The Italian Chapter of the International Humic Substances Society (IHSS)

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Royal Palace of Portici
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Molecular Aspects of Humic Substances and Biological Functionality in Soil Ecosystems

Book of Abstracts

NMR CERMANU

FACULTY OF AGRICULTURE

SICA

SCIENTIFIC PROGRAM

MONDAY DECEMBER 5

12.00-13.30
WELCOME SPEECHES AND NOTES ON THE HISTORY OF THE INTERNATIONAL HUMIC SUBSTANCES SOCIETY-IHSS
Prof. Paolo Masi, Dean of the Agricultural Faculty, Portici.
Prof. Teodoro Miano, President of the Italian Chapter of the IHSS, University of Bari, Italy
Prof. Alessandro Piccolo, Chairman of the Organizing Committee

13.30-15.00
Buffet Lunch

HS CHEMISTRY AND STRUCTURE

Chairpersons: Dr. Riccardo Spaccini, Dr. Claudio Zaccone

INVITED LECTURE
15.00-15.40
Molecular characterization of humic substances in the environment by pyrolysis-GC-MS
José Gonzalez-Perez, CSIC, Seville, Spain.

VOLUNTARY TALKS
15.40-16.00
Modifications of carboxylic functions in a humic acid by a Click Chemistry reaction: preliminary results and potential applications
Antonio Nebbioso, University of Naples Federico II, Italy.

16.00-16.20
Spin labeling as a technique to investifate the molecular scale environment of soil humic substances interacting with chemical contamiants.
Olga Aleksandrova, Ural Federal University, Ekaterinburg, Russia.

16.20-16.40
Major and trace element concentrations in peat humic acid and their complex forming properties.
Diana Dudare, University of Latvia, Riga, Latvia

16.40-17.10
Nature of ferrihydrite-humic substances (Fe-HS) coprecipitate: scanning and transmissions electron microscopy (SEM, TEM) and dynamic light scattering (DLS) analysis.
Claudio Colombo, University of Molise, Campobasso, Italy.

17.10-18.00
POSTER SESSION

TUESDAY DECEMBER 6

HS AND MICROBES

Chairpersons: Edoardo Puglisi, Maria Rita Arenella

INVITED LECTURE

9.00-9.40
Humus genesis reviewed. Microbial biomass as a significant source for soil organic matter formation
Matthias Kästner, UFZ Department Umweltbiotechnologie, Leipzig, Germany

VOLUNTARY TALKS

9.40-10.00
Changes in soil microbial community structure in outdoor mesocosm experiment using three different grasslands in UK
Luisa Massaccesi, Università di Perugia, Italy

10.00-10.20
NMR application to study the interactions between humic substances and alkaline phosphatase and related effects on enzymatic activity
Pierluigi Mazzei, University of Naples Federico II, Italy.

10.20-11.00
Coffee Break

11.00-11.20
Oxidative polymerization of a humic acid by heterogeneous biomimetic catalysis
Assunta Nuzzo, University of Naples Federico II. Italy

11.20-11.40
Spectroscopic and chemical comparison between digestate obtained by domestic organic wastes and sewage sledge under mesophilic and thermophilic conditions.
Maria Rosaria Provenzano, University of Bari, Italy

11.40-12.00
Phytodecontamination of freshwater and NOM-enriched water from the endocrine disruptors bis-phenol A, 17a-ethynilestradiol and linuron
Eliana Gattullo, University of Bari, Italy

12.00.12.20
Soil Organic Matter and silvicultural regime in beech forests in Trentino
Paolo Carletti, University of Padova, Italy.

12.20-12.40
Effects of use of compost at different humification stages on root colonization by arbuscular mycorrhizal fungi in maize plants
Vincenza Cozzolino, University of Naples Federico II, Italy

12.40- 15.00
Buffet Lunch and **POSTER SESSION**

HS AND PLANTS

Chairpersons: Luigi Sciubba, Vincenza Cozzolino

KEY-NOTE LECTURE

15.00-15.30
Is the humic substances structure responsible for different and controversial biological activity?
Adele Muscolo, Università Mediterranea di Reggio Calabria, Italy

VOLUNTARY TALKS

15.30-15.50
Effects of two lignosulfonate-humates on *Zea mays L.* metabolism
Andrea Ertani, Università di Padova, Italy

15.50-16.10
Agroindustrial residues and their biological activity on maize (*Zea mays L.*) metabolism
Diego Pizzeghello, University of Padova, Italy

16.10-16.30
Physical-Chemical characterization of Lignin isolated from three different plant biomasses by oxidative alkaline extraction.
Davide Savy, University of Naples Federico II, Italy

16-30-17.00
Water extractable organic matter, stability, and maturity relationships in biosolids from urban sewage sludges
Luigi Sciubba, University of Bologna, Italy.

17.00-17.30
Coffee Break

17.30-18.30
GENERAL ASSEMBLY OF THE ITALIAN CHAPTER OF THE IHSS

20.30
GALA-DINNER
Miglio d'Oro Park Hotel

WEDNESDAY DECEMBER 7

HS AND ENVIRONMENTAL PROTECTION

Chairpersons: Pierluigi Mazzei, Eliana Gattullo

9.00-9.30
KEY-NOTE LECTURE
Influence of humic substances on plant-microbes interaction in the rhyzosphere
Edoardo Puglisi, Università Cattolica di Piacenza, Italy

VOLUNTARY TALKS

9.30-9.50
Evolution of the peat organic material following smouldering wildfires and possible implications in paleoenvironmental reconstructions
Claudio Zaccone, University of Foggia, Italy

9.50-10.10
Thermal analysis associated to Fourier Self-Deconvolution of infrared spectra applied to the study of anaerobic digestion of municipal solid waste and sewage sludge.
Anna Daniela Malerba, University of Bari, Italy

10.10-10.30
Co-polymerization of 2,4-dichlorophenol in humic substances under oxidative biomimetic catalysis
Barbara Fontaine, University of Naples Federico II. Italy

10.30-11.00
Coffee Break

11.00-11.20
Effects of an exogenous humic acid and oxidative biomimetic catalyst on the remediation of a highly polluted soil
Filomena Sannino, University of Naples Federico II. Italy

11.20-11.40
TPH and PAH removal from a contaminated soil in solid fermentation by a microbial mixture (a mixed culture, humic substances and inorganic nutrients)
Cesar Garcia-Diaz, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico

11.40-12.00
Binding of phenol and differently halogenated phenols to dissolved humic matter as measured by NMR spectroscopy
Riccardo Spaccini, University of Naples Federico II. Italy

12.00-12.20
Cr(VI) reduction capability of humic acid extracted from organic fraction of municipal solid waste
Barbara Scaglia, University of Milano, Italy

12.20-13.00
CLOSING REMARKS

INVITED LECTURE

Molecular characterization of humic substances in the environment by pyrolysis GC-MS

José A. González-Pérez

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Pyrolysis (Py) coupled to gas chromatography (GC) and mass spectrometry (MS) is a powerful tool that is widely appliaed in the characterization of organic mixtures of diverse origin like humified organic material. Py–GC/MS is mainly used for a direct study of materials which, owing to their complexity, are difficult to analyse by conventional methods. Pyrolysis of natural organic matter generates a wide range of products with diverse chemical properties that can be related to their biochemical origin (aliphatic compounds and methoxyphenols derived from lignin, cyclic ketones and furans from polysaccharides, N-containing molecules from proteins, organic acids, etc.). In this communication, a number of examples of the use of Py-GC/MS will be described and will include: a) maturity assessment during composting processes, b) monitoring the effect of fire on soil organic matter affected by wildfires, c) the characterization of humates (modified humic acids) with agricultural use and d) the characterization of humic extracts (Humic, fyulvic and humins) in soils.

The results of a Double-Shot Py-GC/MS analysis used to investigate the molecular features of a HA that enters in solution in aqueous media (pH 8.5) at different extraction times will be also described. Products of total desorption (280º C) and pyrolysis (500º C) were obtained in the original HA (Fr0) and six other fractions (Fr1-Fr6) obtained after re-precipitation of the dissolved HA at pH 1.5. A semi-quantitative assessment of the different precursors that contributed to the HA fractions was achieved.

Modifications of carboxylic functions in a humic acid by a Click Chemistry reaction: preliminary results and potential applications

Antonio Nebbioso and Alessandro Piccolo

Centro di Ricerca Interdipartimentale sulla Risonanza Magnetica Nucleare (NMR) per
l'Ambiente, l'Agro-Alimentare ed i Nuovi Materiali (CERMANU)
Università di Napoli Federico IIVia Università 10. 80055 Portici

The fate of biomacromolecules after cellular death is natural decay. This long term process is believed to play a pivotal role in formation and assembly of Natural Organic Matter or Humic Substances. Conversely, soil chemical modification induced by human activity needs to happen in considerably shorter time scales. Moreover, harsh conditions of most chemical reactions are incompatible with the environment. Nevertheless, proper soil modification would greatly contribute for carbon dioxide reduction in observation of the Kyoto protocol, and the efforts of researchers in this field are accordingly increasing. A viable approach could be found in Click Chemistry. In fact, for a reaction to comply with Click approach it is required: i. high yields (90-95%); ii. favourable kinetics; iii. mild reaction conditions; iv. absence of toxic byproducts; v. cost efficiency of reagents and vi. compatibility with water solutions. In an attempt to modify soil humic substances with a "Click Chemistry" approach, an alkynyl alcohol was esterified with carboxyl goups of an organosoluble fraction from a peat humic acid through Fischer ester formation. The alkyne adduct was then conjugated with a hydrophilic azido amine via 1,3 dipolar cycloaddition, the main Click Chemistry reaction. The result was a hybrid humic molecule with improved hydrophilicity and positive ionizability. Such adducts were characterized via HPSEC hyphenated to Electrospray-Orbitrap Mass Spectrometry. Several formulae were found attributable to the "Clicked" hybrid products from short (C_8) and long chained ($C_{>20}$) carboxylic acids, albeit with low yields. It is odd that humic molecules conjugates of average length (C_{16-18}) were not observed. Tandem mass spectrometry was also used for structural clarification and to rule out amine-ester transposition. We showed that Click chemistry is applicable for cheap, easy, and flexible modification of humic substances and may represent an advanced supramolecular engineering for soil humic matter. A potential application in this direction is currently in development: the formation of a hybrid between humic molecules and anionic binding site. Such engineered Bio-effector could be used *in situ* to modulate release of nitrate, sulphate and other anionic nutrients.

