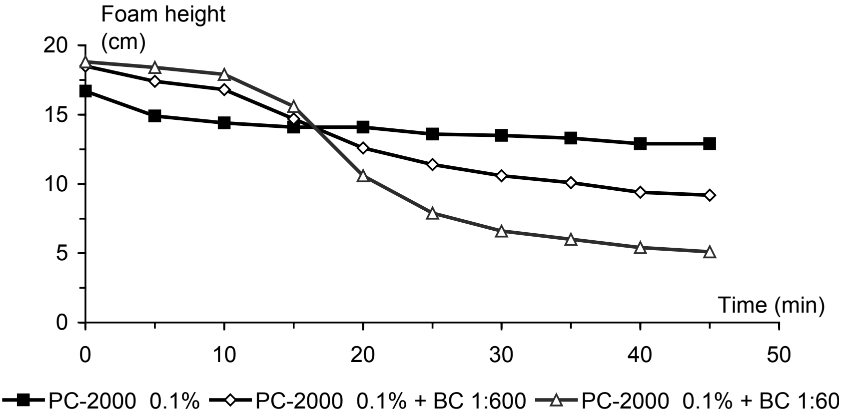

Figure 2. Influence of the peat bath concentrate (BC) on foam formation and foam stability of the detergent Plantacare® 2000 (PC-2000).

The detergent Plantacare® 2000 produced voluminous and very stable foam. BC 1:60 slightly increased the foam height in the first 10 minutes and decreased it markedly 15 minutes after measurements started. In contrast, BC 1:600, the foam height decrease in combination with Plantacare was less pronounced (Fig. 2).

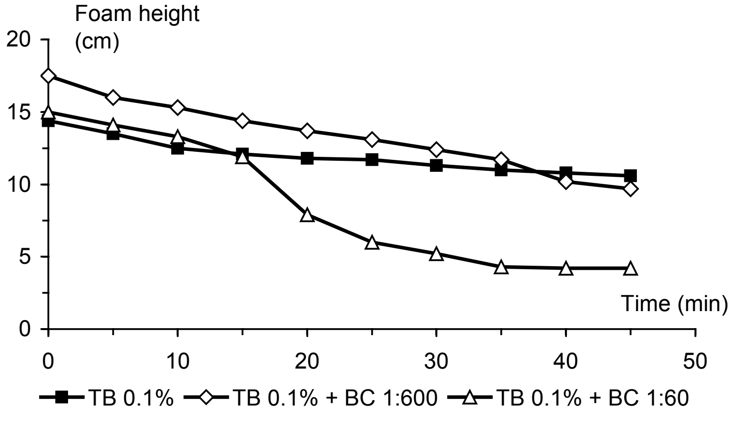

Figure 3. Influence of bath peat concentrate (BC) on foam formation and foam stability of the detergent TEGO®-Betain (TB).

TEGO®-Betain CKD developed stable foam. Within 45 minutes the foam level sunk only about 5 cm. TB in combination with BC 1: 600 surpassed the foam height of TB alone,

in combination with BC 1: 60 the foam height decreased about 5 cm after 15 minutes (Fig.3).

The cytotoxicity of TEGO®-Betain CKD and Plantacare® 2000 (2) is significantly reduced by the Original Altteich® Bath Concentrate (BC) but not by Na-humate isolated from the Altteich peat deposit (Tab. 1). The reason for this behavior could be a higher percentage of aliphatic moieties in the peat HS compared with the isolated Na-humate.

Table 1. Half maximal cytotoxic concentrations (CC_{50}) of detergents with and without humic substances (HS) in U937 cells after 1h exposure

Detergent	Combination	CC_{50} (µg/ml)	Confidence Interval (µg/ml)
TEGO® Betain CKD	without	31.3	27.7 - 35.5
	with Na-humate	42.5	39.8 - 45.3
	with BC	95	93.6 - 96.3
Plantacare® 2000	without	131	117 - 148
	with Na-humate	136	114 - 162
	with BC	414	359 - 478

4. CONCLUSIONS

Solutions of 0.1% sodium dodecyl sulfate, TEGO® Betain and Plantacare® 2000 developed foam heights between 13 and 17 cm, followed by a time-dependent decline of the foam level. The most stabilized foam was shown by Plantacare® 2000, followed by TEGO® Betain. Addition of peat suspensions BC 1:60 and 1:600, respectively, had an influence on foam formation by SDS, TB and PC with the exception of Triton X-100. Foam formation increased in the presence of BC 1: 60 of SDS, TB and PC in the first ten minutes, in presence of BC 1:600 of PC 30 minutes.

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Effect of Different Physiologically Active Substances in Conditions of Greenhouse Industrial Production

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Keywords: humic, lignin-humus acids, greenhouse industrial production

1. INTRODUCTION

Growing of greenery plants under industrial conditions defines the economy of this industry. When producing vegetables under conditions of artificial lightning the problem of increased accumulation of nitrates in vegetables is faced. Different physiologically active substances become more and more popular as means for correction of bioproductive process.

The aim of investigation was to evaluate physiologically active substances of different origins (humates and ethylene-producers) under greenhouse conditions.

2. MATERIALS AND METHODS

In industrial conditions of greenhouse company “Leto” experiments with humic (GA-Na), humus likely substances LGA-Na (lignin-humus product extracted from aspen) and ethylene producer “Regrost” (obtained on the base of calcium carbide) were conducted.

The experiments were carried out in pots on the hydroponics installation produced by companies Agro-forest-group and VEFI. The experimental crops were dill, lettuce, parsley, spice and cucumber.

The concentration of nitrates was measured by potentiometric method with ion-selective electrode. Yield of the crops was determined as biomass of the main output at the regulated dates of harvesting. Average sample number for each treatment was from 40 up to 540 pots.

Doses of substances were optimized according to their physiological efficiency. Humus substances were used in concentrations 10….10^{-4}%. In some cases the seeds were treated, in some cases the treatments were carried out on leaves.

3. RESULTS AND DISCUSSIONS

All investigated substances positively influenced on crops, but the efficiency of their influence was different for different crops. Thus, more effective for dill plants was LGA-Na (leaves were treated by LGA-Na in concentration of 10^{-5}%). For the treatment of 540 pots with dill plants 4 g of "Regrost" were used. As a result there were 34 % less of ill plants and nitrates concentration decreased on 226 mg kg^{-1}. The growth rate of plants increases on 5-8 days after treatments with those substances.

The efficiency of the substances on lettuce was nearly the same. After treatments with LGA-Na and Regrost the increase in fresh biomass was nearly 40%. The improvement in yield quality was observed.

The most effective substance for parsley plants was Regrost. Nearly double increase of the biomass was observed after treatments with this substance. The percentage of ill plants was lower. After treatments with LGA-Na preparation the biomass increased by 40% and less than 4% were wastes.

The experiments showed that treatments with humic substances not only positively influence yield of the crops, but also decrease content of nitrates in plants. In lettuce, dill, parsley the concentrations of nitrates decreased more than two times (from 582 to 294 mg kg^{-1}) when ГК-Na was applied and more than 3 times (from 582 to 143 mg kg^{-1}) when LGA-Na preparations were used. The same relationship was observed in the variants with cucumber. Concentrations of nitrates decreased from 464 to 222 mg kg^{-1} in the treatments with GA-Na and to 133 mg kg^{-1} when LGA-Na preparations were applied.

4. CONCLUSIONS

The investigated substances considerably influenced bioproductivity and quality of greenery crops and significantly corrected economical and ecological indexes of greenhouse production.

To evaluate the effect of these concentrations 10…10^{-10}% several peaks of stimulated and inhibited influence of different gradients of the concentrations have a wave character.

Humic substances and ethylene producers are not only activators of growth processes. They might be used as means of management of quality of crops yield. It is especially important for the production of dietetic foodstuff.

Efficacy of Silicious Lignin Products Application for Biological Agriculture Exemplified by Experiments with Red Clovers

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Keywords: lignosilicon, organic farming, red clover, green mass yield and quality, soil fertility

1. INTRODUCTION

Lignosilicons (LSi) belong to the group of the biologically active, ecologically safe compounds for the farming, which has been synthesized in the Institute of Wood Chemistry. They are derived from the different lignocellulosic materials and are used as growth activators for the cereals cultivation (oats, wheat, maize). LSi showed favorable influence on the plants development, increased their productivity and improved quality of the plants and soils, both. Earlier results of the LSi testing indicated its perspectives for the oats and winter rye the biological farming with red clover undersow (1, 2). Small LSi dosages (40-120 kg/ha) were added during sowing alongside with oats and clover seeds and have activated root system development for both types of plant species (2). In the case of clover not only root volume, but also root mass and number of tubercles increased. Harvest of oats with red clover undersow was considerably higher than in the case of control.

Aim of current work is to evaluate LSi influence on the clover green mass harvest, its qualitative parameters and soil fertility as well.

2. MATERIALS AND METHODS

LSi is the powder-like compound with 5% silicon contents. It is being synthesized on the base of ethanol production wastes using sulfur acid percolation hydrolysis of wood.

Tested species: two varieties of red clover *Trifolium pratense L.*, Latvian selection: fusariosis and cancer resistant brand “Divaja”, and brand “Jancis”, which is known for high seeds productivity, cold resistance and ability to grow on non fertile soils for the 2-3 years. Vegetation and field tests were done in the soil corresponding to *Orthieurtic Mollic Gleysol* with organic matter content 3.9%, pH_{KCl} 5.3-5.6, medium content of mobile K_2O and P_2O_5. For the evaluation of LSi influence on the agrochemical soil parameters soil samples were taken using probe almost twice per vegetation period. The following data were acquired:

pH_{KCl}, organics content (C, %), total nitrogen contents (%), nitrates content (NO_3, mg kg^{-1}), K_2O, P_2O_5 and Ca contents (mg kg^{-1}). Analysis of plants for the macro and micro elements contents has been performed as well. Root system was tested using calibrated scanner Epson STD-1600^+ and Win Rhizo 2002 C software package.

Microfield experiments in the years 2004-2007 were made at the certified biological field. Plot size – 5 m^2, distance between rows – 15 cm. LSi was manually introduced into rows before the sowing in 40 kg/ha, 120 kg/ha and 240 kg/ha dosages. Mineral fertilizers were not used. Normalized seeds consumption – 20 kg/ha (10 g per plot). For the plant material sampling frame (0.5 x 0.5 m) was used. Each sample was taken four times. Vegetation experiments were carried out in 6.5 l vessels, filled with screened soil taken from the biological fields. Vegetation pots were placed on open air close to experimental biological fields.

3. RESULTS AND DISCUSSION

Results of the vegetation experiments in the 6.5 l vessels and in the open fields have shown that considerable differences in the development of the red clovers “Divaja” and “Jancis” on the background of the LSi and without additional introduction of the mineral fertilizers can be observed in 40-70 days after sowing and depend on the weather conditions. Results of the microfield red clover “Jancis” experiments (July 7, 2004 – July 21, 2005) have shown that LSi did not influence on the seeds germination (70-72%, the same result as in control). Differences in the plants development appeared 40 days after sowing. The best development was observed in the case of 240 kg/ha LSi addition – average green raw mass of single plant was up to 1.5 times higher than control, tubercles amount was 28% higher than in control. Scanning of roots has shown 30% increase in total roots length, 26% increase in roots volume and increase in forks and tips numbers.

After 70 days amount of tubercles in the samples grown on the background of 240 kg/ha LSi addition was 37% higher than in control, total root length – 65%, roots volume – 63%, tips and forks amount was more that two times higher. These results indicate that LSi exhibits activating influence on red clover root system development, especially taking in the view fact, that for control month after the first test root system additional growth was insignificant, whereas on the background of the LSi it was higher than after first 40 days. In the conditions of microfield experiment introduction of 40 kg/ha LSi into the upper soil layer did not influence on harvest. On the background of 120 and 240 kg/ha LSi addition increase of raw green mass of clover “Jancis” was 5% and 13%, correspondingly (16% and 22% on dry matter). In the case of 240 kg/ha LSi amount of protein was 29% higher than in control, and in the green mass of this clover plants the share of leaves and flowers increased comparing to control (Table 1).

Table 1. Influence of the different LSi dosages on green mass “Jancis” clover harvest and its quality, 2 hay-crops. Microfield experiment (July 7, 2004 – July 21, 2005)

Variant	LSi dosage, kg ha^{-1}	Harvest, t ha^{-1}		Protein harvest, t ha^{-1}	Botanic anylsis of green mass, %	
		Green mass	Dry mass		Leaves + flowers	stem
Control	0	57.8±1.3	13.4±1.2	1.4±0.2	35±1	65±1
LSi	40	57.6±1.5	13.1±1.2	1.4±0.1	33±4	67±4
LSi	120	60.7±1.4	15.5±0.9	1.6±0.1	40±3	60±3
LSi	240	65.3±1.2	16.4±1.0	1.8±0.2	39±2	61±2

Soil analysis was performed in the spring of year 2005, 10 months after „Jancis” clover sowing. Amount of organic matter in soil has increased from 4.7% to 5.0% and 5.1% correspondingly for the variants with 120 and 240 kg/ha LSi introduction.

Results of microfield experiments with „Divaja” red clover (July 7, 2005 – September 25, 2007) have shown that considerable differences in the plant development are being observed 40-50 days after sowing, as in case of „Jancis” clover.

The highest results were obtained on the background of 240 kg/ha LSi addition: yield of green and dry mass were 30% and 50% higher than in control, correspondingly, protein amount was 60% higher. On the background of 120 kg/ha LSi addition yield of for green and dry biomass, as well as protein, were 22%, 27% and 31% higher, correspondingly. Clover seeds harvest in the year 2007 September was 18% (120 kg/ha) and 27% (240 kg/ha) higher than for control, so clover plants grown on the LSi background are characterized with higher reproductive ability.

Macroelemental content in the “Divaja” clover biomass did not change on the background of LSi addition, but considerable changes are being observed in the microelements contents. Iron contents in leaves and flowers increased up to 30-40%, and boron – 40% comparing to control. It should be noted 3-5 times increase of iron and 2-3 times increase of silicon in roots. Increase of iron and molybdenum contents in roots can be explained by nitrogen fixation activation on the background of LSi. It is also being proved by total increase of organics and total nitrogen contents in soil, as well as protein contents in green mass of plants. Soil analysis was performed in the spring of year 2006, 10 months after sowing. It has shown increase of organic matter from 3.9% up to 4.4% (120 kg/ha) and 5.1% (240 kg/ha). Total nitrogen content increased from 0.17% up to

0.23% and 0.25%, NO_3 decreased from 33.7 mg kg^{-1} to 20.0 mg kg^{-1} and 23.3 mg kg^{-1} for 120 kg/ha and 240 kg/ha LSi dosages, correspondingly.

Soil analysis performed in the fall of year 2007, after seed harvesting, showed favorable and prolonged LSi influence: 2.2 years after sowing organic mater in the soil increased from 3.9% up to 4.3% in control and up to 5.3% for 240 kg/ha LSi dosage.