Spin Labeling as a Technique to Investigate the Molecular Scale Environment of Soil Humic Substances Interacting with Chemical Contaminants

Olga N. Aleksandrova; Ural Federal University, Ekaterinburg, Russia

The ability of electron paramagnetic resonance (EPR) spectroscopy to monitor and elucidate directly the different molecular-scale environments of paramagnetic spin probes has been demonstrated in model soils and soil samples taken from the environment. The hyperfine spectrum of nitroxide spin label (SL) molecules originates from the unpaired electron density at the nitrogen atom (Figure 1). It results in the characteristic three-line hyperfine structure of the SL spectrum with the splitting constant a_0. The value a_0 decreases with solvent polarity declines to low values in hydrophobic solvents. Since a_0 is a polarity indicative of the environment, in which SL is situated, this parameter could be used to probe hydrophobic environments of soil molecules. Investigating the problem of an influence of humic substances (HS) on the irreversible binding of organic chemical pollutants in soils revealed the splitting and following shift of the left and right spectral lines of the paramagnetic probe 4-Hydroxy Tempo that was incubated with the soil during some days (Figure 1).

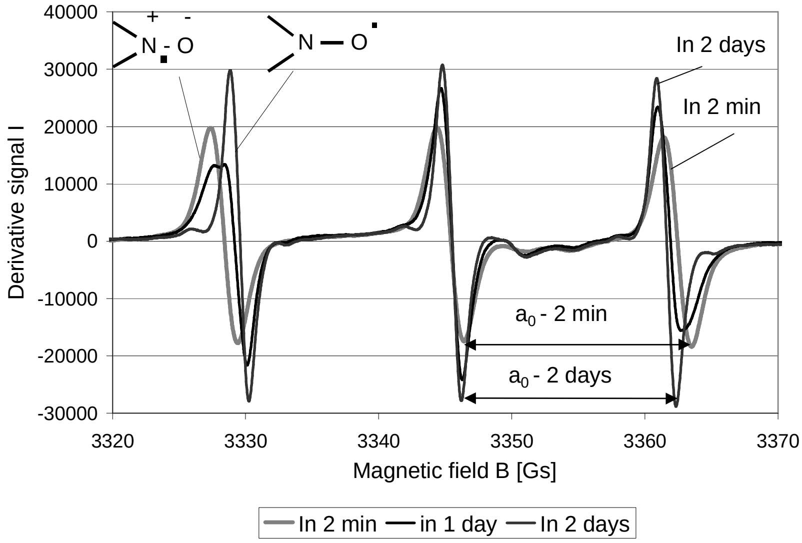

Figure 1 – EPR spectra of SL 4-Hydroxy Tempo incubated with soil during 2 days

As shown in Figure 1 after 1 day of the incubation with soil, molecules of SL with hydroxyl functionality are already partitioned in the sites of different polarity and in 2 days, almost all SL molecules are situated in the environment with the less polarity whereas a shift of spectral lines of SL Tempo with other functionalities was not observed during the same incubation with soil. At the same time, the SL spectral lines were not broadened. However, the g - value of soil organic radicals slightly decreased. These results allowed for hypothesizing the plasma-chemical processes that took place between soil components under the influence of SL hydroxyl functionality and was accompanied with an electron transfer to quinoid substructure of HS. In turn, it resulted in a shift of the thermodynamic balance between quinones, semiquinones and quinone radicals of HS and an increased ability of quinones to form the cycloproducts.

Quinone; semiquinone; spin labeling; molecular scale environment

Major and trace element concentrations in peat humic acids and their complex forming properties

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Humic substances (HS) are a general category of naturally occurring, biogenic, heterogeneous organic substances that can be characterized as yellow to black in colour, of high molecular weight, and refractory. It has been hypothesized, that the main factor affecting metal accumulation in peat profile are humic substances. Peat ability to accumulate major and trace elements depends on the character of element supply (whether in particulate or ionic form), potency of metal ions to bind functionalities in the peat structure, pH, oxygen presence, presence of complexing compounds, inorganic ions and many other factors. Major and trace element presence in peat are of importance as an indicator of peat genesis and organic matter humification processes and for industrial use of peat. Trace element accumulation in peat profiles has been studied to reconstruct changes of human pollution and track down sources and characterize intensity of anthropogenic pollution. HS form most of the organic component of peat and they play a major role in the biogeochemical cycling of many trace elements due to significant complex forming ability. The character of the complex formation between humic acids and major and trace elements is an object of intensive studies during last decades. The aim of this study was to assign major and trace element distribution between peat and peat humic acids from two well characterized raised bog profiles and analyse factors affecting element concentrations in peat humic acids, as well to determine the Cu (II) and Pb (II) complexing capacity and complex stability constants with humic acids in respect to their properties and humification characteristics of studied peat and their humic acids.

In our study the concentrations of 17 elements - Fe, Pb, As, Cu, Ca, Mn, Cr, Ni, K, Zn, Ti, Se, V, Sr, Co, Rb, Br was determined in humic acids of Dzelve and Eipurs Bogs. Our study indicates the impact of human-induced pollution on element concentrations in the upper layers of the studied bogs, both in humic substances and in peat. Trace element concentrations in peat and in peat humic acids depend not only on human-induced pollution, furthermore, for several toxic trace elements (for example, As, Pb and others), natural processes are of key importance. An increased concentrations of many elements (As, Fe, Ni, Cu, Pb, Cr) have been found in the deeper layers of the bog, and their source is the natural weathering of bedrock and supply with groundwater. The total concentration levels of major and trace elements in the studied peat and humic acid samples can be considered as relatively low.

Complex forming properties of isolated humic acids were studied as well. Found complex (Cu^{2+} - humic acid) stability constants (logK) are within a range of stability constants found for humic substances isolated from other sources (soil, coal, water, logK=3.94 – 5.15), and similar to complex stability constants found for peat humic acids (logK=5.17 – 5.29). The values of the obtained stability constants were ranging from 4.06 to 5.73. Stability constants significantly changes within studied bog profiles are well correlated with age and decomposition degree of peat layer from which humic acids are isolated.

Keywords: humic substances, peat, trace and major elements, complexing capacity, stability constant

Nature of ferrihydrite-humic substance (Fe-HS) coprecipitates: scanning and transmission electron microscopy (SEM, TEM) and dynamic light scattering (DLS) analysis

Ruggero Angelico[1] , Andrea Ceglie[1], Ji-Zheng He[2], Giuseppe Palumbo[3], Claudio Colombo[3]

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Key Words: Fe-Humic complexes; Dynamic light scattering (DLS); ferrihydrite.

Humic substances (HS) may act to mobilize Fe from insoluble Fe minerals. HS are negatively charged polyelectrolytes of varying molecular weight and can strongly react with surfaces of poorly ordered iron oxides. Coprecipitation between HS and iron oxides in soil was demonstrated by the presence of poorly ordered Fe minerals that was explained by the site distortion of the octahedral Fe, possibly due to partial removal of interacting organic molecules. To compare the chemical interaction of HS with iron minerals during coprecipitation, we prepared ferrihydrite-HS associations by coprecipitation using a leonardite. The reaction products were studied by transmission (TEM), scanning electron microscopy (SEM) and dynamic light scattering (DLS) analyses. DLS data suggest that the particle distribution of Fe-HS complexes were characterized by the prevalent presence (62 %) of very large particles (micron size range of 255 nm with a size distribution standard deviation of 272 nm) and by a 38 % of particles with 85 nm of size (with a size distribution standard deviation of 14 nm). Two particle morphologies are evident in the TEMs and SEMs observation (Fig. 1-8). The TEM images showed an irregular subangular macroparticles with colloidal structure, with rough or irregular surfaces rounded by very small rounded particles. Most of the rounded and angular particles could be small ferrihydrite aggregate that are also observed as discrete particle association. The smooth surfaces of the rounded and angular particles suggest that they are poorly crystalline Fe phases. The SEM images of the ferrihydrite-HS show the presence of indented particles with similar shapes and dimensions, recalling the original structure of the Fe-HS observed with TEM covered by nanoparticles of ferrihydrite. The structure of Fe-HS complexes show a woven network of two class of particles into a micelles structure, which can include nanopartcile of iron phases into the cavity of the HS as an intermediate characteristics from ferrihydrite-coprecipitates products and iron-humic substances. This is important not only for the knowledge of factors affecting Fe availability in soils but also to characterize the particle structure and the possible HS interaction in the coprecipitated Fe-HS complex.

INVITED LECTURE

Humus genesis reviewed – microbial biomass as a significant source for soil organic matter (and non-extractable residue, NER) formation

M. Kästner

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Carbon from any biodegradable organic compound in soil partitions into parent compound, metabolites, non-extractable residues (NER), CO_2, and microbial biomass. This distribution must be known to assess the fate of the compound in soil. For example, NER from pesticides are considered to consist of adsorbed and sequestered parent compounds or metabolites and thus as hazardous residues. However, they may also partly derive from bacterial biomass, resulting in harmless biogenic residues. In addition, the formation of soil organic matter (SOM) or humic compounds has long been a dominating topic in soil science because the amount and composition of SOM determines soil quality but the processes are still not yet really understood. The so-called humic substances were regarded for a long time as a novel category of cross-linked organic materials. However, proper management of soil organic matter (SOM) is needed for maintaining soil fertility and for mitigation of the global increase of the atmospheric CO_2 concentration.

Microbial biomass residues could be identified as a significant source for SOM. We incubated ^{13}C-labelled bacterial cells in an agricultural soil and traced the fate of the ^{13}C label of bacterial biomass in soil by isotopic analysis. In this presentation, we summarise the mass balance data and visualise the microbial biomass and its residues by scanning electron microscopy (SEM). Our results indicate that a high percentage of the biomass-derived carbon remains in soil, mainly in the non-living part of SOM after extended incubation. The SEM micrographs only rarely show intact cells. Instead, organic patchy fragments of 200-500 nm size are abundant. These fragments are associated with all stages of cell envelope decay and fragmentation. Similar fragments develop on initially clean and sterile in situ microcosms during exposure in groundwater providing evidence for their microbial origin. Microbial cell envelope fragments thus contribute significantly to SOM formation. The results provide a simple explanation for the development of the small, nano-scale patchy organic materials observed in soil electron micrographs. They suggest that microstructures of microbial cells and of small plant debris provide the molecular architecture of SOM adsorbed to particle surfaces. This origin and macromolecular architecture of SOM is consistent with most observations on SOM, e.g. the abundance of microbial-derived biomarkers, the low C/N ratio, the water repellency and the stabilisation of microbial biomass. The specific molecular architecture determines carbon mineralisation and balances as well as the fate of pesticides and environmental contaminants. These conclusions were confirmed by studies on the biodegradation of isotope labeled 2,4-D and ibuprofen in soil which quantified the contribution of microbial residues to the NER in soil. The amount of label found in biomolecules indicated that virtually all of the NER of the compounds are derived from microbial biomass.

Changes in soil microbial community structure in outdoor mesocosm experiment using three different grasslands in UK.

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Globally, soils are the largest terrestrial carbon (C) reservoir, but there is compelling evidence that over the last few decades large amounts of C have been lost from soils of natural and agricultural ecosystems through erosion, leaching and accelerated soil respiration. In UK, the conversion of native grassland to cropland causes a rapid decline of soil organic carbon stocks, which is released into the atmosphere as CO_2. Further, also the grassland management and in particular the variety of plants that compose the grassland can affect the soil capacity of accumulate organic C and, consequently, mitigate CO_2 concentration in atmosphere. The aim of this work was to test if the enhancing of the grassland botanical diversity improves the soil C sequestration, through the altering of the C inputs to soil and the related microbial processes. To test the impact of dominant species identity, evenness and spatial distribution on ecosystem functioning, we set up an outdoor mesocosm experiment using three grassland plant species that differ significantly in their traits: Lotus corniculatus (a N-fixing forb), Anthoxanthum odoratum (a slow-growing grass), and Plantago lanceolata (a fast-growing forb). Mesocosms were arranged in a blocked randomised design with 5 replicates, giving a total of 120 experimental units. Mesocosms were set up in June 2009 at the Lancaster University Field Station, in England. Changes in rhizosphere microbial community structure were assessed by the analysis of the ester-linked phospholipid fatty-acid (PLFA) composition of the soil. Specifically, the technique was used to measure the relative abundance of active fungi and bacteria, which constitute about 90–95% of total heterotrophic metabolism in most soils. To the aim of exploring the single response of variables three-way ANOVA analysis was carried out. The three-way ANOVA results shown that there were significant differences ($p<0.05$) in the total PLFAs within the different evenness of the different dominant species between pots planted with patches or random. In particular, the amount of the total PLFAs was higher in the patches plots than in the random plots. The same differences were found for the total fungal and total bacterial. In the amount of total fungal biomass were also found significant differences ($p<0.01$) between the dominant species. This means that in the plots in which *Plantago lanceolata* was the dominant species planted in patches we had the highest amount of total fungal. The *Plantago lanceolata* is a fast-growing forb and so it needs nutrients quickly in the early stages of growing. In the competition with microbes for nutrients in soil solution, this specie appears to be an advantage. The fact that the total fungal amount was higher than total bacterial in the mesocosms, in which the *Plantago* is the dominant specie, was also in accordance with the recent evidence about the fungal seasonal activity, in a cold soils, that seems to be dominant in winter (the samples collection were made in October) while bacterial were more active in the summer. Changes in community structure will also summarize using Principal Component Analysis (PCA)

Keywords: grassland, PLFA, total fungal, total bacteria**.**

NMR applications to study the interactions between humic acids and Alkaline Phosphatase and related effects on enzymatic activity

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The phosphorous biogeochemical cycle and the bioavailability of the inorganic form of phosphate compounds for plants is strictly depending by the activity of the enzyme Alkaline Phosphatase (AP), in soil alkaline conditions. Nevertheless, when AP is introduced in soil, it interacts with a wide range of biotic and abiotic factors that can influence its catalytic activity. In this work, the interactions occurring between a model AP and ubiquitarian aggregates, such as humic acids (HS), were investigated by a composite NMR approach. The enzyme was tested with an increasing amount (0, 0.5, 1, 2 and 6 mg mL^{-1}) of two different humic acids, extracted from a volcanic soil (HV) and from Lignite (HL), respectively. The increasing resonance broadening of protonic AP spectrum was detected as a function of both HV and HL concentrations. The enzyme ^{1}H spectrum was parcelled out in 22 buckets, and both relaxation (T_1 and T_2) and correlation times (τ_c) were analysed for each bucket. As the humic amount was raised, the relaxation and correlation times decreased and increased, respectively, because of restricted translational and rotational motion of humo-enzymatic complex (AP-HS). Diffusion experiments provided further evidence of AP-HS interactions since self-diffusion constants progressively decreased when humic concentration was augmented. In addition, by both correlation times and diffusion experiments, emerged that HL exhibited a higher affinity with AP than HV. When the AP catalytic activity was tested, adopting *p*NPP as substrate, a progressive slowing down of substrate hydrolysis, as a function of reaction time, was detected as an increasing humic amount (0, 6, 8, 12 mg mL^{-1}) was added and the highest inhibition was identified when AP was treated with HL.

Keywords: Alkaline Phosphatase, Humic Acids, NMR, Relaxometry, DOSY, Enzyme Interactions.

Oxidative Polymerization Of A Humic Acid By Heterogeneous Biomimetic Catalysis

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A heterogeneous biomimetic catalyst was synthesized by immobilizing a meso-tetra(2,6-dichloro-3-sulfonatophenyl)porphyrinate of manganese(III) chloride [Mn-(TDCPPS)Cl] immobilized on kaolinite clay mineral previously functionalized with a molecular spacer, and employed to catalyze the oxidative polymerization of a lignite humic acid. The humic acid polymerization under heterogeneous biomimetic catalysis induced by either H_2O_2 oxidant or irradiation with UV light, was followed by high-performance size exclusion chromatography (HPSEC), with both spectrophotometric and refractive index (RI) detectors. A significant increase of apparent weight-average molecular weight (Mw) of the humic acid occurred with the oxidative polymerization catalyzed by the synthesized heterogeneous catalyst in a 72 hours reaction time. The enhancement in the apparent molecular mass of humic matter following the catalyzed oxidative polymerization was also confirmed by HPSEC chromatograms recorded after acetic acid addition, at pH 3.5. These findings indicated that the heterogeneous biomimetic catalysis increased the molecular mass of a humic acid by formation of intermolecular covalent bonds, thus suggesting its potential use in the ecological and environmental control of soil organic matter processes.

Key words: oxidative polymerization, heterogeneous, biomimetic catalysis, humic acid

Spectroscopic and chemical comparison between digestates obtained by domestic organic wastes and sewage sludge under mesophilic and thermophilic conditions.

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[2] Centro Interdipartimentale di Ricerca per le Scienze Ambientali (C.I.R.S.A.). Università di Bologna, Ravenna, Italia.

The conversion of organic wastes into methane and carbon dioxide by anaerobic digestion (AD) is becoming an increasingly attractive means of waste treatment and resource recovery. In addition, during AD, the waste is converted to a valuable effluent that can be used in agriculture because of its high nutrient as well as organic matter contents.

The temperature of the process has been reported as one of the most important factors in AD since the degradation rate of organic components is mainly dependent on the nature and concentration of microorganisms. Thermophilic digestion, with respect to mesophilic, has additional benefits including improved biogas production, more thorough destruction of viral and bacterial pathogens and a high degree of waste stabilization and oxidation of readily degradable organic materials.

FTIR spectroscopy and fluorescence spectroscopy in the emission, excitation and synchronous modes and excitation-emission matrix (EEM) represent useful tools in order to characterize organic matter of different nature and origin.