4. CONCLUSIONS

According to the results of microfield experiments LSi showed favorable influence on the plants development, increased their productivity and improved qualitative parameters of the plants and soils, both. The best results for the biomass harvest and seeds (2.2 years after sowing), as well as increase in organic matter in soil, were observed for the LSi dosage 240 kg/ha, introduced once into the upper soil layer before clover sowing. On the background of LSi the share of leaves and flowers in green mass increased.

Increase of “Jancis” and “Divaja” red clover dry mass harvest was from 22% to 50%, and increase of protein content was from 29% to 60%, for control and 240 kg/ha LSi dosage, correspondingly.

Activity of microflora in soil is being increased on the background of LSi, as well as nutrients and microelements uptake from soil and their transport into the plant.

Prolonged influence of LSi can be observed over two years – after 2.2 years after sowing organic mater in the soil increased from 3.9% up to 4.3% in control and up to 5.3% 240 kg/ha LSi dosage. Clover seeds harvest was 18% (120 kg/ha) and 27% (240 kg/ha) higher than control.

ACKNOWLEDGMENTS

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Inhibitory Action of New Organic Substrates for Ornamental Plants and Their Humic Fractions on the Growth of the Phytopathogenic Fungus *Pythium ultimum*

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Keywords: organic substrates, humic fractions, ornamental plants, compost, phytopathogenic fungi, *Pythium ultimum*

1. INTRODUCTION

The production of ornamental plants is a relevant segment of worldwide agriculture, and it has great economical importance in Italy. Peat and coconut fibres are considered conventional organic growing media in container cultivation of these plants. Nevertheless, new organic substrates which include composts have been recently used for at least partial replacement of traditional plant growing matrices. In the last years, a number of investigations have demonstrated the effectiveness of composts of various origin and their humic-like fractions in suppressing soil-borne plant pathogens (1-3), and their application to soil has been proposed to control many different diseases. Numerous fungal species such as *Pythium ultimum* can cause severe damages to horticultural and ornamental plants, especially at early growth stage and in greenhouse conditions. The organic fraction is definitely the main component of growing substrates, and the major responsible of the pathogenic-suppressive properties of these matrices. Few recent studies report a significant action of humic and humic-like fractions in the growth inhibition of different fungal species (4-6). The mechanisms by which composts or their humic fractions inhibit plant pathogens are not fully understood, even if an evident relationship occurs between physical, chemical and biological properties of these materials and their capacity to suppress pathogenic fungi.

The objectives of this work were to test: (i) different new organic growing substrates on the pathogenic activity in vivo of *P. ultimum* on plants of *Impatiens walleriana*; and (ii) the humic and humic-like acids (HAs) isolated from these substrates, at two concentrations, on the growth in vitro of the fungal mycelium.

2. MATERIALS AND METHODS

Plants of *I. walleriana* were grown for 14 weeks in a climatic chamber using a peat (P) or a coconut fibres (CF) as conventional substrates, and a compost (C) or mixtures at 20, 40 and 60% (v/v) of C with P (C20+P, C40+P and C60+P) or C with CF (C20+CF, C40+CF and C60+CF) as new organic substrates. During the growth period, the fungus was inoculated three times in the substrates and pathogenicity was monitored by observing the apparent symptoms of plant suffering and by measuring plant biometric parameters, such as height, number of leaves and the length of the main leaf vein at 10, 12 and 14 weeks after transplanting.

In a second set of experiments, the HAs extracted from the 9 substrates by conventional methods (7) were tested at concentrations of 50 and 300 mg/L on the growth in vitro of *P. ultimum* on PDA (potato dextrose agar) medium according to the procedure previously reported (5). All in vivo and in vitro experiments were replicated 6 times and data obtained were statistically analysed by one-way analysis of variance (ANOVA), and and the means of treatments separated by the least significant differences (LSD) test.

3. RESULTS AND DISCUSSIONS

The various substrates caused differentiated plant growth response. The treatments with C alone or its mixtures at 40 and 60% with P or CF resulted lethal for plants even before the first fungal inoculation (6 weeks after transplanting). The substrates C20+P and C20+CF resulted the most effective in controlling the fungus and increasing the growth of *I. walleriana*. In fact, the number of leaves per plant, plant height, and the average length of the main vein increased respectively of 234, 95, and 33% for C20+P with respect to P alone, and 192, 136 and – 3% for C20+CF with respect to CF alone (Fig. 1).

In the in vitro experiments, no morphological changes were observed for the fungal mycelium as a function of the presence of any HA at 50 and 300 mg/L in the growing medium, with respect to the control (PDA alone). However, the presence of the various HAs in the PDA medium produced different specific growth responses by the fungus. The higher dose of HAs isolated from C and P showed the maximum inhibitory effect on the growth of *P. ultimum*, with respect to the control, causing a general decrease of the radial growth of mycelium during the entire experimental time with a maximum of 55 and 39%, respectively, at 18 h after inoculation (Fig. 2). Similar results were obtained in recent studies (3, 5-6) that showed that composts or their HA-like fractions inhibited significantly the growth of some soil-borne plant pathogenic fungi. In general, HAs isolated from all mixtures of C with P or CF caused a significant inhibition of the fungus at both concentrations only at 42 h from inoculation.

Finally, in the attempt to relate the biological action of HAs with their main chemical and functional properties, it appeared that C-HA, i.e., the most effective in the fungal inhibition, presented, among the 9 HAs examined, the highest H and C contents, the lowest O content, and a quite low total acidity and COOH group content, thus confirming the occurrence of specific correlations already observed in our previous studies (5-6).

P C20+P CF C20+CF

Figure 1. Effect of different substrates on the growth of *I. walleriana* at 13 weeks after transplanting.

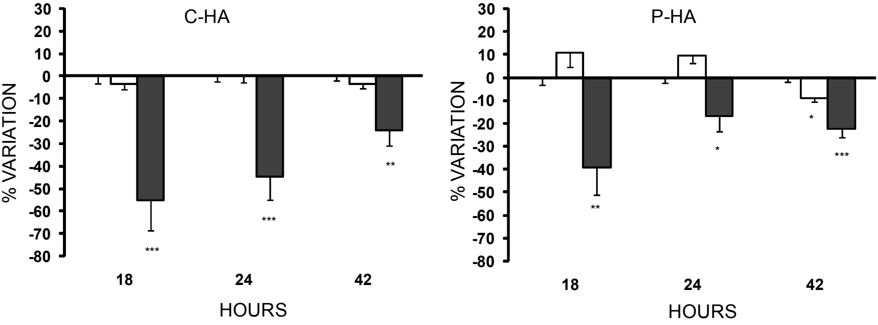

Figure 2. Effect of C-HA and P-HA at concentrations of 50 mg/L (white bars) and 300 mg/L (black bars) on the variation (%) of radial mycelial growth of *P. ultimum* on PDA with respect to control (only error bars).

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Artificial Humic Substances as Possible Compensators of the Destroyed Links in Carbon Cycle

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Keywords: humic substances, carbon, soil organic matter, ecological solution

1. INTRODUCTION

One of the global ecological problems is the depletion of humus in intensively exploited soils. Soil humic substances (HS) are important depot of carbon, which is comparable to depot of oil and coal, but unlike the latter two, humic substances are renewable. From the ecological point of view, to prevent further destruction of the environment, optimal organic fertilizers must be produced not from natural resources, but from materials already included into anthropogenic carbon cycle, such as organic wastes and garbage, and cattle manure. Necessity of optimal utilization of wastes is unquestionable. Previously use of cattle manure was good, until it became the problem in Europe due to the lack of interaction between farms, producing manure, and end users. Collection, storage and composting of animal wastes causes emission of large amounts of greenhouse gases. The oxidative-hydrolytic destruction (OHD) technology was developed by Biocorrection A/S to accelerate processes of humification of biogenic wastes and prevent formation of CO_2, CH_4, NH_4 and N oxides. This is extremely important, as carbon, preserved in the stable soil organic matter, does not come into atmosphere. The matter in OHD reactor is exposed to oxidative-hydrolytic destruction under high pressure and temperature. Product, resulting from the OHD reaction, is represented by liquid part (BioC-HS) and solid part with non-hydrolyzed residues (in ratio 10:1-2). BioC-HS is chemically close to natural HS. The object of this research was to show physiological activity of BioC-HS preparations, potential and limiting factor of their application.

2. MATERIALS AND METHODS

Laboratory experiments were conducted at constant temperature, optimal moisture and in the absence of light. Analyzed soil samples comprised: humus-free mineral materials (sand and loam, pH_{KCl} 4.9 and 6.0, C 0.6 and 0.3%, correspondingly) and materials from A1 horizon of soddy-podzolic loamy soil (pH_{KCl} 4.0, C 3.9%). Cucumber plant seeds were used for bio-indication. HS were used in two modifications: Na and Ca forms (Na-, Ca-HS) produced from cow manure by the OHD method with addition of NaOH and $Ca(OH)_2$ correspondingly. .We used reagents of 50 mg HS / ml. HS consist of 85% of humic acids and fulvic acids were the rest. Microbial activity was estimated by photo film application method. Soil aggregate waterproof was estimated by their destruction in water (Standard method).

3. RESULTS AND DISCUSSION

Experiment 1

Physiological activity was tested on cucumber seeds, which were germinated in Petri dish at 27ºC, wetted with excess concentration of BioC-HS (Na) solutions (fig. 1). The observed results were similar to that of the activity of natural HS. The highest effect was registered at 100 mg/l of HS, the lower and higher concentrations were not as effective. The wider is the range of effective concentrations, the more usable is the product in practical applications. That is why it is important to study further application of different forms of HS in various plants.

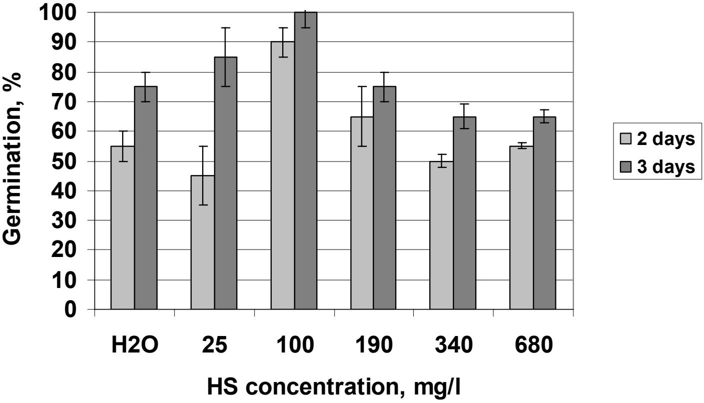

Figure 1. Germination of cucumber seeds wetted with BioC-HS solutions.

Experiment 2

The BioC-HS (Ca) was applied to loam A1, loam and sand soils in doses equivalent to 10 and 50 t/ha. BioC-HS (Ca) was found to reduce acidity of substrates and increase total content of C and N. BioC-HS (Ca) increased microbial activity, when applied in the dose of 10 t/ha and decreased the same, when applied in dose of 50 t/ha (fig 2). High dose (corresponding to 50 tons per hectare) exhibited inhibiting effect. Correspondingly, high doses of humic preparations will be quite efficient on early stages of remediation of humus-free soil, since they inhibit activity of destructors, which eventually promotes accumulation of organic matter in the soil.

Table 1. Chemical properties of 1 month-old composts

Variant	$\mathrm{pH_{H2O}}$	$\mathrm{pH_{KCl}}$	C total, %	N total, %	C/N
A1	4.96±0.01	3.97±0.02	3.44±0.01	0.37±0.01	9.44±0.09
A1+10 t/ha	5.13±0.01*	4.10±0.01	3.48±0.01	0.38±0.01	9.23±0.14
A1+50 t/ha	5.26±0.01	4.33±0.01	3.55±0.02	0.42±0.01	8.47±0.21
Loam	6.69±0.03	5.68±0.03	0.53±0.01	0.09±0.00	6.27±0.08
Loam +10 t/ha	7.06±0.01	6.14±0.01	0.66±0.01	0.04±0.00	15.5±0.58
Loam +50 t/ha	7.06±0.01	6.24±0.01	0.78±0.01	0.12±0.01	6.81±0.17
Sand	7.02±0.03	6.45±0.01	0.30±0.01	0.04±0.00	7.38±0.24
Sand +10 t/ha	7.07±0.01	6.73±0.01	0.35±0.01	0.04±0.00	8.75±0.32
Sand +50 t/ha	7.16±0.01	6.75±0.01	0.43±0.01	0.06±0.00	7.83±0.31

* Values in bold have significant difference with control on the level of 95%.

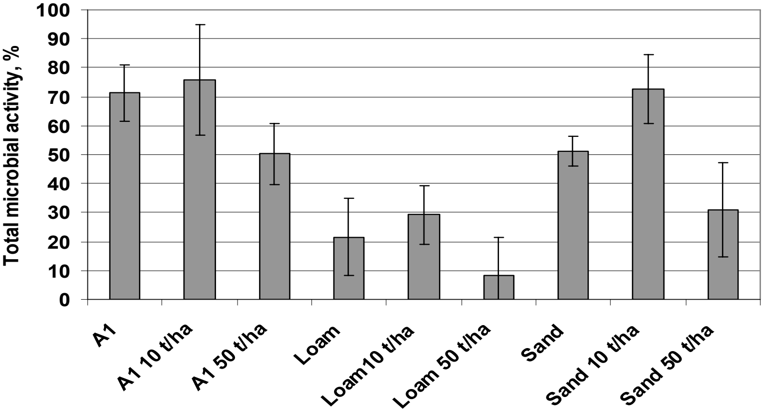

Figure 2. Total microbial activity in Experiment 2.

The most promising results, in terms of structure of the soil, were registered in the groups treated with BioC-HS (Ca). Used light loamy soil (variant “Loam”) in control group had an unfavorable blocky structure, with prevalence of fractions with size larger than 10mm. Application of the humic preparation dramatically reduced the amount of block aggregates larger than 10mm and thus improved the share of agriculturally important fractions (from 10 to 0,25mm). The effect was more pronounced in groups, treated with large amounts of BioC-HS humic substance preparation (corresponding to 50 tons per hectare). As it is shown on the diagram (figure 3), BioC-HS (Ca) preparation had a significant effect on water-stability of clay aggregates: the index increased from 13 to 70% approaching that of park soil (90-92%).

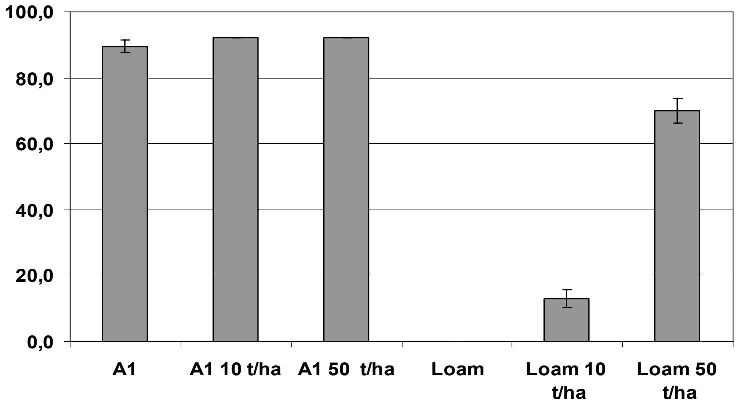

Figure 3. Waterproof aggregates, %.