The aim of the present work was to compare chemical and spectroscopic characteristic of digestates (D) obtained by the organic fraction of domestic solid wastes (OFDSW) and sewage sludge (SS) under mesophilic and thermophilic conditions. The OFDSW and SS samples were collected in the municipality of Bagnacavallo (RA), in the Emilia-Romagna region, Northern Italy. Substrates were mixed in 1:1 ratio and submitted to AD. A horizontal, partly mixed, plug-flow experimental apparatus was used under mesophilic (35 °C) and thermophilic (55 °C) conditions with an initial organic loading of 3-4 Kg $VS^{\cdot}day^{-1}$ and successively of 6 Kg SV/d. In this experiment the 1:1 ratio was adopted in view of a composting stage, subsequent to anaerobic digestion, during which the addition of other biomasses would lower the relative SS percentage.

The average biogas production was 2.5 m^3/m^3*day under mesophilic conditions and 4 m^3/m^3*day under thermophilic conditions and the CH_4 percentage in the biogas was about 70%. Under thermophilic conditions, the highest reduction of VS and HA+FA was obtained and the TOC contents in digestates decreased as temperature increased.

Results of fluorescence spectra of substrates considered in this work were similar for both series whereas spectra of D samples were characterized by peaks occurring at longer wavelengths than the input substrates indicating the occurrence of molecules structurally more complex in the final products. Further, spectra of D under thermophilic conditions exhibited peaks located at longer fluorescence wavelengths whit respect to D under mesophilic conditions. This shift toward higher wavelengths could be associated to a major complexity of molecular structures of digestates obtained under more drastic conditions. SS samples presented great contents of inert materials deriving from the city sewer system which affected the final products as proved by FTIR spectra

Keywords: anaerobic digestion, thermophilic, mesophilic, fluorescence spestroscopy. FTIR

Phytodecontamination of freshwaters and NOM-enriched water from the endocrine disruptors bisphenol A, 17α-ethynilestradiol and linuron

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Endocrine disruptors (EDs) represent a heterogeneous group of organic chemicals which interfere with the hormonal system of animals and humans acting as hormone-like substances. Among EDs, bisphenol A (BPA), 17α-ethynilestradiol (EE2) and linuron (LIN) cause concern because of their wide presence and persistence in waters. BPA is employed for the production of polycarbonates and epoxy resins and it is present in food and drink packaging and many industrial goods. EE2 is a component of oral contraceptives, and LIN is one of the most used phenylurea herbicides. The removal of these compounds from aquatic systems is a priority goal in order to protect wildlife and human health. Several remediation techniques are nowadays available, among them phytoremediation is one of the most powerful and sustainable and consists in the use of plants to uptake, transform, volatilize or stabilize pollutants present in the environment. This study considered real aquatic systems, where the presence of microorganisms, natural organic matter, salt content and other contaminants may interfere with the phytoremediation process. The potential of ryegrass (*Lolium perenne*) to remove BPA, EE2 and LIN from Suwanne River NOM solution at a concentration of 20 mg L^{-1}, and from two freshwaters collected in Puglia from Sassano Lake (SL, Conversano) and Morelli River (MR, Ostuni) was tested.

Forty ryegrass seeds were germinated for 6 days on a cotton gauze overlying each of the above media in the absence or presence of a mix of BPA, EE2 and LIN at concentrations of 1, 0.1 and 1 mg L^{-1}, respectively. At the end of experiments, germination percentage, root and shoot length, and seedling fresh weight were measured in order to evaluate plant response to EDs. In addition, BPA, LIN and EE2 were extracted from each medium and concentrated by solid phase extraction (SPE). The residual product was measured by high performance liquid chromatography (HPLC) and diode array detector (DAD). Biometric data of all treatments were statistically compared to those obtained in pure bidistilled water (control) by ANOVA and LSD test.

In the absence of EDs, ryegrass germination was highly stimulated by SL (marked increase of biometric parameters), slightly promoted by NOM solution (increase of shoot length) and dramatically inhibited by MR (decrease of all biometric parameters). In the presence of the three EDs, the germination was inhibited scarcely by NOM (reduction of root length) and considerably by MR (reduction of all biometric parameters). The high carbon content and low salinity in SL and the opposite in MR could explain results. EDs concentrations did not decrease in the absence of plants, whereas significant removal of products were observed in the presence of ryegrass. Removals of BPA ranged from 62% (SL) to 43% (MR), of EE2 ranged from 73% (SL) to 26% (NOM), and of LIN resulted about 15% in all treatments. Results confirm the phyto-decontamination potential of this plant even in slight phytotoxic conditions, and encourage further insights of this technology at real scale.

Keywords: endocrine disruptor, natural organic matter, phytoremediation, freshwater

Soil organic matter and silvicultural regime in beech forests in Trentino

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Beech (*Fagus sylvatica* L.) forests characterize the landscape of many mountain areas in Italy, from the Alps down to the southern regions of the Mediterranean area. According to the National Forest Inventory (2005), the total area covered by beech in Italy corresponds to 9.4% of the country's total forest area.

Coppices still represent 46.1% of total beech forests but in the last 50 years, areas where beech coppices remain unmanaged are increasing. Forest policies have been increasingly directed to favoring the conversion of beech coppices to high forests which are considered both more productive and ecologically more functional. Thus many unmanaged coppices have been governed to obtain a conversion to high forest. Little information exists on the consequences of this management choice on soil ecosystem properties, such as soil organic matter and soil humic substances. This study consists in a quantitative and qualitative characterization of soil organic matter as a function of different management and pedoclimatic conditions in beech coppices in Trentino, Northern Italy.

Study area belongs to the SIC Dolomiti Brenta (code BioItaly IT3120009) in the Adamello-Brenta Natural Park. The study was conducted in four beech coppices belonging to the Natura 2000 sites. According to the Habitats Directive (92/43/EEC) two sites were classified as calcicolous mesic typical beech forests (9130 *Asperulo-Fagetum* beech forests) and two calcicolous xeric beech forests (9150 Medio-European limestone beech forests of the *Cephalanthero-Fagion*). For each forest type one unmanaged coppice (over the last 40 years) and one coppice stand converted to high forest by releasing a limited number of standards were selected. Four soil profiles were opened in each study site and undisturbed samples of O and A horizons were collected. Soil pH, organic carbon content, organic nitrogen content, total humic carbon content, humic and fulvic acid contents were determined in each sample. The qualitative features of humic substances were examined through gel-permeation and Diffuse Reflectance Infra Red Spectroscopy.

Results show that the two forest types respond in a different way to the conversion treatment. In mesic beech forest, humification parameters evidence lower values in treated stands with humic/fulvic acids ratio dropping from 1.01 to 0.58. In xeric beech forest, a stronger polycondensation of humic compounds was observed in the site treated for conversion, with higher humic/organic carbon ratio (0.24 vs 0.18) and more high molecular weight fraction (35.75% and 28.44% in treated and untreated sites respectively).

Overall soil organic matter turnover appears as a complex balance of factors such as atrophic activity, site characteristics and time. Conversion to high forest appears to change soil organic matter evolution in a site-specific manner. However the short time elapsed (four years) after conversion treatment doesn't allow to predict soil organic matter features at the end of the conversion process.

This work is part of the "INHUMUSnat2000 - Forme di humus-indicatori di funzionalità per i siti di Natura 2000" project, supported by "Fondo per i Progetti di Ricerca della Provincia Autonoma di Trento", n. 1587/2004.

Keywords: Soil Organic Matter; Fagus sylvatica L.; DRIFT; Coppice.

Effects of use of compost at different humification stages on root colonization by arbuscular mycorrhizal fungi in maize plants

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The arbuscular mycorrhizal fungi (AMF) are an important component of soil microbial community that can significantly affect plant growth and soil stability. In recent years it greatly increased the interest on the use of AMF as biofertilisers, in the context of sustainable agriculture. The application of compost represent an agricultural practise of ecological and environmental relevance, with the input of organic matter at elevated biochemical stability. Since soil is supplied with decomposing organic materials of various origin, mycelium of inhabiting AMF is in permanent contact with complex products of decomposition possessing considerable biological activity.

Despite the importance of this farm management practise in agroecosystems, limited information on the effect of compost inputs on AMF is available. It is essential to increase our knowledge on the management of agroecosystems in the context of sustainable agriculture as these practices can affect the viability of the AMF and the microbial community composition, hindering /favouring the presence of strains, which in turn, play an important role in plant growth, soil health and quality. This is because of certain unknown inhibitory moieties which may be present in the organic amendment that deleteriously or positively can affect the AMF's ontogenic cycle.

In this study we examined the effects of application of compost, derived from organic fraction of municipal solid waste, at increasing decomposition stages (60, 90, 120 days, named C60, C90, C120 respectively) on AMF colonization of roots in maize plants (*Zea mays* L., cv. Limagrain 3321) and plant growth, linking organic compounds generated by decomposition process with colonization of host plants.

In particular, a pot experiment was carried out using a soil characterized by silty-clay texture, alkaline pH, 1.2 g kg^{-1} organic C. The plants were grown for two months, without (control) and with addition of compost in two different doses (50 and 100 t ha^{-1}), for each stage of decomposition. All plants were fertilized according to the needs of the crop and taking into account the characteristics of the soil. Six replicates were set up for each treatment. The compost used in the experiment, has been characterized by elemental analysis (CHNS), solid-phase NMR and gas chromatography (GC-MS) after pyrolysis. For the evaluation of the percentage of root colonization, we used the method of Giovannetti and Mosse (1980).

Application of compost did not increase significantly plant growth with respect to the control, in all treatments. In contrast, there was a significant reduction of biomass production, particularly when C120 was added. Only, the addition of C90 did not affect plant growth. AMF root colonization of *Z. mays* was significantly reduced by C120 addition. NMR results did not highlight significant differences in the spectra. Other analysis are still being completed (GC-MS) to can relate phenological with biological, chemical and spectroscopic data.

KEY-NOTE LECTURE

Humic substance: relationship between structure and activity:

Is its changing structure responsible for different and controversial biological activity?

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Abstract

The complexity of humic substances and their remarkable properties have attracted and continue to attract the attention of many investigators, bringing over the years new knowledge on their structure, physicochemical and biological properties pointing to the use of these interesting natural compounds in many practical applications. The numerous investigations produced, up to now, controversial results, because of the lack of detailed knowledge on the composition of HS that makes very difficult to identify the relationships between the structure and the activity of these substances. Evidence presented here showed that the effects of HS on plant growth depends on the source, concentration and molecular weight humic fractions and mainly by different chemical compounds contained into them. Humic matter appears also to display an hormone-like activity. It is not clear if this activity is strictly linked to the chemical structure of HS or whether it depends on hormones of microbial origin entrapped into them. In any case, HS exhibit stimulatory effects on plant cell growth and development. In this review, the relationship between humic substances structure, chemical composition and physiological effects on plant growth and metabolism are examined. In particular, the responses of plants to humic substances are described, the regulatory circuits which allow plants to cope with humus are presented, and how the present findings can add new information to the humic substances world, is discussed.

Effects of two Lignosulfonate-Humates on Zea mays L. Metabolism

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Two lignosulfonate-humates (a and b), derived by an industrial process from lignin, were chemically characterized and their effects on maize plants in terms of growth, nitrogen metabolism and photosynthesis were investigated. The biological effects of lignosulfonate-humates were compared with those induced by a humic acid extracted from leonardite in order to better understand the complexity of their biological effects.

Our results displayed that both lignosulfonate-humates (a and b) had a hormone-like activity and increased root and leaf growth. Moreover, all treatments increased the content of chlorophyll, glucose and fructose and enhanced the activity of Rubisco, glutamine-synthetase and glutamate-synthase. The observed intense biological activity indicated that lignosulfonate-humates and leonardite humic acid had a positive role on photosynthetic process of maize plants. In the light of these results, lignosulfonate-humates may be used as biostimulants to increase crop yield.

Keywords: Lignosulfonate-humate; leonardite; biological activity; *Zea mays*; nitrogen metabolism; Rubisco enzyme.

Agro-industrial residues and their biological activity on maize (*Zea mays* L.) metabolism

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One way to counteract the increasing amount of organic residues which derive from agro-industrial processes is their use, after functional transformation, as soil additives. The present study focuses on the effects of four different agro-industrial by-products from rape (*Brassica napus* L.), castor-oil plant (*Ricinus communis* L.), linen (*Linum usitatissimum*), and digestate, on maize (*Zea mays* L.) growth and metabolism. The products were first analyzed for their content in carbon, nitrogen, sulfur, and total flavonoids and phenols. The presence of indoleacetic acid and isopentenyladenosine was also determined both via immunoassay and bioassay methods. Maize plantlets were grown in the absence (control) or presence of 0.1 and 1 mL L^{-1} by-product doses. From our results, both the concentration and treatment, as well as their interaction, were statistically significant in influencing the maize growth. The products increased root and leaf biomass and had a positive effect on nitrogen metabolism, as resulted from the increasing activity of nitrate reductase, glutamine synthetase, and glutamate aminotransferase enzymes. From these results it can be assumed that the agro-industry by-products tested in this study may be successfully used as bioactive plant growth enhancers.

Key words: agro-industrial residues, bioactive substances, auxin, cytokinin, nitrogen metabolism.

Physical-chemical characterization of Lignin isolated from three different plant biomasses by oxidative alkaline extraction

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Lignin extracted from plant biomass can be converted in various basic chemicals for industry[1] to produce either industrial chemicals (vanillin, toluene, ferulic acid) or biostimulants for agriculture (humic-like substances).

Several methods have been applied to extract Lignin from biomasses. One is based upon alkaline treatment that provides an effective delignification and chemical swelling of fibrous cellulose. Studies on the treatment of straw with alkaline reagents, particularly NaOH, showed that up to 50% of *Poaceae* Lignin, was readily solubilized by 1.5% aqueous NaOH solution at room temperature. The solubilization of Lignin can be further increased by adding a solution of hydrogen peroxide (H_2O_2) to the reaction mixture. Approximately one-half of Lignin and most of hemicelluloses in wheat straw and corn stalks were solubilized by their treatment at 25° C with an alkaline solution of H_2O_2 and delignification was most effective at pH 11.5[2]. Lignin can subsequently be easily separated from the solubilized hemicellulose, by adding ethanol and lowering pH[3].

In this work, we considered two perennial rhizomatous herbaceous plants: miscanthus (*Miscanthus χ Giganteus*) and giant reed (*Arundo donax*, L). The perennial plants are assumed to provide an significant contribution to reduce anthropogenic CO_2 emissions, since the CO_2 released by their combustion does not exceed that fixed by photosynthesis during plant growth.

Our objective was to compare the physical-chemical charateristics of Lignin extracted from these two different biomass, by following an oxidative alkaline procedure. The Lignin isolates from the two different biomasses were characterized by Attenuated Total Reflectance Fourier Transform spectroscopy (DRIFT-IR), and by both liquid-state (DOSY spectra) and solid-state ^{13}C-CPMAS-NMR (mono-dimensional and T1ρH spectra). Moreover, their solubility in water was quantitatively assessed.

Keywords: Lignin; Humic-like sustances; Alcaline peroxide extraction; *Arundo donax*; *Miscanthus χ Giganteus*

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Water Extractable Organic Matter, Stability And Maturity Relationships In Biosolids From Urban Sewage Sludges

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Large amounts of sewage sludge are yearly generated worldwide, reaching the amount of $9^{\cdot}10^6$ tons in 2006 in the EC [1]. Sewage sludges are rich in organic carbon and nutrients for plant growth, and, if properly managed, they could improve soil fertility mainly in intensively cropped degraded soils [2]. Its agricultural use is widely recommended as the most common disposal to preserve or increase soil organic matter (SOM) [3]. Mediterranean soils are often subjected to severe degradation processes accompanied by a decline in SOM content, and thus soil fertility. The use of organic amendments restores SOM content and soil physical, chemical and biological functions [4]. However, sewage sludges could contain hazardous contaminants, such as heavy metals, organic xenobiotics and patogens, and, as fresh organic waste, they can also negatively affect soil properties and plant growth. Thus, raw sewage sludge should be subjected to appropriate treatment, such as composting, before land application, to avoid possible phytotoxic effects.

In this work, biosolids deriving from composting municipal sewage sludges (aerobic and anaerobic plans) with rice husk in the ratio 1/1 v/v (72/28 w/w), treated in a forced ventilation plant for stabilization and then in a static pile for curing. Water extracts, obtained through the method reported in UNI 10780 [5] was employed for the study of stability (oxygen uptake rate, OUR) and maturity (germination index, GI) of the biosolids.

As the first results, we found a good stability of all biosolids (OUR < 10 mmol O_2 kg^{-1} SV h^{-1}), and a good maturity for aerobic and anaerobic based biosolids (GI > 80%), only the mixed (aerobic + anaerobic) sample was phytotoxic (GI = 1%). The stability of biosolids samples was positive correlated to electrical conductivity (EC), total organic carbon (TOC) and nitrogen (TON), total protein, total phenols, ammonia (NH_4^+-N), and negative with the degree of aromaticity (AD), and the GI. The maturity was negative correlated to pH, total phenols, total proteins, ammonia (NH_4^+-N), and positive with nitrate (NO_3^- -N) and AD. These results confirm the hypothesis that: (i) the stability can be predicted on the basis of the presence of fermentable substrates in the water extract; (ii) and the maturity on the basis of pH, ammonia, phenols and AD. Therefore, a simple acid-base titrimetric approach was applied at the water extracts and an interpretative model of titrimetric curves was developed in order to provide the stability and the maturity of the biosolids.

Keywords: Biosolids, stability, maturity, water extractable organic matter

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KEY-NOTE LECTURE

Influence of humic substances on plant-microbes interactions in the rhizosphere

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Humic substances are known to play a wide range of effects on the physiology of plant and microbes. This is of particular relevance in the rhizosphere of terrestrial environments, where the reciprocal interactions between plants roots, soil constituents and microorganisms strongly influence the core process of soil fertility, i.e., the plants acquisition of nutrients.

Chemical advancements are constantly improving our knowledge on humic substances: their supra-molecular architecture, as well as the plentitude of their chemical constituents, many of which are biologically active. One straightforward approach for linking the structure of humic substances with their biological activity in the rhizosphere is the use of rhizoboxes, which allow applying a treatment (e.g., an amendment with humic substances) in an upper soil-plant compartment and take measurements in a lower isolated rhizosphere compartment that can be sampled at desired distances from the rhizoplane.

The rhizobox approaches can be adopted to assess the effects of several humic substances, as well as composted materials, on maize plants rhizodeposition of carbon, and in turn on the structure and activity of rhizosphere microbial communities. In order to gain a complete understanding of processes occurring in the complex soil-plant-microorganisms tripartite system, rhizobox experiments can be coupled with bacterial biosensors for the detection and quantification of bioavailable nutrients, chemical analyses of main rhizodeposits constituents, advanced chemical characterizations of humic substances, DNA-fingerprinting of microbial communities, and multivariate statistical approaches to manage the dataset produced and to infer general conclusions.