4. CONCLUSIONS

1. Application of HS preparation, produced by BioCorrection A/S aided in accumulation of nitrogen and carbon in soils, stabilization of pH near neutral values. This attests increased stability of the soil organic matter and, correspondingly, reduced emission of greenhouse gases from the soils.
2. Prevalence of calcium humates within the BioC-HS (Ca) preparation provides substantial improvement of the structure of clay soils. Water-stability of aggregates is seen substantially improved, opening wide perspectives for application of the BioC-HS in remediation of this type of soils.
3. It was revealed that biological activity of preparations is to a large degree dependent on the dosage: low concentrations of the preparation may be inefficient, average – can have stimulating effect and high may possess inhibiting properties. Thus, humic substance preparations may be used both for inhibition and stimulation of soil processes, related to activity of microorganisms; that is why selection of dosages and administration regimens is an important task for researchers, conducting investigation of applied aspects of utilization of humic substances.

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Isolation of Humic Substances from Underground Brine Water of Chiba, Southeast Japan

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Keywords: underground brine, fulvic acid, electrodialysis

1. INTRODUCTION

The underground brine water is the fossil seawater confined to Pliocene-Pleistocene stratum with sedimentary and diastrophisms. The largest hydropressured natural gas field in Japan is the Minami-Kanto gas field, in Chiba prefecture, South-Eastern Japan. The brine contains humic substances (HS), other than methane and iodine. These precious natural resources, CH_4 and I, are used for commercial purpose (the production of I_2 accounts for 30% of the world), but HS, including fulvic acid (FA) at a concentration of 40-70 mgL^{-1} in brine, are not utilized effectively as yet (1).

The physiological functions of FA on plants, especially as plant growth stimulations, root stimulation, nutrient absorption and photosynthesis ability for embryo plant have been examined (2-4). In addition, FA have various additional functions, including redox and complexation abilities for metal ions (5-6) and hygroscopicity (7) caused by carboxylic and/or other functional groups.

There are various procedures to isolate HS from natural water, of which the IHSS method (8) is regarded as a de-facto standard procedure for isolation of HS. However, it involves a considerable amount of hydrochloric acid and sodium hydroxide for the elution from the resin column. Furthermore, it is not suitable for brine because of its high salinity and iodine content.

Therefore, electrodialysis was applied to desalination and recovery of HS from high salinity brine water. This alternative method is able to reduce the environmental loads, and can be regarded as a "*green process*".

2. MATERIALS AND METHODS

The FA from brine was prepared by the electrodialysis method. The electrodialyzer used was the micro-acilyzer S1 equipped with a NEOSEPTA ion-exchange cartridge (ASTOM, Japan). The molecular weight cut-off (MWCO) of this ion-exchange membrane was 300 Dalton. The FA were characterized by elemental analysis and functional group contents. Furthermore, the plant physiological activities of the FA were studied with flowering kale (*Brassica oleracea* L. var. *acephala* DC. f. *tricolor* Hort) cultivated by hydroponics with various concentration of FA and heavy metal ions (Cu, Cr) in cultivation medium.

3. RESULTS AND DISCUSSION

During the progress of the electrodialysis (Figure), the FA concentration in the desalination stream remained almost constant, while virtually all of the chloride anion was removed from the desalination stream. These FA from brine have aliphatic structure from the results of characterization.

It was observed in hydroponics, both of shoot and root of plant growth inhabitations and chlorosis caused by exposure free heavy metal ions or metals with FA. While in case of exposure metal-FA complex, extracted from brine water, it was observed no chlorosis and inhibition of plants.

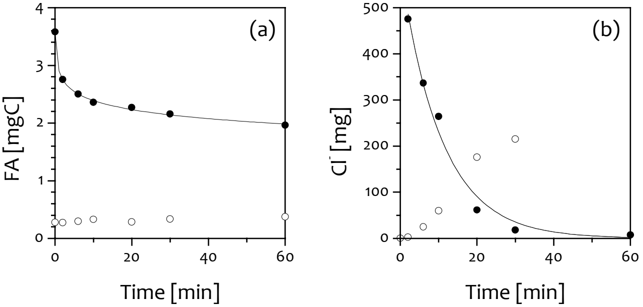

Figure. Variation of the content of FA (a) and chloride (b) in desalination (dot) and concentration (circle) streams as a function of time.

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Biotic Control of Humic Substances Ecotoxicity and their Remediation Effect in Contaminated Environment

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Keywords: bioassay, detoxication, test-organisms, humic substances, water and soil remediation, ecological standardization, certification

1. INTRODUCTION

The elaboration of program for biotic control of ecosystems is a crucial question in ecology. The indexes of toxicant concentrations as well as any other chemical and physical parameters are only symptoms, but not reasons for determination of good health or «unhealth» of ecosystem. The modern biotic conception of environmental control is based on the evaluation of disturbance in biota.

There are different approaches to evaluation of environmental deterioration depending on the directions for use of territories in agricultural, industrial, house-building or reserves purposes. These approaches consider different biodiagnostic systems and special lists of biotic responses. The biodiagnostic includes the methods of bioindication *in situ* and bioassays in laboratory.

The Russian State Register of Environment Methodologies includes the list of about 10 standard ecotoxicological methods recommended for biological control of environment contamination and waste safety. It inscribes the most commonly used acute toxicity tests with *Daphnia magna* and *Ceriodaphnia affinis,* very popular luminescent bacterial Microtox (R) bioassay. Green algae *Chlorella vulgaris* or *Scenedesmus quadricauda* are also recommend as test-organisms in experiments on bio-testing to analyze the population cell number in respect to the control performing direct counting with Goryaev camera as well as using less labor-intensive biophysical technique of spectral measurements, etc.

Official statements strongly recommend including at least two biotest-systems for environmental purpose. The responses of two test-organisms from different taxonomic groups must be considered in ecological certificate and decision of a commission of experts in Russian Federation. The aqueous extracts from wastes and soils are recommended by state institutions to test for eco-toxicological control. The tests are carried out with the use of aquatic organisms (Criteria for determination of harmful level of dangerous waste…, The Order of Russian Ministry of Natural Resources # 511, 2001).

However there is a big problem in control of ecotoxicity because the sensitivity of test-organisms is very flexible and often ambiguous depending on kind of toxicants [8].

In this paper we attract attention to two aspects of laboratory bio-assays: 1) control of humic substances toxicity and selective sensitivity of biotest-systems 2) control of remediating effects in contaminated environment.

2. MATERIALS AND METHODS

Different biotest-systems are used in our laboratory (Laboratory for Soils Ecotoxicological Analysis of MSU, Certificate # POCC RU.0001.513050, www.letap.ru). They include the following types of organisms: (1) hydrobionts - *Daphnia magna*, *Ceriodaphnia affinis, Paramecium caudatum*, *Scenedesmus quadricauda* and *Artemia salina,* which are officially recommended for natural ecosystems and waste biotic control, (2) plants, which are usually used to control pesticides in agrocoenosis, and (3) soil habitat microorganisms, which are represented by micromycetes. In experiments were compared their sensitivity to dissolved humic substances.

We investigated the interactions of hazardous–waste chemicals with humic substances. Laboratory experiments were performed to estimate the detoxication effect of commercial dissolved humic-mineral concentrate (HMC) on toxicity of phosphogypsum by mixing two substances in ratios 1:1 and 1:10. In special experiments the reactions of different pigmentation microscopic fungi on potassium humate Powhumus (Humintech Ltd) were studied. It was tested in concentrations of 0.1 and 0.02%. To analyze the growth and spectral characteristics of micromycetes the humic substance was added in the nutrient Chapek-medium.

3. RESULTS AND DISCUSSION

Humic substances are the main component of organic matter in all terrestrial and aquatic ecosystems, comprising a variety of molecular structures and functional groups. On one hand they bind different kind of pollutants, thereby decreasing the bioavailability and consequently the toxicity of these substances in most instances. On the other hand recent studies describe direct interactions and effects on organisms (4-6). We summarized here the results of our studies in these directions.

The remediation effect of humate in polluted soils is measured with terrestrial and aquatic organisms. Early our experiment indicated that phosphogypsum, as a chemical waste belongs to IV dangerous class because of high concentrations of F- and P-content toxic compounds (3). Detoxication of phosphogypsum upon addition of humic-mineral concentrate (HMC) was shown using survival-tests performed on *Daphnia magna* and growing seeds of radish *Raphanus sativa* and rice *Orysa sativa*. It was revealed that mitigation toxic effect was stronger at lower concentrations of HMC. At the same time the toxicity of mixture of two components increase with increased HMC concentrations.

The sensitivity of test-organisms depends on kind of toxicants. Our results showed that for instance *Paramecium caudatum* are useless for ecotoxicity control of substrates exposed to oil pollution (or polluting). It wasn’t appropriate to estimate the colored humic substance toxicity using algae-fluorescence tests. It is difficult also to study toxicity of humic substances using some species of organisms because their response to humic substances depends on the adaptation process as well as functional and physiological state of organisms. For instance hydrobionts living in oligotrophic waters have the best sensitivity to presence of humate than any organisms from waters rich of organic matter (1).

The estimation of the toxicity of humic substances on soil microorganisms showed that microscopic fungi may be useful for elaboration of biotest-systems. Some species of fungi, as it was reported earlier, are able to decompose humic substances. Micromycetes showed different sensitivity to hymates in the nutrient medium depending on the pigmentation of mycelium. Some decrease of fungal biomass for dark-colored strains of micromycetes in liquid medium with potassium humate Powhumus was marked [2]. The results showed that the investigated concentration of humate in Chapek-agar did not affect the development of the non-colored (apigmented) *Fusarium* colonies - neither toxic nor stimulation affect on the growth in solid medium, whereas the dark pigmented strains of micromycetes change their growth and spectral characteristics in presence of humic substances. Thanks to that phenomenon we recommend to use *Fusarium* as a test-culture to estimate the toxicity in contaminated soil with different humus content (7). Phytotests in the control of humate toxicity and its bioremediation effect by chemical pollution discover good results (3, 6). The application plant as test-organisms makes a sense to include in the list of effective bioassay methods.

4. CONCLUSIONS

To sum up the brief review our studies we should stress that the combination of different tests including both aquatic and terrestrial test-organisms is required to get precise result in many cases, especially by the ecotoxicological estimation of the contaminated soils. We suppose that the situation with standard methods in ecological legislation will progress more quickly. In future the range of authorized aquatic test-systems will supplement with the test-systems based on the reaction of terrestrial organisms at different levels of biota: microorganisms, plants, invertebrate and vertebrate animals. The improvement bioassay for the evaluation of ecotoxicity of humic substances, chemical waste and contaminated natural material is in full accordance with the biotic conception of the environmental control. Step by step this conception put into practice and replaces the sanitary-hygienic control regulations in preservation of the environment (8).

ACKNOWLEDGEMENTS

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Extraction and Exchange Behavior of Metal Species in Therapeutically Applied Peat Characterized by Competitive Extractants

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Keywords: Balneological peat, metal extractants, extraction kinetics, dissolved organic matter

1. INTRODUCTION

Natural peats are continuously formed by complex microbiotic humification processes of plant residues during thousands of years. There are about 500 million hectares of peatlands in the world (nearly half of Europe area). Due their high content of humin, humic acids (HA) and fulvic acid (FA), peats exhibit favorable chemical-physical properties enabling their application in various technical areas, for instance wastewater treatment, pollution monitoring, fuel production, enzyme immobilization, as well as in veterinary and human medicine (1).

According to some speculations the medical and veterinary effects of peat are probably associated to their high content of humic substances (HS) and humic-bound metal species (2). As yet, the natural enrichment of metals in balneological peats (3) and their possible (bio)-availability during peat therapy has scarcely been investigated, excepted Beer et al. (4) having studied the possible uptake of heavy metals by patients pre-treated by peat mush baths and peat tampons, respectively. At best, heavy metals in medically applied peats and their aqueous extracts have occasionally been characterized (5). For this purpose, improved metal speciation procedures, differentiating heavy metals in balneological peats as a function of their extractability and availability, respectively, are desired.

Therefore, in the present work the extraction behaviour of dissolved organic matter (DOM) and metal species from therapeutically used peats (Großes Gifhorner Moor, Sassenburg/North Germany) were characterized by means of a versatile analytical approach. In particular, the availability and exchange behaviour of peat-bound metal species towards both competitive chelators (DTPA, EDTA). In environmental science, the chelators DTPA and EDTA, respectively, are conventionally used as extractants to assess “(bio)available” metal fractions, for instance in soils and organic-rich sludge samples.

2. MATERIALS AND METHODS

The medical peat samples (Vitamoor®, H. Wulfes KG, Sassenburg/Germany) were collected from "Großes Gifhorner Moor", Sassenburg/North Germany.

To characterize the peat, 100 g of the sample (wet) were suspended in 250 mL of high-purity water and some mL of 2 mol/L NaOH solution were added to adjust a pH value of 4.5. Then, the mixture was magnetically stirred (250-300 rotations per minute) in a room temperature (23-25^{o}C). The pH was maintained using a conventional glass electrode. After different periods of time (5, 10, 15, 30, 60 and 120 minutes) small aliquots (about 50 mL, each) of the peat suspension were filtered through a coarse paper filter and, finally, through a 0.45 µm flat membrane to remove particles. The fractions collected this way were characterized by their UV/VIS absorbance at 254 nm and 436 nm, respectively, as well as by metal determinations using atomic spectrometry. Applying an analogous extraction procedure and different extractors (0.01 mol/L $CaCl_2$, 0.01 mol/L ethylendiaminetetraacetic acid (EDTA) and 0.01 mol/L diethylenetriaminepentaacetic acid (DTPA), respectively, were also studied. Before peat extraction the pH value of the EDTA and DTPA solutions was adjusted to 5.0 by adding 2 mol/L NaOH solution. The standard deviations of the metal extraction procedures applied were assessed from 5 different batch runs extracting 100 g samples of peat (wet), 250 mL extractant, equilibration for 1 h; metal determinations by ICP-OES and AAS, respectively.

3. RESULTS AND DISCUSSION

Typical metal exchange curves, obtained by EDTA and DTPA from medical peat as a function of time, are presented in Figures 1 and 2. Both complexants exhibit more or less comparable kinetics of metal and DOM remobilisation, respectively. The remobilisation equilibria for the studied metals are approached within 60 minutes, except Al and Fe needing up to 2 hours. The EDTA and DTPA solution have a similarly strong capability to remobilize major fractions of heavy metals from peat. Most probably, the chemical extraction mechanisms of both chelators are quite similar.