By such an approach it was found for instance that humic substances are significantly affecting the amount of carbon deposited by plant roots. This induction effect is more evident for substances with more hydrophobic and complex structure, thus supporting the scientific hypothesis of the “microbial loop model”, which assumes that plants feed rhizosphere microorganisms with carbon in order to support their metabolic activity towards organic materials. This hypothesis is further confirmed by changes in the sugar and organic acids composition, as well as in the fingerprinting of microbial communities obtained by DNA-based phylogenetic analyses. Multivariate statistical analyses can also allow the identification of chemical constituents more related to the observed microbial diversity changes, thus giving useful indications for the design of further experiment aimed at understanding one of the most complex and fascinating micro-environments, the rhizosphere.

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Evolution of the peat organic material following smouldering wildfires and possible implications in paleoenvironmental reconstructions

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Organic matter (OM) burning in wildfires affects different ecosystems but it is poorly studied. By large, the most affected ecosystem are peatlands, which are particularly flammable in dry conditions. When ignited by self-heating, or biogenic or anthropogenic heat sources, peat smoulders and is difficult to extinguish despite extensive rains or weather changes. It persist for very long periods of time (months, years), and spreads over extensive areas. Smouldering is the slow, low temperature, flameless form of combustion of OM in porous form. It becomes self-sustained from the heat released from the ambient heterogeneous oxidation. Smouldering is the most persistent type of combustion.

Most studies on smouldering focused on ignition, C losses or emissions, without full understanding of evolution of OM in smouldering fires. Identification of ancient peat fires by their chemical signature is extremely important for paleoenvironmental reconstructions, especially considering that peat soils (and bogs in particular) are often used as archives of environmental changes of past millennia.

The present work studies the physical, chemical and spectroscopic changes in OM features following a smouldering fire within three *Sphagnum* peat columns (26 cm deep) having different initial moisture contents (MC), i.e., from dry conditions (50%MC), to undisturbed conditions (100%MC), and also wet conditions (200%MC). The dry and undisturbed peat columns spread the fire after ignition from the top to a depth of 5 cm and 11 cm, respectively. The fire in the wet peat column did not spread.

Chemical analysis of columns after fire shows a consistent variation of the chemical markers across the interface between burn residue and undisturbed peat. The depth variation of chemical markers extends the whole residual column of 5 cm in 50%MC, and 8 cm deep in 100%MC. No relevant variation of any of the chemical parameters is observed in 200%MC peat column. The results of this study show that: *i)* smouldering fires could occur either when peatlands are drained (<50%MC) or in undisturbed conditions (e.g., near 100%MC); *ii)* there is a higher production of aromatic and condensed molecules a few centimetres below the fire front, as suggested by the higher C/H values and by fluorescence spectra; *iii)* the increase of the total N and decrease of C/N ratio a few centimetres below the fire front suggest the incorporation of, and the relative enrichment in, N during charring and underlines the importance of "black N" as an integral part of the char; *iv)* smouldering events should be at least considered in paleoenvironmental reconstructions. For example, previous studies have generally interpreted peaks in ash content as ascribed to an increase of either dust depositions or mineralization, whereas variations in C/N to changes in either vegetational or humification degrees. The present work propose additional possible interpretations.

Further research is in progress to find proxies allowing reconstruction of ancient smouldering events along peat profiles.

Keywords: peat, wildfire, ash content, N-enrichment, charcoal

Thermal analysis associated to Fourier Self-Deconvolution of Infrared spectra applied to the study of Anaerobic Digestion of municipal solid waste and sewage sludge

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The organic fraction of domestic solid wastes (OFDSW) and sewage sludge (SS) are the most frequently wasted renewable source of energy. These substrates can be subjected to anaerobic digestion (AD) that generates biogas (CH_4 and CO_2) and a residue known as digestate (D) which is characterized by high contents of recalcitrant organic molecules.

The aim of this work was to verify the applicability of Differential Scanning Calorimetry (DSC) associated to Fourier Self Deconvolution (FSD) of infrared spectra in order to characterize substrates (OFDSW1, SS1, OFDSW2 and SS2) collected in the municipality of Bagnacavallo (RA), in the Emilia-Romagna region, Northern Italy and digestates (D1 and D2) obtained under mesophilic conditions (35 °C).

The higher organic content of OFDSW samples were expressed by higher total organic carbon (TOC) and volatile solids (VS) values with respect to SS samples. The higher total solids (TS) and the lower VS and TOC values of SS2, with respect to SS1, confirmed the greater content of inert materials. Regarding digestates, TS and TOC values were lower than those of substrates.

Oxidative combustion of organic compounds causes exothermic reactions. In fact, common features to all thermograms were two exothermic peaks located at: 282 °C for SS1 and SS2, 344 °C for OFDSW1 and at 383°C for OFDSW2 and 509 °C for OFDSW1, 510 °C for OFDSW2, 506 °C for SS1 and 513 °C for SS2. D1 and D2 samples showed a typical three-step pattern comprising two endotherms at 150 °C and 215 °C and an exotherm at 525 °C.

In the FDS trace of OFDSW1 heated to 400 °C a strong reduction of bands of COOH (1740 cm^{-1}), amide II (1580 cm^{-1}) and alcohols, esters, ethers and polysaccharides (1170-1040 cm^{-1}) was evident whereas FDS trace of OFDSW2 heated to 400 °C showed a stronger reduction of the peak at 1400 cm^{-1} as compared to that of OFDSW1. In the FSD traces of SSs heated to 400 °C a reduction of the relative intensity of peaks at 1740 cm^{-1} and at 1550 cm^{-1} was evident. FSD traces of Ds samples heated to 400 °C revealed a relative reduction of peaks intensity in the range 1750-1630 cm^{-1}; at 1380 cm^{-1} (aliphatic groups) and at 1120 cm^{-1}.

The notable difference observed between the ΔH values of the high temperature exotherm peak of OFDSWs and Ds thermograms reflected the high degradability of the OM in the substrate. On the contrary, similar values of ΔH were obtained for the high temperature exotherm peak of SSs and Ds presumably because of the high content of inert materials in sludge that remained unchanged in the final product.

DSC provided evidence of different thermal stability of samples investigated; the shift of the exotherm toward higher temperature with respect to substrates indicated stabilization attained by OM during the process. The high content of inorganic material in SSs samples affected the digestates composition.

Keywords: Anaerobic Digestion, Differential Scanning Calorimetry, Fourier Self Deconvolution, FTIR spectroscopy.

Co-polimerization of 2,4-dichlorophenol in humic substances under oxidative biomimetic catalysis

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The catalytic activity of a water-soluble iron-porphyrin was evaluated in oxidative co-polymerization reactions intended to form covalent bonds between an halogenated phenol (2,4-dichlorophenol) and humic molecules.

The catalyzed oxidative coupling reaction was tested upon different incubation conditions (with and without humic acids, dark and daylight) in presence of H_2O_2 as oxidizing agent, and several analytical methods, such as High-Performance Liquid Chromatography (HPLC), Gas-Chromatography/Mass-spectroscopy (GC/MS) and Nuclear Magnetic Resonance (NMR) were used to follow the structural changes in both halogenated phenol and humic matter.

The results showed that iron-porphyrin had a good catalytic efficiency in all the analyzed conditions. Moreover, its activity was enhanced in daylight incubations, by inducing the complete disappearance of the tested phenols, despite a certain inhibition of reactivity at large amounts of humic acids. The reaction products were mainly identified as dehalogenated and hydroxylated phenols. Dimers/trimers of dechlorinated phenol were found when the reaction was conducted in absence of humic acids, while in their presence it was also observed the formation of co-polymerized products. Further confirmation of the occurred covalent interactions between phenols and humic substances was achieved by DOSY-NMR spectroscopy.

These findings show that iron-porphyrin is an efficient catalyst for the covalent binding of polyhalogenated phenols to humic molecules, thereby suggesting that the co-polymerization biomimetic reaction may become an useful technology to remediate soils and waters contaminated by halogenated phenols and their analogues.

Keywords: biomimetic catalyst, halogenated phenols, humic substances.

Effects of an exogenous humic acid and oxidative biomimetic catalyst on the remediation of a highly polluted soil

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The soil environment becomes contaminated with hazardous chemicals of different structure and toxicity due to polluting industrial activities. Several organic compounds (pesticides, hydrocarbons, dyes, polycyclic aromatic hydrocarbons, polyalogenated phenols and biphenils) may accumulate in soils and sediments, thus endangering human health and environmental quality. The use of oxidative biomimetic catalysts, such as metal-porphyrins, appears promising as a soil remediation method. Water-soluble iron-porphyrins are non-toxic biomimetic catalysts which have been successfully applied as to dechlorinate and detoxify chlorophenols and other organic compounds.

The aim of this study was to assess the oxidative capacity of an iron-porphyrin synthetic water-soluble (FeP) in the presence of an exogenous humic acid from lignite (HA) for the decontamination of a highly contaminated soil from the site of ACNA (Aziende Chimiche Nazionali Associate), an industrial area near Cengio (Savona), included in the list of national priorities for environmental remediation. A first series of experiments were conducted by placing 40 g of soil sample (2.0 mm sieved) in Petri dishes, and adding the following solutions to the soil: I) 20 mL of a 1.09×10^{-4} M aqueous solution of FeP catalyst (control 1); II) 3.2 mL of a 8.1 M freshly prepared H_2O_2 solution (control 2); III) both the amounts of FeP and H_2O_2 solutions added in the preceding experiments. A second series of experiments were conducted by first treating 40 g of soil in Petri dishes with 20 mL of 1 mg mL^{-1} of HA aqueous solution. The soils were left to air-dry for 10 days, and then the samples were treated with the following solutions: IV) 20 mL of a 1.09×10^{-4} M aqueous solution of FeP catalyst; V) 20 mL of a 1.09×10^{-4} M aqueous solution of FeP catalyst and 3.2 mL of a 8.1 M freshly prepared H_2O_2 solution. The treated soils were incubated at room temperature for 30 days in the dark, in order to avoid photocatalytic oxidations, then subjected to Soxhlet extractions, and the extracts analyzed by GC-MS.

Results showed that polycyclic aromatic hydrocarbons were abundant in the contaminated soil (4562 mg kg^{-1}), followed by monoaromatic halogenated and nitrogenated compounds, sulphones, biphenyls and, thiophenes in decreasing order. The treatment with FeP in the presence of oxidizing agent (H_2O_2) allowed removal of pollutants by 50% compared to control, while the removal reached 69% after addition of the exogenous humic acid. The increase in removal efficiency is attributed to the oxidative co-polymerization reaction of aromatic contaminants in the humic matrix. In conclusion, the use of biomimetic metal-porphyrin catalysts seems to represent an innovative approach to efficiently and rapidly remediate contaminated soils.

Keywords: Humic substances, soil remediation, biomimetic catalysis, co-polymerization

TPH and PAH Removal from a Contaminated Soil in Solid Fermentation by a Microbial Mixture (a mixed culture, humic substances and inorganic nutrients)

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The biodegradation of total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAH) is carried out in the nature by microbial successions. Generally a single microbial species does not own the capacity to mineralize certain a compound one (Rieser-Roberts, 1998). In such sense, it is necessary to consider that in the efficient elimination of hydrocarbons of petroleum, single compound, a crude complex mixture or same, are required more than a microbial species with capacity to degrade each one of the initial polluting agents (Kotterman, et al, 1998). Degradation of such molecules is possible by co-metabolism with the inclusion of microorganisms such as those having the capacity to transform aromatic molecules. These microorganisms could be the ones present in cellulosic organic wastes or humic substances. Hydrocarbon desorption from soil particles has been carried out with synthetic surfactants. However, an interesting alternative of TPH desorption process can be the use of surfactants of organic origin, such as humic acids (Conte and Piccolo, 2005). PAH can be incorporated to them by forming structures around the humic acid hydrophobic pole, thus forming structures similar to micelles (Wijnja et al, 2004).

A factorial experimental design was carried out to investigate the effects of four parameters on TPH and PAH removal on artificially contaminated soil with fuel oil. These parameters have been chosen according to some field experiment needs *in-situ*. Thus, the influence of exogenous hydrocarbonoclast microorganisms (EHM, isolated from sugar cane bagasse and humic acids), humic acid (HA), fulvic acid (FA) and contaminant concentration (5000, 20000 and 50000 mg/kg-1 of a complex mixture of TPH from fuel oil (Aliphatics, 35±2%; PAH 9±3%; Saturates, 32±2% and Asphaltenes, 24±3%)) have been tested. Within the parameters investigated, the presence of EHM and FA were the most important parameters affecting TPH removal from soil, up to 57% 46% and 32% in 34 days for the treatments with 5000, 20000 and 50000 mg/Kg-1 respectively. The results of the removal rate of PAH of the system with 5000 mg/Kg-1, showed a decrease of the initial content in fuel oil of acenaphtene (92% from 38mg/Kg), phenanthrene (66% from 314mg/Kg), fluoranthene (45% from 218mg/Kg), chrysene (100% from 16.5mg/Kg) and benzo[a]pyrene (36% from 85mg/Kg) and 100% removal of aliphatic hydrocarbons until 30C. These results show the good interaction among all the components studied. On one hand, the EHM which offers the possibility of removing the most recalcitrant and carcinogenic hydrocarbons, on the other hand the humic substances properties that could have solubilized hydrocarbons and make them more accessible to microorganisms and mitigate their toxicity in the system. Although, we found that the presence of both humic substances not improve the TPH removal. The results obtained in this study will contribute to understanding the interaction between microorganisms and humic substances in hydrocarbons' removal processes.

Keywords: humic acid, fulvic acid, hydrocarbonoclast microorganisms, polycyclic aromatic hydrocarbons, soil

Binding of Phenol and Differently Halogenated Phenols to Dissolved Humic Matter As Measured by NMR Spectroscopy

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Proton (1H-) and Fluorine (19F-) NMR measurements of spin-lattice (*T*1) and spin-spin (*T*2) relaxation times and diffusion ordered spectroscopy (DOSY), were applied to investigate the association of non-substituted (phenol P) and halogen-substituted (2,4-dichlorophenol DCP; 2,4,6-trichlorophenol TCP; 2,4,6-trifluorophenol TFP) phenols with a dissolved humic substances (HS).

The *T*1 and *T*2 values for both 1H and 19F in phenols decreased with enhancing HS concentration, indicating reduction in molecular mobility due to formation of non-covalent interactions. Moreover, correlation times (τC) for different hydrogen and fluorine atoms in phenols showed that anisotropic mobility turned into isotropic motion with HS additions. Changes in relaxation times suggested that DCP and TCP were more extensively bound to HS than P and TFP. This was confirmed by diffusion measurements which showed full association of DCP and TCP to a less amount of HS than that required for entire complexation of P and TFP. Calculated values of binding constants (Ka) reflected the overall NMR behavior, being significantly larger for DCP- and TCP-HS (10.04 ± 1.32 and $4.47\pm0.35 M^{-1}$, respectively) than for P- and TFP-HS complexes (0.57 ± 0.03 and $0.28\pm0.01\ M^{-1}$, respectively). Binding increased with decreasing solution pH, thus indicating a dependence on the fraction of protonated form (α) of phenols in solution. However, it was found that the hydrophobicity conferred to phenols by chlorine atoms on aromatic rings is a stronger drive than α for the phenols repartition within the HA hydrophobic domains.

Keywords: Humic substances, Organic contaminants; NMR-relaxation times; DOSY

Cr(VI) reduction capability of humic acid extracted from organic fraction of municipal solid waste.

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A humic acid (HA) extracted from organic waste (HAw) at a yield of 87.5 ± 2.1 g kg^{-1} dm was tested for its capacity to reduce Cr(VI) to Cr(III) in aqueous solutions at pH 2.5, 4 and 6, in comparison with coal-derived commercial-HA (HAc). Both HAs tested were able to reduce Cr(VI) at pH 2.5 and 4, being HAw ability, much higher than that of for the HAs; no Cr(VI) reduction occurred at pH 6. Cr(VI) reductions depended strongly on pH and theirs kinetics can be described by the model proposed previously:

$$\frac{dCrVI}{dt} = \frac{-(k_0 + k[H^+]) * [CrVI]_0^{0.5}}{X_e}$$

in which the term $[CrVI]_0$ is the starting Cr(VI) concentration, and the term Xe is the equivalent fraction of HA oxidized. Apparent rate coefficients: $k' = (k_0 + k[H^+])$, that were, as log k'_{HA}, of -8.45, -9.63 for HAw, and of -9.41 and -10.41 for HAc, at pH 2.5 and 4, respectively, indicated the HAw was more performant than HAc but, also, of soil and peat HAs.

The reduction ability of HAs depends on functional groups such as thiols and phenols, rather than the presence of free radicals. HAw was more performant than HAc, because of the presence of more reactive phenols, i.e. methoxy- and methyl-phenols.

Reducing properties of waste-HA coupled to the high HA yield, prefigured the possibility of future industrial development of the use of HA in wastewater treatment.

Keywords: hexavalent chromium; humic acid; reduction kinetic; organic fraction municipal solid waste; phenol molecules.

POSTER SESSION

Interactions between proteins and soil humic substances: evidences from electrophoeresis and mass spectrometry

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Humic substances are ubiquitary in the environment, and represent a large and poorly characterized fraction of the soil organic matter. Proteins in soil are stabilized by interactions with inorganic solid phases such as clays and organic matter after their active or passive release from living organisms. Protein stabilization in soil has relevant environmental effects, representing for example a large fraction of the soil N, an extracellular pool of enzymes, and a source of potentially bio-active peptides. Our previous research showed that interactions between proteins and soil organic matter may induce modifications in the extractable proteins, thus limiting the development of soil proteomics (Giagnoni et al., 2011, 2012). While protein-clay interactions have been relatively well studied, to date interactions between proteins and humic substances are considerably less characterized.

We adopted a multidisciplinary approach to better understand the main factors leading to the formation of humic-enzymes complexes under various reaction condtions. The studied proteins were commercially available β-glucosidase (β-G) from *A. niger* and horse muscle myoglobin, whereas humic substances (HS) were extracted by a sandy loam soil.

Experiments were conducted under different chemical conditions and for different contact times. At the end of each incubation period, native and SDS page were performed to evaluate eventual changes in protein structure. The obtained gel bands obtained were analyzed by tandem MS spectrometry.

The main results were that in some cases contact with HS varied the electrophoretic mobility of the tested proteins, and reduced β-glucosidase activity detectable on native PAGE. The contact between proteins and HS also affected the protein identification by MS, in terms of peptide coverage.

The possible mechanisms of protein/humic substances interations will be discussed.