Therefore, EDTA and DTPA might be useful for a simple removal of undesired high metal loads of peat prior to its therapeutic use. The UV absorbance A_{254nm} of DOM, co-extracted by EDTA and DTPA solutions, respectively, exhibits significantly higher but similar values compared with those obtained by pure water as extractor (results not shown). This raise is probably caused by an increasing mobilisation of HS molecules from peat by these complexants.

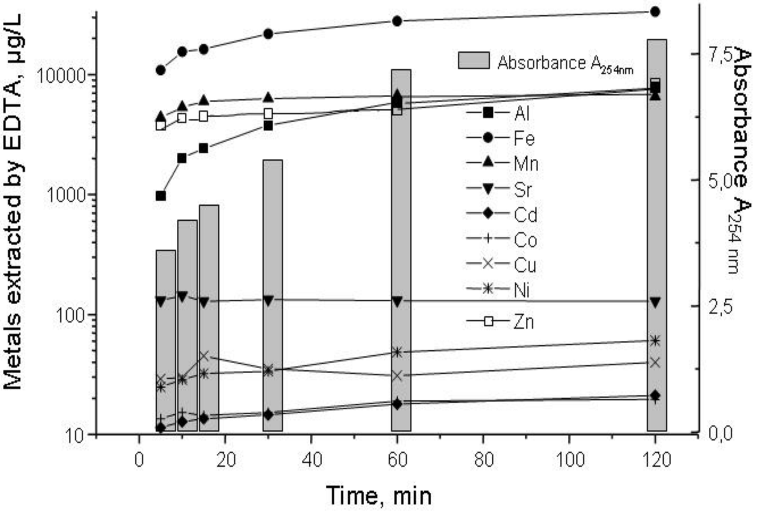

Figure 1. Exchange of peat-bound metal species by EDTA solution.

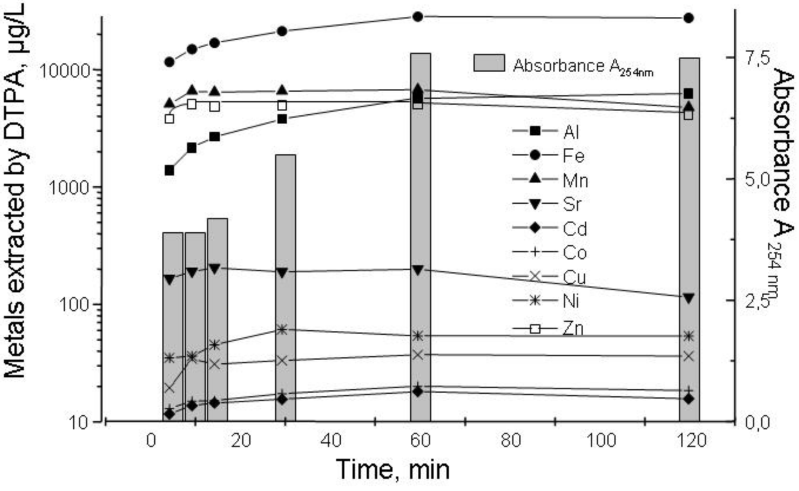

Figure 2. Exchange of peat-bound metal species by DTPA solution.

4. CONCLUSIONS

DTPA and EDTA exert a strong, well comparable and reproducible extraction capability towards peat-bound heavy metals, similar to the operational DTPA and EDTA availability of metals in soils. All in all, the present study, based on a versatile extraction approach, improves the knowledge of metal species and their chemical availability in balneological peats. Additional medical investigations, however, are required to clarify in more detail the positive and negative effects of metals in peat therapy.

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Study of the Parameters that Influence on the Process of Alkaline Extraction of Humic Substances from Peat

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Keywords: humic substances, humic acid, fulvic acid, extraction, structural characterization

1. INTRODUCTION

Humic substances (HS) are formed by biomolecules transformation during the decomposition process of present vegetable and animals residues in the environment. To understand the behavior in the environment, the HS has been studied under different aspects: partial characterization of structures (1), determination of the equilibrium constant of metal-HS species (2) and lability of metals (3). Meanwhile, the first step for these studies is the extraction of HS and the future interpretations will depend on the experimental conditions used in the extraction procedure. Therefore it is important the development of analytical methods to extract HS from soils with minimal changes in the originals structures, in relatively short time, high-income and low-contaminants.

The International Humic Substances Society (IHSS) recommends a procedure based on four hours extraction with NaOH 0.1 mol L^{-1} solution at room temperature in the soil/extractor ratio 1:10 (g/mL) under nitrogen atmosphere. However, according to (4), several factors influence the extraction procedure and a lot of subjects are still for being answered. Some authors have used softer extractors as sodium pyrophosphate, formic acid and organic solvents while other authors prefer an extraction more complete of HS using alkali. Then, this work studied the parameters that influence the alkaline extraction of HS from peat as alkaline extractor type, concentration, time and peat/extractor ratio.

2. MATERIALS AND METHODS

The sample of peat was collected (30 cm depth) in an area located in the Ribeirão Preto city, at Sao Paulo state-Brazil. After drying on air, the sample was grind in porcelain grail and sieved in 2 mm (70 mesh).

The extractions were performed varying the extractor solutions (NaOH, KOH), and their concentrations (0.1, 0.5 and 1.0 mol L^{-1}), in four hours, peat/extractor ratio 1:10 (g/mL), room temperature (25-30°C), under nitrogen atmosphere and mechanical agitation. After extraction the sample was centrifuged at 2000.*g* for 10 minutes to separate the humin, insoluble fraction. The humic extract was concentrated in evaporator at 55 °C to approximately 10 mL, moved for a Petri plate and dried in stove with air circulation. That procedure was repeated varying the time of extraction between 1-8 hours.

The extractions were performed using KOH 0.5 mol L^{-1} solution, during four hours at room temperature under nitrogen atmosphere and mechanical agitation with different ratio peat/extractor: 1:5, 1:10, 1:20, 1:30, 1:40 and 1:50 (g/mL).

3. RESULTS AND DISCUSSION

In according to Figure 1 by reducing the concentration of alkali, the extraction of HA increases and the FA reduces. These evidences were also characterized by viewing the difference in color (brown, yellow, and almost colourless) of fulvic fraction extracted according to the concentration of the solution used as extractor. This occurs due to the alkali concentration increasing, that causes a disruption of intermolecular forces that connect the FA to HA, such as hydrogen bridges or links-type ester (5).

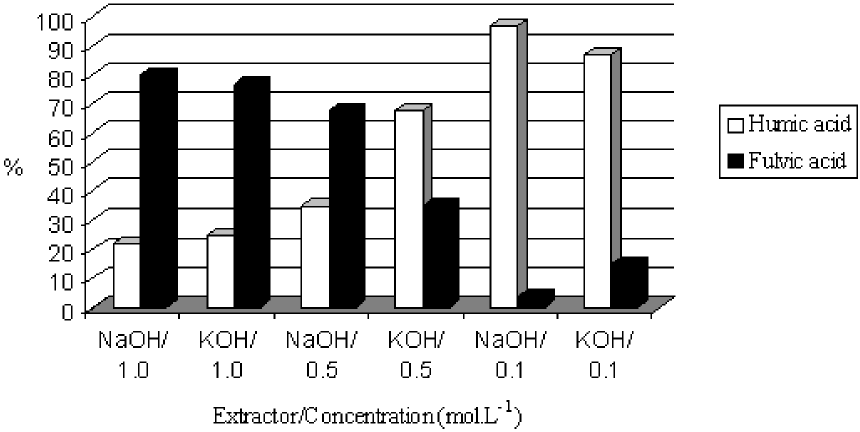

Figure 1. Influence of extractor solution and concentration in the content of HA and FA extracted from peat.

The amount of organic matter (OM) extracted with alkali solvents increases with the extraction time (Figure 2) due to the slow depolymerization of complexes with high-molecular weight. These complexes are part of humin and are associated with silicates. Thus, the time needed for extraction depends on the rate of OM humification and the characteristics of the inorganic material present in the matrix. In the sample used in this study, the depolymerization of OM present in humin is verified up to 240 minutes, after that time there is not a considerable increase in the content of extracted OM.

About the peat/extractor ratio, it was observed that the yield of organic material extracted was higher in the ratio 1:20 (g/mL) (results not shown). In biggest proportions it was just observed increase of the inorganic material content, but not of the organic material.

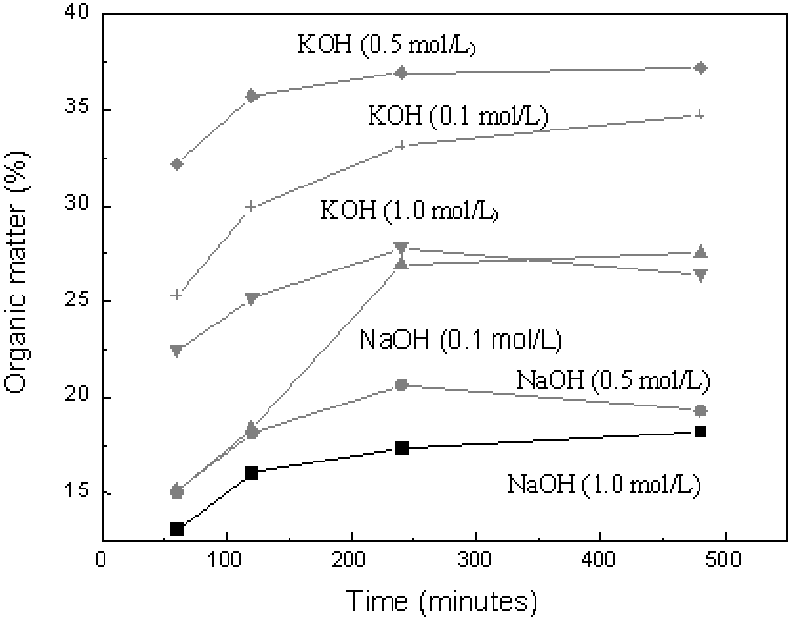

Figure 2. Influence of extraction time in the content of extracted organic matter from peat.

4. CONCLUSIONS

The best conditions for extraction of humic substances in the studied peat sample were: KOH extractor solution (0.5 mol/L), extraction time of 4 hours, peat/extractor ratio 1:20 (g/mL), nitrogen atmosphere and mechanical agitation. The KOH extractor solution

(0.5 mol L^{-1}) showed advantages on NaOH for the studied sample, due to higher yield and reduction of contaminants in the extraction process.

ACKNOWLEDGEMENTS

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Evaluation of Tropical Peat as a Potential Medium for Bioremediation of Vanadium

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Keywords: Brazilian peat, vanadium, bioremediation

1. INTRODUCTION

Petroleum exploitation is the main economic activity of Sergipe State, in Northeast Brazil, location of the country's largest terrestrial oilfield. The industry generates diverse by-products, such as volatile aromatic oil fractions, organic acids and metals (1). Vanadium is recognized to be one of the most abundant metallic constituents of petroleum, and can cause undesirable side effects in oil-fired power plants, as well as potential environmental contamination (2). In recent years, interest has increased in the use of low-cost adsorbents, such as peat, for environmental remediation purposes; however application of this material for remediation of vanadium remains largely unexplored. According to a Mineral Resources Research Centre report, Sergipe State contains 21 peat deposits, distributed across almost the entire State, with an estimated total resource of 800,000 t (dry ash) of peat. The present study evaluates some of the parameters influencing bioremediation of vanadium using these tropical peats.

2. MATERIALS AND METHODS

A peat sample was collected from the Santo Amaro das Brotas region of Sergipe State. The raw material was air-dried at room temperature, and then sieved through a 115-mesh grid. Batch adsorption experiments were carried out as follows: 20 mL aliquots of aqueous, 10 mg L^{-1} V (V), solution were added to 40 mg of peat, in stoppered polyethylene flasks which were then shaken at a speed of 175 rpm for 74 h in a constant temperature shaker bath at 25 ± 0.2 ^{o}C. Different initial pH values were employed (2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0). After filtration through a 0.45 μm membrane filter, the concentrations of V (V) in the supernatant solutions were determined by flame atomic absorption spectrometry. For the adsorption isotherm experiments, the adsorbent (40 mg) was added to 20 mL of different concentrations of V (V) solution (5, 10, 50 and 100 mg L^{-1}), at pH 5.0. The bottles were shaken at 25 ± 0.2 ^{o}C for an equilibration time of 50 h, after which the concentrations of V (V) in the supernatant solutions were determined.

3. RESULTS AND DISCUSSION

Parameters that influence the adsorption of metal ions to peat include pH, contact time and the metal ion concentration.

Figure 1 illustrates the removal of vanadium by peat (0–10 cm depth) at different pH values (2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0), after 72 hours, the time necessary to ensure that equilibrium conditions had been reached. Data show that adsorption of vanadium increased with pH, reaching a maximum at pH 5.0–7.0, at which the adsorption capacity was 4.5 mg V g^{-1} of peat, with decreased adsorption at pH 8.0.

Adsorption results using different vanadium concentrations, at pH 5.0, are shown in Figure 2. Equilibration times were 12 h (5 mg L^{-1} solution), 30 h (10 mg L^{-1} solution) and 48 h (50 and 100 mg L^{-1} solutions). The vanadium concentration influenced the contact time required to reach equilibrium, and the adsorption capacity increased at higher vanadium ion concentrations (3).

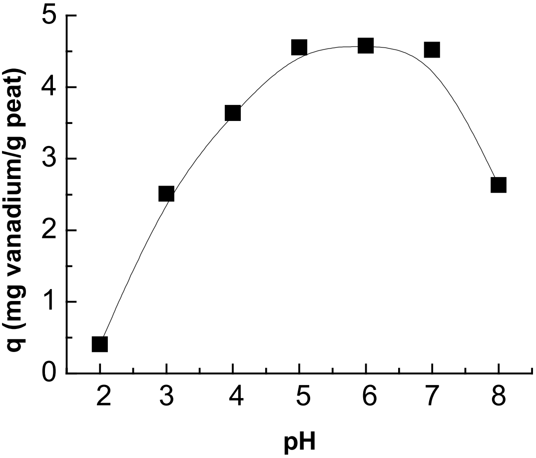

Figure 1. Effect of pH on vanadium adsorption (q) onto peat.
Conditions: 10 mg (V) L^{-1}; T = 25 ± 0.2 ^{0}C; t = 72 h, m = 40 mg peat.

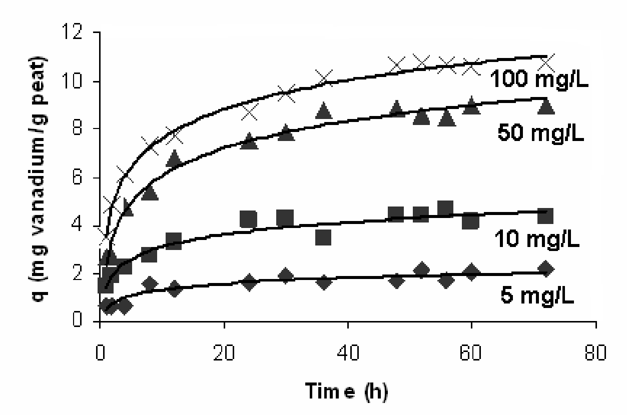

Figure 2. Influence of solution concentration on vanadium adsorption (q) onto peat. Conditions: T= 25 ± 0.2 0C; pH = 5.0; m = 40 mg peat.

A pseudo-second order model for the adsorption of vanadium onto peat was adopted from (3). The reaction rate expression can be described by $\frac{t}{q_t} = \frac{1}{h} + \frac{1}{q_e} t$ (Figure 3), where q_e is the amount of metal ion adsorbed at equilibrium, q_t is the amount of metal ion on the surface of the adsorbent at any time, t, and h is the initial adsorption rate. The adsorption rate constant (k) is given by $h = kq_e^2$.

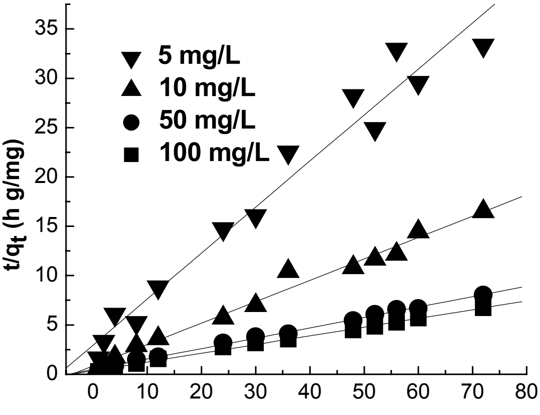

Figure 3. Vanadium adsorption (t/q_t) *versus* time (h), according to vanadium ion concentration. T = 25 ± 0.2 ^{0}C; pH = 5.0; m = 40 mg peat.

The value of the rate constant, *k,* decreased from 7.4 x 10^{-2} to 5.5 x 10^{-2} g mg^{-1} h^{-1}, for 5 and 10 mg L^{-1}, respectively. Subsequently, at 50 and 100 mg L^{-1}, values of *k* were constant at 2.2 x 10^{-2} g mg^{-1} h^{-1}.

The results demonstrate that the amount of vanadium adsorbed increases at higher initial metal ion concentrations. At higher concentrations there is a more efficient utilization of the adsorptive capacities of the peat, due to the greater driving force across a higher concentration gradient.

4. CONCLUSIONS

Peat from Santo Amaro das Brotas, Brazil, has clear potential for removal of vanadium from aqueous solutions. The results presented here can be used to optimize pilot scale experiments on removal of the metal from aqueous by-products of the petroleum industry.

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Influence of Soybean Chelates on Chemical and Biochemical Properties of Humic Substances from Biotransformed Lignite

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Keywords: chelate, humic, fungi

1. INTRODUCTION

The role of fungi in the degradation of complex carbon compounds such as starch, cellulose, pectin, lignin, lignocelluloses, inulin, xylan, araban, etc., is well known. *Trichoderma ssp.* are known to possess the complete set of enzymes required to breakdown cellulose to glucose. Fungi are good in accumulation of heavy metals such as cadmium, copper, mercury, lead and zinc. Certain low rank coals are partially solubilized by fungi (1,2). Early research showed that lignin degrading fungi have the ability to solubilize these coals and a substantial amount of the research interest is focused on the role of lignin degrading enzymes in this process. Although some of the early researchers suggested that extracellular oxidases produced by lignin degrading fungi have a role in coal solubilization, it is now generally accepted that the main mechanism by which such coals are solubilized involves chelation of metals in these coals by oxalate ion (and possibly by other metal chelators). Chelation breaks the ionic linkages between coal macromolecules (*i.e.,* coal-derived humic acids and other materials) which are relatively polar and soluble in water once these linkages are broken (3)

The aim of this study is to determine the influence of soybean chelates on some chemical and biochemical properties of biotransformed lignite.

2. MATERIALS AND METHODS

Procedure for lignite treatment:

The soybean chelates were prepared as water solutions with a final content of elements shown in Table 1. Lignite was treated with this 20% solution at a weight proportion of -lignite:chelate solution = 100:5. Inoculation of *Trichoderma viride* was done with suspension of conidia 5,2x10^{7}cells/ml - 10 ml /0,75 kg lignite, which was the only carbon sourse for fungal development. Cultivation was done at 60% humidity of lignite in a thermostat at a temperature of 33° C the first 3 days and 40°C until the end (14th day). Magic Green is a commercial product of ROMB Ltd – an enzyme accelerator .

The samples were dried at 48°C before analyses. The organic carbon composition was measured according to the method of Kononova-Belchikova (1966). Polyphenoloxidase and peroxidase enzyme activities of the samples were also measured.

Table 1. Chelate forms used

Chelate	Content of elements in chelate solution,%	pH
Fe	0,60	2,75
Mn	0,96	5,3
Zn	1,19	4,6
Cu	0,96	4,9
Co	0,60	4,9
Ca	0,96	10,7
Mg	0,60	9,59
Magic Green 5ml/0,75 kg lignite		
Composition 1-8 + Magic Green	0.84	5.6

3. RESULTS AND DISCUSSION

The data presented in Table 2 show that the humification is pH dependent with a slight influence of acidity (Ch r = 91,5136 + 0,585984*pH; r=0.508; Cf r = exp(2,88597 - 0,269016*pH; r=-0.580). Ca and Mg chelates relatively inhibit *T.viride* and the formation of total humic substances is low, but they are mainly in the form of humic acids form. Lignite (brown coal) can be liquefied/solubilized with several fungi by different mechanisms. When applied industrially, only catalytic mechanisms can compete with chemical methods. The well-known fungal ligninolytic peroxidases show disadvantages such as they are relatively expensive and hydrogen peroxide must be used as a cofactor (2). The catalytic function of Cu- and Zn chelates influence more strongly coal biotransformation.

Table 2. Organic carbon composition of biotransformed lignite

Treatment	Total organic C,%	Total Ch&Cf,%	Ch %	Cf %	E4:E6
Control – without chelates	39,97	24,95^a 62,42b	23,37^a 93,67^c	1,58^a 6,33^c	7,27
1	39,06	24,92 63,80	24,28 97,43	0,64 2,57	7,03
2	39,11	24,33 62,21	23,04 94,70	1,29 5,30	7,03
3	39,49	24,45 61,91	22,11 90,43	2,34 9,57	6,74
4	39,54	24,18 61,15	22,94 94,87	1,17 8,61	7,05
5	39,86	23,47 58,89	21,45 91,39	2,02 8,61	7,08
6	39,73	23,57 59,33	22,87 97,03	0,70 2,97	6,58
7	39,83	23,55 59,13	23,50 99,79	0,05 0,21	6,48
8	38,55	24,08 62,46	23,06 95,76	1,02 4,24	6,98
9	38,14	22,70 59,67	22,45 98,90	0,25 1,10	6,72

a-mass of sample; , b- total organic C; C- organic C of Ch+Cf; Ch- organic C of humic acids; Cf- organic C of fulvic acids

Table 3. Polyphenoloxidase and Peroxidase activity of the samples
(mg PPG/100g /30`)

No	Polyphenoloxidase	Peroxidase
Control	7,7	1,8
1	+5,73	-
2	+3,46	+0,6
3	+2,57	+0,17
4	+4,23	+3,0
5	+4,37	+4,97
6	+5,57	+2,1
7	+4,83	+3,93
8	+28,25	+40,75
9	+31,75	+10,25

The polyphenoloxidase and peroxidase activity of the formed humic substances show that Co and Ca increased twice the peroxidase activity, whereas the catalitic function of Ca, Mg, Fe chelates increased by 50% the polyphenoloxidase activity.

Cf inhibited the enzyme action and Ch favoured the polyphenoloxidase:

a) Influence of Cf on the polyphenoloxidase activity in chelate treated lignite

PPHA = exp(1,75785 - 0,263684*Cf t);r= -0,75195;

b) Influence of Ch on the peroxidase activity in chelate treated lignite

POA = exp(72,6148 - 3,22921*Ch t); r = -0,632509

Magic Green is shown to be a suitable activator of the measured enzymes. However, the full chelate composition slightly depresses the peroxidase. The level of condensation of humic substanses is better in these samples. The relative content of Cf increased in the presense of Magic Green.

4. CONCLUSIONS

The chelates of Fe, Mn, Zn, Cu, Co, Ca, Mg favour the biotransformation of lignite and increase the measured enzyme activity. Magic Green sharply activates the enzymes.

ACKNOWLEDGEMENTS

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In Vitro Effect of Potassium Humate on Terminal Drought Tolerant Bread Wheat

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Keywords: wheat, coleoptile, potassium humate, PEG 6000

1. INTRODUCTION

Water is the important limiting factor for crop production in arid and semi-arid areas of the world. If sufficient water is not available, the use of fertilizers and high yielding crop varieties are useless. Drought stress, in general, reduces nutrient uptake by roots and transport from roots to shoots, because of restricted transpiration rates and impaired active transport and membrane permeability resulting from reduced root-absorbing power. Coleoptile length (CL) is of great importance when considering variable seeding depth, soil surface temperature and moisture, which affect the coleoptile development, and in turn seedling emergence and crop stand establishment. Coleoptile length is a highly heritable character and could be efficiently used in programs designed to segregate generations at early stages of plant development (3). The emergence capability of winter wheat was also associated with coleoptile length and stand establishment (7). Water soluble Polyethylene glycols (PEGs) have been widely used as inert, nonionic solutes in the study of water relations for plants, fungi, and animals (5). PEGs are used extensively for the experimental control of water stress in plants growing in nutrient solutions. It is frequently assumed that plant water relations are similar whether the plants are growing in soil or in a PEG' solution having an equal water potential (4). Potassium humate is an active hormone of natural origin produced from the remains of plants and animals (2). Potassium humate increases crop quality and plant tolerance to drought, salinity, cold, diseases and pests stresses (2 and 6). Treatment of tomato seeds with 0.01% Potassium Humate solution before planting for 24 hours increased crop production 20-25%. Cucumber seed treated with 0.01% Potassium Humate solution before planting for 24 hours and increased its production 38%. Bostan et al. (1) investigated time and percentage of germination of hazel-nut seed in three concentrations of Potassium Humate (0.01, 0.02 and 0.03 ml/seed) and for two treatment periods (12 and 24 hours). Germination increased between 37.08 and 64.14%

with the highest germination percentage (53%) observed in a concentration of 0.02 relative to control (43%).

In this study, we investigated effect of Potassium Humate solution on early growth of wheat genotypes under PEG osmotic pressure as a drought factor.

Evaluation of important characters in early growth stages is necessary for selected wheats under terminal drought of farm experiment in our area (Ardabil, Iran). Furthermore, uses of Potassium Humate can help ours to know about its effect on decrease of drought intensity in the most important stage of growth. So we tried to study about response of wheat genotypes to this miraculous natural hormone.

2. MATERIALS AND METHODS

42 bread wheat genotypes were planted under two farm conditions, irrigated and terminal drought stress, using a simple rectangular lattice with two replications in 2006-2007. Seven genotypes were selected as tolerant to drought stresses (see results). To evaluate germination, coleoptile length of these genotypes to that of a sensitive genotype, the experiment was designed by factorial analysis on the basis of completely randomized block in vitro. PEG 6000 was used to create osmotic pressure, and double distilled water was the control. The A factor had three levels (-7 bar PEG; double distilled water; and 1ml/L K-humate + -7 bar PEG). The B factor was wheat genotypes. Ten seeds were cultured in each Petri dish. Papers and Petri dishes were sterilized in an oven at 120 degrees centigrade for 1.5 hours, and seeds in 20% Sodium hypochlorite for 5 minutes. After sterilization, seeds were washed with double distilled water, and then cultured between papers in Petri dishes. The temperature of laboratory was about 24 degrees centigrade during of experiment. Petri dishes were maintained in a dark place for 10 days. Data gathered included number of seeds germinated and measured coleoptile lengths. The total, maximum and mean coleoptile lengths were calculated.

3. RESULTS AND DISCUSSION

Analysis of variance showed significant differences between osmotic pressures for coleoptile length and germinated seed number, between genotypes for mean and maximum coleoptile length, and between interactions of genotypes × osmotic pressures for mean and maximum coleoptile length. Mean comparisons showed the highest total, mean and maximum coleoptile length in -7 bar PEG and 1ml/l Potassium Humate treatments (Table 1). Genotypes Sardari and Sabalan had the highest amounts of total, mean and maximum coleoptile length (Table 2). Examined closely were Interactions between genotype × osmotic pressures, genotypes Sardari, Sabalan and 4057 in -7 bar PEG and 1ml/lit Potassium Humate. In this study, potassium Humate increased the drought tolerance of genotypes. These results were in accordance with the findings of similar investigations (1). Genotypes that had the highest grain yield under normal and terminal drought stress conditions were grouped as tolerant genotypes using tolerance indices. The tolerant genotypes produced the largest total, mean and maximum coleoptile length. Cultivars emerging rapidly are valuable because rainfall after sowing can result in a soil crust that prevents the wheat coleoptile or first leaf to emerge. Additionally, early emerging crops can maximize on water utilization leading to improved stand establishment and grain yield. Variations in coleoptile length are primarily genetic; although, this trait is significantly affected by genotype and environmental interactions (3).

4. CONCLUSIONS

Genotypes had different responses to Potassium Humate treatment. Potassium Humate (1 ml/l) caused to superior tolerance of Sardari, Sabalan and 4057 genotypes than others against drought (-7 bar PEG 6000) in early growth stages.

Table1. Mean and Maximum of Coleoptile and primary roots length in different conditions

Conditions	Mean of Coleoptile Length	Maximum of Coleoptile Length (mm)	Maximum Length of Primary roots	Mean Length of Primary roots
control	4.01	4.51	17.20	13.83
Stress + K-humate	4.25	4.85	17.73	12.01
sterss	3.95	4.73	16.35	11.21

Table2. Mean and Maximum of Coleoptile and primary roots length in different cultivars

Cultivars	Mean of Coleoptile Length	Maximum of Coleoptile Length (mm)	Maximum Length of Primary roots	Mean Length of Primary roots
Mv 17/Zrn	4.28	5.18	21.60	12.91
Viking/5/Gds/4/Anza	4.30	5.01	13.85	11.33
Apollo/Alvd/4/Spn..	2.86	3.58	13.92	10.48
Gaskogen	3.11	3.72	12.88	10.89
Sardari	4.62	5.41	19.25	14.79
Sabalan	4.93	5.56	20.13	15.33
4057	4.76	5.23	16.11	12.69
5041	3.20	3.89	19.02	10.38

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The Effect of Bioaugmentation on the PCP Degradation in Soil Amended with Humic Acids

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Keywords: biodegradation, humic acids, pentachlorophenol, lignite, oxyhumolite, soil

1. INTRODUCTION

Binding xenobiotics to humus constitutes one of the major reactions by which anthropogenic compounds are transformed in nature (1, 2). Elimination of contaminants through the sorption onto soil particles leads to reducing their mobility, thus their toxicity as the contaminants become resistant in relation to the present microflora. They are firmly fixed in the soil structure, i.e., they are immobilized, what may be a cause of a difficult access to degraded molecules. Humic acids (HAs) appear to have a particular activity in interaction with various organic and inorganic contaminants affecting their sorption, mobility, bioavailability, degradation, and phytotoxicity (3-5). Peat, lignite, and oxidized brown coal, so-called oxyhumolite, are the richest sources of HAs (up to 85%) (6, 7). The positive effect of HAs on microorganisms during biodegradation experiments of organic xenobiotics has already been investigated. It was claimed that the HAs are sorbed onto the bacterial surface very quickly and easily. It was hypothesized that this layer protects the cell against unfavourable environment conditions and so it enables the microflora to grow and to degrade the xenobiotics gradually (8).

The aim of this study is a simulation of real conditions at pentachlorophenol (PCP) degradation in soil using HAs addition as a possible alternative decontamination method. Lignite and oxyhumolite were used as the representative sources of humic acids as they both are of a natural origin.

2. MATERIALS AND METHODS

Microorganisms

A bacterial strain *Comamonas testosteroni* CCM 7350 is an isolate from wastewater treatment sludge plant. It was identified and is stored at the CCM in Brno, Czech Republic.

Characteristics of soil, lignite, and oxyhumolite

Soil samples (Gleyic Fluvisol, Calcaro-haplic Chernozem, and Arenic Regosol) were collected from topsoils of key monitoring sites of the Soil Monitoring System Slovakia. They represent common soil types with a wide range of fundamental soil attributes. Lignite and oxyhumolite were obtained from Czech Republic.

Biodegradation and analysis of PCP

Biodegradation of PCP in soil in the presence of natural soil microflora (NSM) or an inoculum of *C. testosteroni* (5 g/kg) was performed under laboratory conditions in the characterized soil samples with addition of lignite or oxyhumolite. The samples were artificially contaminated with PCP, 10 and 100 mg/kg. After degradation, steam distillation of PCP was carried out in soil samples after alkaline hydrolysis with NaOH. Samples were extracted twice with 15 mL *n*-hexane. The solvent was completely evaporated on rotation vacuum evaporator at 40-45°C and PCP was diluted in methanol and analyzed by HPLC.

3. RESULTS AND DISCUSSION

Natural soil attenuation varies according to soil types. The amount and quality of soil organic matter, mainly HAs, play a key role in the elimination of soil contaminants (9, 10). Sorption ability of HAs isolated from different soil types differs due to their different chemical structure. HAs represent only a small part of arable soil compared with other soil parts. Lignite and oxyhumolite were used because they have several times higher HAs amount than agricultural soils. The main characteristics of used soils, lignite, and oxyhumolite are shown in table 1.

Table 1. Characteristics of tested soils (c_{org}) and data of CHN and NMR analyses of HAs (C, H, N, O, COOH, C_{alif}, C_{ar}, α) isolated from the tested soils, lignite, and oxyhumolite

HA	C_{org} (%)	Elemental analyses (atom. %)				COOH (meq/g HA)	C_{alif} (%)	C_{ar} (%)	α (%)
		C	H	N	O				
Calcaro-haplic Chernozem	1.16	47.8	29.5	3.4	19.5	4.2	33.7	42.2	55.6
Gleyic Fluvisol	1.43	39.9	40.7	3.3	16.1	2.5	46.9	32.8	41.1
Regosol	0.75	42.6	37.3	3.5	16.7	3.6	41.8	38.6	48.0
Lignite	27.5	51.1	28.8	1.1	19.0	4.5	34.7	44.0	55.9
Oxyhumolite	22.2	41.2	37.6	0.7	20.5	4.8	29.7	48.4	61.9

In Fluvisol without HAs addition, higher degradation values (up to 63%) were observed in samples with NSM than in those with *C. testosteroni*. On the other hand, in soils amended with lignite or oxyhumolite, higher degradation was determined in samples with the *C. testosteroni* at both initial PCP concentrations. The highest value of degradation in Fluvisol (91%) was recorded at the concentration of 10 mg/kg PCP in the sample with oxyhumolite addition (Fig. 1). The addition of the bacterial strain *C. testosteroni* appeared to have positive effects on the PCP degradation in Chernozem in most samples in comparison with the NSM; the highest value (71 %) was determined in the sample with lignite addition at 100 mg/kg PCP (Fig. 1). The degradation values in Regosol differed slightly. Generally, in samples containing NSM, higher degradation was reached in samples without HAs addition. Lignite and oxyhumolite positively effected the degradation of PCP in soil where the bacterial strain *C. testosteroni* was present; up to 57% of the initial PCP concentration was degraded (Fig. 1).

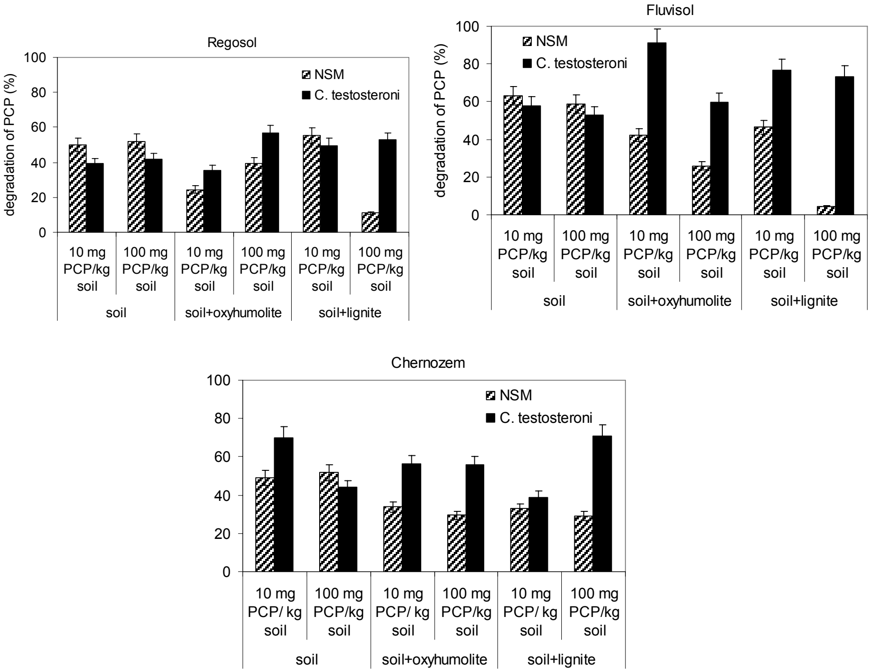

Figure 1. The comparison of PCP biodegradation with natural soil microflora (NSM) and *C. testosteroni* (the initial PCP concentrations 10 and 100 mg/kg) in the real soil samples with and without addition of lignite or oxyhumolite after 21 days of cultivation.

4. CONCLUSIONS

Obtained results of PCP biodegradation indicate that this process depends on the soil type as well as on the organic carbon content. Comparing the addition of lignite or oxyhumolite, it can be assumed that in case of oxyhumolite, immobilization and degradation are more influenced, depend on the soil type and on the initial concentration of PCP more than in experiments with addition of lignite. Biostimulation with lignite and oxyhumolite proves that they had positive effects on biodegradation, mainly when the bacterial strain *Comamonas testosteroni* CCM 7350 was present. Considering the achieved results, in following experiments we will focus on the bioaugmentation of natural soil microflora with allochthonous bacterial strain able to degrade chlorophenols in order to reinforce indigenous microbial consortium and consequently to improve biodegradation.

ACKNOWLEDGEMENTS

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Application of White Rot Fungi for the Removal of Natural Organic Matter

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Keywords: white rot fungi (WRF), natural organic matter (NOM), bioremediation, extracellular enzymes

1. INTRODUCTION

Natural organic matter (NOM), a heterogeneous mixture of organic carbon compounds, affects drinking water quality and causes problems in water treatment processes. Conventional water treatments for NOM removal often generate NOM-containing sludges which can be problematic regarding disposal. There is interest in the application of ‘natural’ biological systems for water and wastewater treatment, and the white rot fungi (WRF) have potential for such processes. The WRF possess lignin-degrading enzymes which have been successfully utilised in the bioremediation of a wide range of recalcitrant compounds and xenobiotics. The oxidative nature and low substrate specificity of these enzymes makes these fungi candidates for the treatment of aquatic concentrated NOM. Although the degradation of humic substances by saprotrophic fungi has been well documented, there appear to be no reports in the literature on the degradation of concentrated aquatic NOM without the addition of nutrients. This paper describes the potential of a newly isolated basidiomycetous fungus *Trametes sp*. from the Dandenong Ranges, Victoria, Australia for the treatment of an aquatic NOM concentrate under nutrient limited conditions. The biodegradability of the treated NOM was then assessed with a consortium of bacteria.

2. MATERIALS AND METHODS

A highly coloured aquatic NOM concentrate obtained from the regeneration of the magnetic anionic exchange resin, (MIEX^{TM}), from Hope Valley Reservoir, South Australia, was used. *Trametes* sp. was isolated from fruiting bodies on dead tree stumps in the Dandenong Ranges, Victoria, Australia, by continuously sub-culturing on malt extract agar, until a pure fungal isolate was obtained. Shake-flask cultures containing 200 mL of concentrated NOM (25-500 mg/L) in sterile tap water were inoculated with 10 g wet weight of pre-grown fungal pellets and incubated at 30°C and 100 rpm for 10 days. Experiments were performed in quadruplicate with appropriate controls and sampled (7 mL) every 10 to 12 hours. All samples were filtered and analysed for pH, decolourisation (colour measured as absorbance at 446 nm, A_{446}), presence of conjugated and aromatic bonds (A_{254}), and ligninolytic enzyme production at the pH of the culture at the time of sampling by the methods of Tien and Kirk for Lignin peroxidase (LiP) (1), Wariishi *et al*. for Manganese-dependent peroxidase (MnP) (2) and Coll *et al*. for Laccase (Lac) (3). High Performance Size Exclusion Chromatography (HPSEC) was used to determine the changes in molecular weight distribution of NOM. The biodegradability of the treated NOM was assessed by change in dissolved organic carbon concentration after bacterial degradation according to Volk *et al*. (4).

3. RESULTS AND DISCUSSION

Trametes sp. decolourised NOM as represented by the reduction in A_{446} (Figure 1). Maximal decolourisation (50%) occurred at 100 mg C/L. For all NOM concentrations, there was a high initial rate of NOM removal which could be due to initial adsorption or uptake of NOM. This was later confirmed by light brown colouration of the pellets, which darkened with increasing NOM concentration. 'Negative decolourisation' occurred shortly after reaching maximal decolourisation for all NOM concentrations (Figure 1). This could be due to fungal disintegration and the release of secondary extracellular proteases on lysis of the cells (5), as indicated by increase in culture pH for all NOM concentrations.

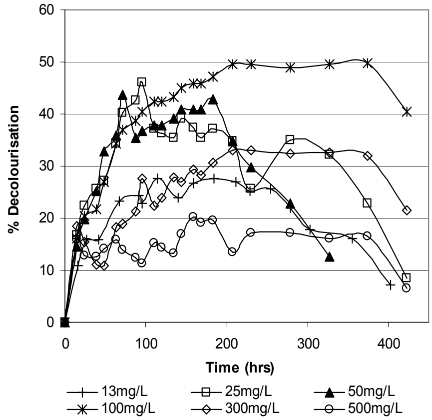

Figure 1. Decolourisation of NOM by *Trametes* sp.

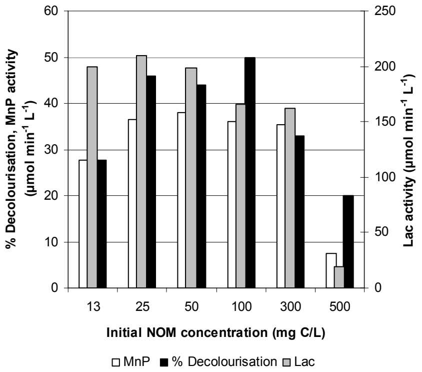

Figure 2. Enzyme activity during NOM decolourisation by *Trametes* sp.

For pH there was a general trend of rapid decrease from initial pH of 7.5 to 4.5 after which it gradually increased to approximately pH 7 coincident with 'negative decolourisation'.

NOM decolourisation coincided with the onset of Lac and MnP activity; no LiP activity was detected. The activity of Lac was greater, suggesting its predominant role in the breakdown of NOM. At greater than 25 mg C/L, the activity of Lac decreased with NOM concentration, but the MnP activity was consistently high at 25-300 mg C/L (Figure 2). These trends suggest enzyme inhibition at NOM concentrations greater than 50-100 mg C/L. This finding is in accordance with Dehorter and Blondeau (6), who found increasing production of MnP and LiP with increasing concentration of humic acid.

Fungal treatment led to increased A_{254} and DOC, the increases being greater for lower NOM concentrations. The biodegradability of the treated NOM sample was examined using the BDOC method as a surrogate biological treatment. This treatment led to reductions in A_{446}, A_{254} and DOC. The changes in the molecular weight distribution of the organic matter after fungal, and fungal plus bacterial, treatment are shown in Figure 3 and Figure 4.

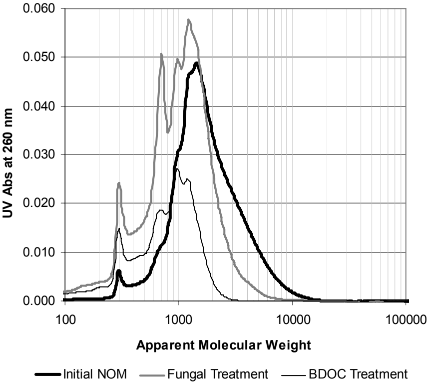

Figure 3. HPSEC of NOM (25 mg C/L) after treatment (detection at 260 nm)

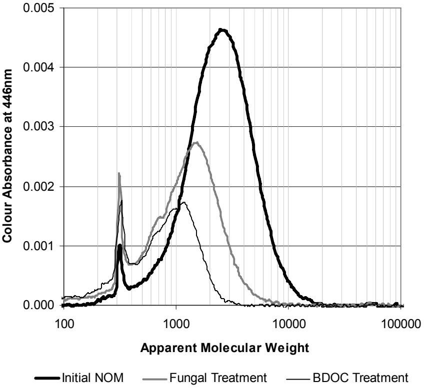

Figure 4. HPSEC of NOM (25 mg C/L) after treatment (detection at 446 nm)

Fungal degradation of NOM resulted in the decrease of higher molecular weight moieties (1,700-10,000 Da) and increase in lower molecular weight moieties (100-1,700 Da) at both 260 nm (Figure 3) and 446 nm (Figure 4). Bacterial treatment led to further reductions in the average molecular weight and removal of some of the lower molecular weight fungal products.

The ability of *Trametes* sp. to degrade NOM was probably due to the synergistic action of MnP and Lac involved in the enzymatic breakdown of medium molecular weight (500-2,000 Dalton) fulvic acids and high molecular weight (2,000-5,000 Dalton) humic acids.

4. CONCLUSIONS

This research demonstrates the potential for the use of white rot fungi, as exemplified by *Trametes* sp., as a source of ligninolytic enzymes for the decolourisation and degradation of concentrated NOM wastes arising from potable water treatment without nutrient addition, thus making these wastes more amenable to conventional biological treatment.

ACKNOWLEDGEMENTS

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Synthesis and Use of Iron Humates for Correction of Iron Deficiency Chlorosis in Higher Plants

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Keywords: humic, iron humate, chlorosis, bioavailability

1. INTRODUCTION

Deficiency of bioavailable iron in soils leads to plant disease known as chlorosis. The most efficient way to correct iron chlorosis is to use synthetic iron (III) chelates such as DTPA and EDDHA. However, application of these chelates may bring about negative consequences for environment as their migration along the soil profile. Therefore, development of iron chelates based on a use of natural macroligands is of great importance. From this prospective, humic substances (HS), which contain large amounts of carboxyl and hydroxyl groups seem to be the most promising compounds. The objective of this study was to develop humic-based iron chelates and to evaluate their efficiency for use as chlorosis correctors.

2. MATERIALS AND METHODS

Iron Humates: Three samples of iron humates were prepared from commercially available potassium humate derived from leonardite (Sakhalin Humate^{TM}) and iron(II) sulfate at different pH in the presence and absence of ascorbic acid. The insoluble part of potassium humate was separated with centrifugation. Iron(II) sulfate with concentration 725 mmol/l was added to 5% solution of potassium humate at pH 11 and 9. The solution of ascorbic acid (2%) was used for stabilization of Fe^{2+} oxidation state. The obtained solution was dried on rotary evaporator. Iron content was determined spectrophotometricaly using complexation with o-phenantroline after oxidative wet digestion. For determination of redox state of iron species, the obtained samples were investigated using Mössbauer spectrometry.

Bioassay: Wheat plants (*Triticum aestivum* L. var. Krestianka) were used as biotarget and Knopp nutrient solution without iron was used as a control. Wheat seeds were germinated in distilled water in the dark at 25°C for 72 hours. Seedlings were grown on

Knopp nutrient solution with iron supplied in the different forms at the concentration of 25 µmol/l. The commercially available chelate Sequestren Fe-EDDHA was used as a positive control. Length of shoots and roots, mass of roots, iron and chlorophyll content in the shoots were measured. Chlorophyll was extracted from shoots with acetone. The contents of a and b chlorophyll were determined spectrophotometricaly. Iron content was determined after oxidative digestion of dried shoots using o-phenantroline technique.

3. RESULTS AND DISCUSSION

The content of iron in all three preparations was 8.8±0.3%, solubility of samples synthesized at pH 9 in the presence of ascorbic acid (AA) was 127±4 g/l, in the absence of AA it was 52±4 g/l. The sample obtained at pH 11 turned out insoluble. Mössbauer spectra of samples synthesized at pH 9 have shown that in the absence of AA, all iron present in iron humate was Fe(III), whereas in the sample obtained in the presence of ascorbic acid, Fe(III) accounted for 94% and Fe(II) - 6%.

The results of bioassay have shown that the both samples of iron humates obtained at pH 9 caused stimulating effect on wheat seeds: the root weight for sample with AA was 112 ± 4 % of control and for sample without AA it was 109±4% of control. At the same time, for Fe-EDDHA the value of this parameter was 106±4% of control. Both Fe-EDDHA and all soluble iron humates promoted accumulation of chlorophyll in shoots. Another indicator of the efficiency of the sample as chlorosis corrector is iron content in shoots. The highest iron content was obtained for iron humate synthesized at pH 9 with AA (243±9 mg/kg that corresponds to 430% of control) and lesser content - for sample without AA (170±7 mg/kg that corresponds to 250% of blank). Of importance is that both iron humates outcompeted Fe-EDDHA: the iron content accounted for 117±6 that corresponds to 173% of blank.

4. CONCLUSIONS

The soluble iron humates with content of iron >8% were prepared. Application of ascorbic acid increased solubility of iron humate and facilitated iron accumulation by plants. The obtained iron humates were shown to be efficient chlorosis correctors.

ACKNOWLEDGEMENTS

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Use of Soil Microorganisms for Producing Standard Samples of Humic Acids

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Keywords: humic acids, standard samples, purification, soil microorganisms

INTRODUCTION

The high solubility of humic substances in alkali aqueous solutions is the basis for their extraction from the soils and other sources such as peat, brown coal, sludges etc. Precipitation (evidence of low solubility) in acid media is commonly used for additional purification of humic substances. The humic substances are a mixture of organic compounds of different chemical classes. The composition of this mixture depends on the natural source from which they are extracted. The standards for humic substances extracted from different sources were proposed by IHSS. These standards in particular comprise data regarding the contents of various aminoacids, sugars and ash content, as characteristics of such mixtures. The composition of humic substances includes various compounds with unknown structure. For instance, the possibility that humic acids engage in pH dependent reversible polymerization and depolymerization (1) was discovered by us and has not been described earlier with regards other classes of organic compounds. Unfortunately, even IHSS does not provide sufficiently definitive descriptions of differences between terms like “humic substances” and “humic acids”. We believe that the term “humic substances” includes all organic substances extracted by alkali water treatment of soil samples and other natural sources. For the purposes of organizing commercial production and application of humic substances, it is necessary to determine the chemical structure of humic acids as the main component of humic substances, which predetermine their role in soil-formation. The first stage to achieving this is the separation of humic acids from the aggregate mass of humic substances. As we have described earlier, humic acids cannot be quickly consumed by soil microorganisms as carbon and energy source. It may be suggested that soil microorganisms have a specific mechanism for the “recognition” of these chemical structures. The “recognition” apparently takes place at neutral pH when humic acids have a form of specific polymer, since in alkali pH the polymer structure is destroyed and microorganisms can use humic acids as a carbon source (2). It is, therefore, possible to use soil microorganisms for the purpose of isolating humic acids from natural sources. Here we suggest a method for isolating humic acids as a fraction of humic substances resistant to transformation by soil microorganisms. Other organics compounds included in humic substances are transformed by the microbial cultures to CO_2 and H_2O (2).

RESULTS AND DISCUSSION

Our investigations demonstrate that samples of humic acids from different environmental sources contain 20% of compounds degraded by soil microorganisms in two weeks and which cannot qualify as humic acids. The comparative study of humic substances and humic acids isolated by way of microbial treatment has shown that humic acids (resistant to microbial transformation) are responsible for intensive absorption of light in visible part of spectra (dark colored) and the formation of water-insoluble complexes with heavy metals. At the same time, in the tests for growth-regulating activity (typical for humic substances), including stimulation of grains germination and stimulations of plants growth and development, humic acids isolated by microbiological method appeared to be inactive. This means that growth-regulating activity of humic substances depends on the part of such substances which is utilized by soil microorganisms.

CONCLUSIONS

Humic substances contain near 80% of humic acids resistant to microbial transformation, which ensure their accumulation in soils. Having such specific features, these compounds have a key role in pedogenesis. We believe that the term “humic acids” should, therefore, comprise this specific group of compounds, standard samples of which can be obtained with help of soil microorganisms.

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Metabolic and Immune-Modulating Properties of Commercial Humic Preparations Produced from Peat

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Keywords: humic preparations, poultry farming, resistance, productivity, mechanism of action

1. INTRODUCTION

Molecular mechanisms of biological activity of humic substances (HS) are at present intensively studied. Peat humic products possess a wide spectrum of biological activity and are widely applied in husbandry and poultry sector. They impact immunity of animals by improving their adaptation to inside and outside environment.

2. MATERIALS AND METHODS

Experiments were carried on chicken-broilers (n = 10000) during five years using different methods of physiology and biochemistry in poultry farms of Dnipropetrovsk region (Ukraine). Commercial humic preparations produced from peat such as Huminate, Hydrohumate, and Oxyhumate were used in the different experiments.

3. RESULTS AND DISCUSSION

The data obtained show that humic substances (HS) stimulate productivity and increase resistance of chickens to different pathogens. In numerous trials conducted under the conditions of poultry farms it has been demonstrated that addition of Huminate as well as of Hydrohumate and Oxyhumate to the basal diet of the fast-growing chicks promoted the protein synthesis and resulted in increased daily gain of the body mass. Changes in amino acid composition were observed both in chick blood serum and in femoral and pectoral muscular tissues. Changes in the content of different metabolites in chicken tissues are shown in Table 1.

The increased contents of G, M, and A immunoglobulins were found using monospecific (in respect to the certain immunoglobulins) antibodies. The level of G-immunoglobulins, which are predominant in chickens, has changed much more as compared to M- and A- immunoglobulins. Of particular importance is that the observed increase in the total content of immunoglobulins characteristic of immunological status of an organism did not exceed the physiological norm.

Table 1. Influence of humic substances on metabolism and immunology status in chicken tissues

Observed changes in liver	Changes in the blood serum	Changes in the pectoral and femoral muscles
↑ cAMP	↑ total protein	Increased formation of bioproduction
↑ proteolysis	↑ fraction of protein as well as lipoprotein, gaptoglobin and immunoglobulins	↑ free amino acids' pool ↑ the content of aminoacids in protein hydrolysate
↑ activity of cathepsin B	↑ IgG, IgM, IgA	↑ activity of cathepsin B
↑ inhibitor of cathepsin B	↑ fibronectin	↑ the content of aminoacids
stimulating the post-translation modification of protein	↓ circulating immune complex	↓ the total lipid content
	↓ free aminoacids	↓ the unbound water

4. CONCLUSIONS

The commercial humic preparations produced from peat were shown to be promising food additives for poultry of different kind and age. They act as growth promoters, immune- and stress- protectors and metabolism correctors.

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Chemical and Plant Growth Stimulatory Properties of Lignohumate

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Keywords: humate, lignohumate

1. INTRODUCTION

Commercial humates (HUM), derived from various natural sources have been reported to be effective soil conditioners and materials for remediation of degraded and polluted soils (1). Today the market of HUM is being actively developed. They are produced by manufactures from various kinds of raw materials and differ considerably in their composition, activity and documented cost. Currently, sufficient data do not exist concerning HUM properties or the efficiency of production or processing as it relates to source raw material and technologies used in production. Furthermore, differences in chemical structure and properties between natural and industrial humates have received limited study. In this paper the theoretical summary of probable mechanisms, due to which HUM display their positive effect on soil and plants are presented. The properties of Lignohumate, a HUM of the new generation, produced by oxidative-hydrolytic destruction of lighosulfonates, are evaluated in comparison with other brands of HUM.

2. MATERIALS AND METHODS

Properties of several grades of Lignohumate are evaluated in comparison with other brands of HUM and natural humic substances. Chemical properties and physiological activity of Lignohumate and a number of other HUM are analyzed, using a standard HA as a reference sample. Plant growth stimulating effect was evaluated in bioassay lab tests.

3. RESULTS AND DISCUSSION

Laboratory studies showed primary chemical parameters of Lignohumates (e.g., elemental content, functional groups, molecular weight, IR- and fluorescence spectra) were more similar to soil humic acids, than HUM produced from peat and lignite. Plant growth stimulating effects were evaluated using bioassay lab tests. From these tests Lignohumate demonstrated biological effects over a wide range of concentrations, with inhibition observed at higher levels as typical for humic substances. Results of field experiments with Lignohumate application are also discussed. Physical and chemical parameters of different Lignohumate samples are shown in the table.

Table 1. Characteristics of different grades of Lignohumate

Parameter	Grades of Lignohumate			
	AM sodium	AM potassium	BM sodium	BM potassium
Appearance	Dark brown scaly powder with a weak special odor		Dark brown suspension with a weak special odor	
Dry matter, % not exceed	-		19	
pH$_{6\%}$	8,5-10			
Organic matter, % from dry matter	58-64			
K, % from dry matter, not less	1	9	1	9
Na, % from dry matter, not less	9	Not regulated	9	Not regulated
Humic acids, % from OM	60-85			
Acid-soluble fraction, % from OM	15-40			
S, % from dry matter, not less	3			
Optical density of 0,02 % solution D$_{440}$	0,250-0,570			
Microelements, % from dry matter:				
Iron	0,01-02			
Manganese	0,01-0,12			
Copper	0,01-0,12			
Zinc	0,01-0,12			
Molybdenum	0,005-0,015			
Boron	0,01-0,15			
Cobalt	0,01-0,12			

4. CONCLUSIONS

According to the chemical data set, Lignohumate can be produced as a Na or K humate; however, due to its lignin-based origin, it possesses a number of special properties, different from peat or coal humates. Lignohumates contain sulfur (more than 3%), a lignin-derived carbon backbone, a low-molecular acid-soluble fraction and humic acid. This composition may serve as a source of plant pigments and hormone-like substances that may in turn give Lignohumate a competitive advantage over other HUM.

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New Express Technique for Determination of Antioxidant Activity of Humic Substances

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1. INTRODUCTION

Interest of analytical chemists to humic substances (HS) is instantly growing. First of all, it is connected to the current trend in agriculture directed toward application of organic and organic-mineral fertilizers and necessity in the corresponding quality control techniques. Another reason is that humic substances bind toxicants and cause disturbing impact on their determination in air, water, and soil. This research is devoted to a use of amperometric technique for determination of humic and fulvic acid. These natural compounds contain phenolic units that makes feasible amperometric detection.

2. MATERIALS AND METHODS

Standards. Quercetin (pure for analysis) was purchased from Fluka. Humic materials: three IHSS standard samples of humic materials from Suwannee River were used including fulvic acid (SR-FA), humic acid (SR-HA), and dissolved organic matter (SR-DOM). Commercial humate was potassium humate (PowHumus) produced from leonardite by Humintech Ltd (Germany). Peat sample was obtained from Likhoslavl (Russia) and extracted with distilled water using volume ratio of 1:5.

Amperometric analysis. Amperometric detection (AD) is based on measuring electrical current produced by oxidation of analyzed compound on the surface of working electrode upon applying the certain electrode potential. Working electrode used was made of glassy carbon, which is the most universal electrode for determination of polyphenolic compounds. Electrode potential varied from 0 to 2 V. The signal was registered in the form of differential curves. The obtained data were processed using the specially designed software which enables calculation of peak areas, heights, and calibrations.

AD has a whole number of advantages for determination of antioxidants. First of all, this is express method: excluding sample preparation, time of one analysis takes only few minutes. Analysis (registration and data treatment) occurs during real time. Reproducibility of analysis is high due to precise injection using six-channel valve. Minimum detectable concentration of polyphenols using AD technique lays in the range of nano- and picograms (1.10^{-9}-1.10^{-12} g). The principal scheme of the device is given in Figure 1.

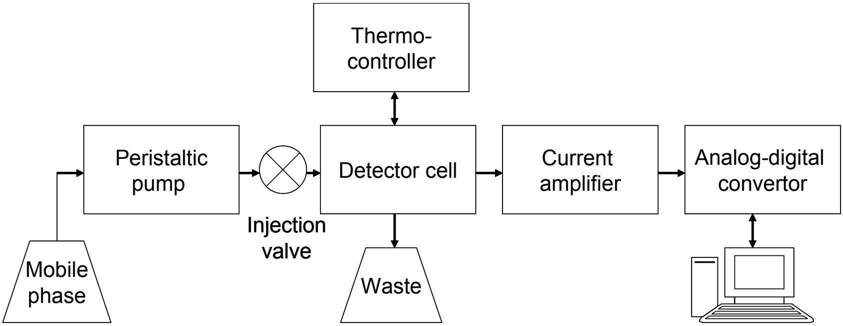

Figure 1. The block-scheme of the device Tswet-Yausa-01-AA.

The device represents a flow-injection system which enables express determination of antioaxidant activity of HS. No other reagents except for standard are needed for this analysis. Quercetin (Fluka) is usually used as a standard fir this determination.

RESULTS AND DISCUSSIONS

The results of antioxidant activity measurements are shown in Table 1. The samples of standard and commercially produced humic substances were used as well as water extract from peat.

Table 1. Antioxidant activity of humic materials and peat extract

Humic materials	Antioxidant activity (by quercetin), mg/g
SR-HA	15.4
SR-DOM	14.6
SR-FA	13.4
Potassium humate (PowHumus, Germany)	5.96
Water extract from Likhoslavl peat	0.50

The proposed method can be also used for express-determination of antioxidant activity of water extracts from soil, peat, sapropel, etc. The quantitative determination is feasible after calibration of the device using standard solutions of quercetin.

4. CONCLUSIONS

The amperometric technique is promising approach for automatic analysis of HS. The device “Tswet Yausa -01-AA” in included into the State List of measuring devices #20706-05, and the measuring protocol of HS is certified (# 31-07 from 04.05.2007).

The Metal Ions Sorption by the Humic-Containing Anion Exchangers

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Keywords: exhausted ion exchange resins, NOM, metal sorption

1. INTRODUCTION

As a result of exploitation of ion exchange resins in processes of water demineralization a gradual deterioration of their structural, physical and chemical, and the operational characteristics is occur. These changes are caused mainly due to accumulation of natural organic matter (NOM) in a phase of the sorbents (1). Such ion exchangers are unusable for the further operation in processes of water demineralization and represent industrial wastes.

Our preliminary researches have shown that ion exchangers exhausted in water demineralization processes in most cases not only keep quite satisfactory level of operational and capacitive characteristics, but also acquire a number of new positive properties which can be used in other areas of water treatment (2).

The purpose of the present work was the research of physical and chemical properties of exhausted ion exchangers, and also the estimation of a presence of acquired properties allowing to develop on their basis new multifunctional sorbents for purification of water with various composition from undesirable impurity of metals. Such properties are the ability to the contact coagulation and complexing with ions of metals. For an estimation of these ones the comparative research of sorption of iron II and copper by fresh and exhausted ion exchangers was carried out.

2. MATERIALS AND METHODS

Researches were spent on the samples of anion exchange resins with different term of operation in processes of industrial water treatment and also on the same ones fresh.

Information about investigated ion exchangers and also the content of NOM accumulated in exhausted sorbents are presented in the Table 1.

NOM content in exhausted ion exchangers was determined as permanganate oxidability of a filtrate decanted after treating of ion exchanger by a solution containing 2% NaOH + 10% NaCl at 45^{o}C during the 24 hours under the thermostatic conditions.

Table 1. Information about investigated ion exchangers

Ion exchange resin	Type	Matrix	Cross-linking, %	Functional group	Bead size distribution	Term of usage, years	NOM content in exhausted exchanger, $mgO_2 \cdot mL^{-1}$
1	SBAE type I	SDVB gel	2	Trimethyl amine	Poly-disperse	7	7.0
2	SBAE type I	SDVB gel	8	Trimethyl amine	Poly-disperse	10	12.5
3	SBAE type II	SDVB gel	8	Dimethyl–ethanol amine	Mono-disperse	3	2.7
4	SBAE	SDVB gel microporous	-	Quaternary amine	Poly-disperse	1	2.9
5	SBAE	SDVB macroporous	-	Quaternary amine	Poly-disperse	1	1.8
6	WBAE	SDVB macroporous	-	Tertiary amine	Poly-disperse	3	10.1
7	WBAE	SDVB macroporous	-	Tertiary amine	Poly-disperse	2	6.0
8	WBAE	PEPA condensing	-	Secondary and tertiary amine	Poly-disperse	7	3.3
9	WBAE	EPA condensing	-	Secondary and tertiary amine	Poly-disperse	8	4.2
10	WBAE	PA gel	-	Tertiary amine	Poly-disperse	2	2.6

SBAE – strong base anion exchanger, WBAE – weak base anion exchanger, SDVB –sterene-divinylbenzene, PEPA - polyethylen-polyamine, EPA– epoxy-amine, PA – polyacrylic

For comparison of sorption characteristics of fresh and exhausted ion exchangers the some series of dynamic experiments were conducted. 1000 specific volumes of model solutions I or II were passed through each glass column filled by above-indicated ion exchangers accordingly. Flow rate of a model solutions passing through sorbents were 20 BV per hour.

Model solutions were prepared on tap water, preliminary dechlorinated and purified from natural organic matter, and had following composition on macrocomponents:

I – solution containing Cu^{2+} - 6.1 mg L^{-1}; Na^{+} - 11 mg L^{-1}; K^{+} - 2.9 mg L^{-1}; Ca^{2+} - 73.6 mg L^{-1}; Mg^{2+} - 10.8 mg L^{-1}; HCO_3^{-} - 173.2 mg L^{-1}; Cl^{-} - 63.6 mg L^{-1}; $SO4^{2-}$ - 50 mg L^{-1}; pH - 6.7.

II – solution containing Fe^{2+} - 6.0 mg L^{-1}; Na^{+} - 11 mg L^{-1}; K^{+} - 2.5 mg L^{-1}; Ca^{2+} - 63.6 mg L^{-1}; Mg^{2+} - 11.5 mg L^{-1}; HCO_3^{-} - 202.5 mg L^{-1}; Cl^{-} - 12.3 mg L^{-1}; SO_4^{2-} - 65,7 mg L^{-1}; pH - 7.2.

3. RESULTS AND DISCUSSION

Researches have shown that decrease in total exchange capacity of ion exchangers correlates with a level of NOM accumulation in exhausted resins (Table 1, 2) and depends on matrix nature, basicity, porous structure of anion exchangers.

Table 2. The change of sorption properties of exhausted resins in comparison to initial ones*

Ion exchange resin	Specific NOM content, mg O_2 per functional group	Change of total exchange capacity, %	Change of specific sorption capacity regarding to metals, % **	
			Cu	Fe (total)
1	5.55	- 7.5	+ 84.0	+ 3.2
2	10.72	- 14.5	+ 107.4	+ 36.8
3	2.08	- 2.7	+14.8	+ 11.0
4	2.38	-1.6	Not fixed	+ 57.0
5	1.53	-1.7	Not fixed	+ 26.6
6	5.16	- 15.3	+ 111.8	+ 8.7
7	3.51	-8.2	+ 70.3	+ 11.2
8	1.26	- 8.1	+ 8.6	+ 25.2
9	1.87	- 18.3	+ 22.1	+ 24.4
10	1.46	-2.2	- 0.1	+ 0.1

* Initial and exhausted ion exchange resins were treated by 5 BV of NaCl.

** Specific volume of model solution passed through the ion exchangers was 1000 BV

Maximal decreasing of total exchange capacity for resins with NOM accumulation is noted for anion exchangers with condensing polyamine matrix. Weak base and gel strong base SDVB anion exchangers are subject to a "poisoning" by NOM to a lesser degree and the minimal decreasing of capacity at NOM accumulation was observed for polyacrylic weak base anion exchangers and strong base porous on the basis of SDVB.

The comparison of data about change of the specific sorption capacity in relation to copper (Table 2) has shown that copper extraction by exhausted strong and weak base SDVB ion exchangers, and also by condensing polyamine anion exchangers increases in comparison with fresh ones, and that increasing correlates with growth of NOM-content in a sorbent. At the same time, copper extraction by exhausted polyacrylic anion exchangers practically didn't change in comparison with fresh ones.

The increasing of iron II extraction by exhausted gel strong base and porous weak base SDVB anion exchangers, and also by condensing polyamine anion exchangers (Table 2) wasn't so considerably as in a case of copper. At the same time for exhausted SDVB anion exchangers with micro- and macroporous structure the substantial increasing of iron extraction in comparison with the samples of fresh ones was marked. The sorption of iron by exhausted polyacrylic anion exchanger practically didn't change.

4. CONCLUSIONS

Investigations have shown that anion exchangers "poisoned" by natural organic matter during water demineralization processes lose somewhat capacitive properties, but increase ability to complexing with ions of transition metals and acquire properties of contact coagulants. The acquired properties are most obviously shown in a case of exhausted anion exchangers on the SDVB-base, to a lesser degree – in a case of polycondensing polyamine anion exchangers and practically were absent for polyacrylic resins. An ability to contact coagulation is most obviously shown in a case of strong base porous anion exchangers on the basis of SDVB.

On the basis of exhausted anion exchangers exposed to certain treatment the multifunctional sorbents suitable for high level of water purification from ions of heavy and nonferrous metals can be developed.

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Humic Acids of Pelosilt (Sapropel) as Prospective Biogenic Stimulators for Medicine and Cosmetology Use

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Carried out since the 1970s, the extensive researches of composition of the fresh-water lakes sediments, i.e. sapropels, (with the content of organic matter (OM) up to 70-90%) have shown the sapropels to be perspective raw materials for the extraction of biogenic stimulators of various compositions (2, 3).

The majority of biogenic stimulators (1) produced by the medical industry using organic solvents are known to be the extracts of organic components from the seas, estuaries, and salt lakes silts, poor in organic matter (2-5%). The medical biogenic stimulators like FIBS, peloidodistillate, and Torfotum are the distillates of biogenic amines, simple phenolic structures, and organic acids volatilizing with steam. The content of lipid and humic fractions in silts makes only shares of a percent while the sapropels from the lake Sudobl contain 8-9% of lipids and 10-30% of humic substances depending on the degree of biochemical decomposition and transformation of the initial biomass in different layers of the deposit (4, 20).

The comparative analysis of the chemical nature of HA of sapropels and HA of the salt lakes, estuaries, and seas silts shows that they have the same structure being natural specific new formations generated from the initial biomass of reservoirs, mainly from zoo- and phytoplankton, algae and living organisms under oxygen deficit (6). The only difference is that HA of sapropels being generated in fresh-water reservoirs (with water mineralization up to 1 g/l) unlike HA generated in salt-water reservoirs don’t form so strong organomineral complexes with metals. It allows preserving the stable solutions of HA of sapropels in ampoules for more than 10 years.

Combination of chemical and instrumental research methods has shown (5) that humic acids (HA) of sapropels differ from HA of soils, peats and coals. Humic acids of sapropels basically consist of hydrolysable polypeptide-carbohydrate complex (16 aminoacids and 5 carbohydrates) in a combination of fatty compounds with low content of simple aromatic units. Condensed aromatic structures such as those present in HA of soils and coals are not found in HA of sapropels. This fact is proved by benz(a)pyrene testing (the more condensed, the more carcinogenic the product is).

Humic acids of sapropels are the mixture of acid substances formed as a result of biochemical transformation of the initial biomass of the reservoir. They represent a group of amorphous polycarboxylic acids with various content of polyfunctional groups (amine, amide, carbonyl, carboxyl, phenolic hydroxyl, etc.) soluble mainly in the alkaline solutions and precipitated when the solution is acidified by mineral acids (down to pH 1) due to the suppression of weak organic acids dissociation. Upon drying, HA make amorphous (or pseudocrystalline) dark-brown or black powder. Because of high content of aliphatic structures, the HA molecules are hyperhydrated and when dissolved, keep a lot of bound water (up to 14-20 g/l). As a result, the system is transformed into paste when only 3-4% of HA are condensed. If the concentration of HA increases, the solution stability needs the medium reaction (pH) to be more than 3.0 for 1% solution, 6.0 for 2% solution, and 7.0 for 3% solution. Decreasing pH for the solutions of the above mentioned concentrations transforms the solutions into the colloidal systems (7).

It has been proved that humic acids of sapropels have the properties of biogenic stimulators (3 mg/kg); viz. they stimulate the macrophage defense reaction, promote nervous tissue regeneration, stimulate tissue reparation, and produce anti-inflammatory effect (8) in case of tissue burns and cornea diseases. They stimulate mitochondrial respiration (9, 10) and inhibit free radicals. By their radioprotective properties various fractions of HA of sapropels show the evident radioprotective effect (11). HA molecules are polydispersed (molecular mass is 1000-100000). Certain part of them penetrate the human skin by diffusion and electrophoresis (12, 13).

The results of clinical trials of a preparation made from the sapropels from the lake Sudobl with 1% of HA content have proved that humic acids block the excess of hyaluronidase resulting in improvement of joints' covering surfaces right up to the full recovery.

Application of the sapropel therapeutic muds with increased content of HA in the solution (14) or taking baths with a water-soluble extract (where HA is the main reactant) helps patients with skin diseases (psoriasis, lichen planus, atopic dermatitis, seborrhea, acne vulgaris). The improvement (itch and desquamation disappear) can be observed beginning from the 6th procedure (15). Applications of sapropel HA solutions in treatment of patients with catarrhal gingivitis and mild periodontitis showed better results than Propolis (1% solution) therapy (17).

Applications of the solutions of HA of sapropels to rabbits cause the changes in all skin layers. In the samples of skin with hypodermic tissue the following changes can be observed: loosening and thickening of the stratum corneum with increased desquamation;

moderate cells enlargement in the stratum granulosum; the stratum spinosum is characterized by focal proliferation, reduced nuclei basophilia, and appearance of small vacuoles in the cytoplasm; in the dermis, there is an increased number of dilated blood vessels of the deep net including microcapillaries, hair bulbs are enlarged, and sebaceous glands have signs of proliferation. All these signs are evidence of intensification of proliferation processes and functional activity of the skin (14, 16).

The aim of our research was to get an effective biogenic stimulator for tissue therapy which would excel its analogs both in efficiency and in a period of storage (up to 10 years) with stable chemical structure and biological activity.

We have licenses for the exploitation of the ecologically clean deposit of the therapeutic sapropel muds from the lake Sudobl (Minsk region) which is unique in Europe (18). We also have licenses from the Public Health Ministry of Belarus for manufacturing of products for medical and cosmetic use. We have patented (19, 20) the methods of products preparation from the lake deposits, as well as the processes and equipment for excavation of the raw material from the deposit (21). Having a research laboratory of our own we have formulated and introduced new preparations on the basis of HA that are widely used in spa institutions in Belarus and abroad. Nowadays, HA, both separate and combined with other bio-active substances extracted from sapropel muds, are extensively used in manufacturing of cosmetic products of new generation having proved their effectiveness.

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