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ORGANIC MATTER EFFECT ON BIOCHEMICAL TRANSFORMATIONS IN URBAN PARK SOILS INFLUENCED BY VARIOUS ANTHROPOGENIC CHANGES

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The test objects were the humus horizons of soils in 12 park gardens located in cites of eastern Poland and Upper Silesia. Gardens situated in city centres and suburbs were included in the test. The activity of the enzymes tested (dehydrogenases, phosphatases, proteases and urease) was several times lower in the soils of the gardens located in city centres than in the gardens situated in the suburbs. The correlations between the enzyme activity and organic carbon contend in urban soil were of a various nature, depending on the location of the gardens, which indicates the fact that they are mainly determined by the intensity of the anthropogenic impact.

Keywords*:* urban soils, organic matter, enzymatic activity.

Fluorescence quenching method for determining pyrene partition coefficient to dissolved organic matter from compost

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The importance of dissolved organic matter (DOM) in determining the fate of hydrophobic organic pollutants in aqueous environments has already been recognized. In particular, several researchers have reported the affinity of various pollutants for DOM, and suggested that, upon association, rates of chemical and biological degradation, photolysis, and volatilization may be altered. For compounds that possess high fluorescence efficiencies, such as policyclic aromatic hydrocarbon (PAH), it is possible to measure the equilibrium constants for the association of these molecules with DOM on the assumption that free PAHs fluoresce in aqueous solution but not if associated with DOM. As a consequence, the fraction of PAH associated with DOM may be determined directly by the Stern-Volmer plots based on the fractional decrease in fluorescence intensity (FI) upon addition of DOM.

The aim of the present study, which is part of a wider research activity on phytoremediation, was to determine the partition coefficient of pyrene to dissolved organic matter (DOM) from compost by means of the fluorescence quenching method.

The compost was obtained from a mixture of coffee chaff and pruning residues (305 and 600 kg, respectively), with an initial C/N ratio = 28. The composting process was performed for 120 days during which a set of chemical parameters were monitored. The DOM sample was isolated from the mature compost using a saline solution, and was analyzed by chemical and spectroscopic techniques, in order to evaluate its nature, which resulted closely resembling that of “native” soil humic substances.

Aliquots of 25 μl of aqueous solution of pyrene were added to five DOM extracts to give final concentrations of pyrene and DOM of 10μg L^{-1} and 5-25 mg L^{-1}, respectively. The solutions were throughly mixed for 5 min, and then allowed to stand for 10 min. Fluorescence data were obtained by means of a Perkin Elmer spectrofluorimeter, mod. LS55, by setting operating parameters previously optimized. Fluorescence intensity (FI) values were collected at 368-370 nm (excitation wavelength, 271 nm). Fluorescence measurements were made both in each DOM solution in the absence of pyrene and in pyrene solutions in the absence of DOM (three replicates).

The FI values of pyrene in the presence of DOM (FI_{DOM}) and in the absence of DOM (FI_0) were used in the Stern-Volmer equation. Values of FI_0/FI_{DOM} versus DOM concentration gave a linear plot with a $r^2 = 0.9957$. The partition coefficient calculated from the slope resulted of 2.08 x 105 (l kg^{-1}). This value was greater than those usually obtained for soil humic components, and indicates a very high affinity of pyrene for compost DOM. This result was reasonably ascribed to the tertiary structure of DOM which is very rich in hydrophobic cavities and/or pseudo-micelles. This property appears very important in indicating that compost could be used, besides for its amendment capacity, also as a potential tool in contaminated soils remediation.

Key words: DOM; compost; pyrene; fluorescence quenching; partition coefficient

Effect of natural organic fractions on the ability of two herbaceous plants to remove the endocrine disruptor 4-nonylphenol from water

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The endocrine disruptor 4-nonylphenol (NP) is an organic compound able to mimic the estrogen 17β-estradiol and consequently alter the function of the hormonal system of animals and humans. This molecule is mainly used for the production of nonylphenol polyethoxylates (NPEO), a group of surfactants commonly used for cleaning products and pesticides. NP is released by NPEO in anaerobic conditions, especially during wastewater treatment. Through the discharge or reuse in agriculture of wastewaters, sewage sludge and other polluted matrices, NP enters soils and waters reaching in many cases hazardous levels. Its removal by means of phytoremediation has been recently explored. This technology consists in the exploitation of plants to uptake, transform, volatilize or stabilize pollutants present in the environment. It is well known that all polluted media contain organic matter which interferes with plants and microorganisms and certainly plays a role in the bioavailability of contaminants. However, the influence of the organic fraction in the phytoremediation from NP is almost unknown. The goal of this study was to investigate the influence of the IHSS products Elliot soil humic acid (HA) and Suwannee River natural organic matter (NOM) on the ability of ryegrass (*Lolium perenne*, RY) and radish (*Raphanus sativus*, RA) to remove NP from water.

Three plants of 21-day growth of each species were separately placed in pots containing the following aqueous solutions: NP at 1 mg L^{-1} (NP_1), the combinations between NP_1 e HA at 10 and 200 mg L^{-1} (NP_1+HA_{10}; NP_1+HA_{200}), the combinations between NP_1 e NOM at 10 and 200 mg L^{-1} (NP_1+NOM_{10}; NP_1+NOM_{200}). For each treatment, a blank without plants was performed in triplicate in order to assess NP degradation in the various solutions. After 1 and 2 days of growth under controlled conditions, the residual NP was measured in each solution by using high performance liquid chromatography (HPLC) analysis and a fluorescence detector (λex= 230 nm, λem= 310 nm). Then, the experiments were stopped and NP was extracted from plants. All treatments were replicated 4 times and data were statistically analysed by ANOVA and LSD test.

No significant variation of NP concentration was observed in all blanks during the experiments. In the absence of organic fractions, after 1 and 2 days both plants removed a considerable amount of NP, respectively 71% and 97% (for RY) and 74% and 96% (for RA). This high phytoremediation efficiency was slightly affected by HA and NOM at the two concentrations tested. In particular, HA_{10} and NOM_{10} increased NP removal by RA and RY, probably by stimulating both the plant enzymatic activity and the microbial activity, whereas HA_{200} and NOM_{200} reduced NP removal, perhaps by adsorbing it and reducing consequently its bioavailability. After 2 days, in NP_1 treatment only 3% and 5% of NP initially applied was extracted by plant tissues of RY and RA, respectively. Because of the low NP phytoaccumulation, it is expectable that this compound is transformed by plants. With respect to NP_1, a lower amount of product was recovered by plants grown in the presence of all combinations between NP_1 and HA or NOM, probably because both organic fractions stimulated the metabolization of NP more than its phytoaccumulation.

Keywords: endocrine disruptor, humic acid, natural organic matter, 4-nonylphenol, phytoremediation

Study on soil fertilizer Powhumus application in roseroot (*Rhodiola rosea* L.) cultivation

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Rhodiola rosea L. (from Crassulaceae family) is a dioecious, slowly growing, herbaceous perennial plant, originated in the mountain regions of South West China and native around whole of the northern hemisphere north of the 50th latitude. It grows as a natural plant in the Alps and the Carpatian mountains and in the Northern parts of Europe. This plant has been used since antiquity as a remedy for numerous health disorders: improvement of physical condition, treatment of anemia, depression, asthenia, impotence, scurvy, gastro-intestinal and nervous system disorders and also as a stimulant and anti-inflammatory. Phytochemical investigations effected on *Rhodiola rosea* rhizomes and roots evinced six different groups of important pharmaceutical substances: salidrosides, cynnamyl alcohol glycosides (rosine, rosavine, rosarine), flavonoids, (rhodionine, rhodiosine, rhodioline), terpenoids, etc. Modern phytotherapy considered this species a vegetal source with an antioxidant and antistress-adaptogene action, due to influence of some of its compounds on the level of monoamine and peptide (ß-endorphine type) in the human body. This action is mainly due to rosavine and salidroside. The rapidly growing demand and also high prices for raw material for industry could cause increased pressure on natural habitats. Due to intensive collection, the natural populations are seriously threatened and nowadays roseroot is registered as an endangered plant in many European countries. The aim of the study is finding optimal culture conditions for roseroot plants in order to enable their successful field cultivation.

In the field experiment located on experimental fields of University of Life Sciences in Lublin, the effects of two methods of plantation establishment (autumn and spring sowing) and organo-mineral fertilizer Powhumus application in two soil conditions were studied. Roseroot plantation was established by autumn and spring seeds sowing (November 2009 and April 2010) on two soils: heavy loamy sand and silt-loam. Before seeds sowing and every spring Powhumus WSG-85□ (produced by Humintech, Düsseldorf, Germany) in a dose 6 kg·ha-1 was applied. Powhumus WSG-85® is organo-mineral fertilizer, gained through alkaline extraction from highly oxidized lignites (known as leonardite). It contains 85% humic acids, 12% K2O and 1% iron and has a certificate of qualification for use in organic farming in Poland. At the end of each vegetation period 5 randomly chosen plants from each plot were dug and measured and its raw material was dried at 50ºC in a drying chamber. What is more, an enzymatic activity of soil samples were performed. There were used 5 tests: dehydrogenase (the TTC test), urease, protease, acid phosphatase and alkaline phosphatase.

Better plants growth and higher underground parts yields of roseroot were observed in objects with autumn seeds sowing, growing on silt-loam Generally, Powhumus WSG-85 application was especially effective on sandy soil with low humus content. Roseroot fertilisation with Powhumus was the factor that affected yielding and quality parameters of plants as well as increase in activity of all studied enzymes in comparison to control objects..

Key words: *Rhodiola rosea* L., root yields, Powhumus, enzymatic activity of soil

Biodecontamination of water from bisphenol A by using ligninolytic fungi and the modulation role of humic acids

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Endocrine disruptor compounds (EDCs) are a wide group of chemicals of both natural and xenobiotic origin possessing the capacity of altering or inhibiting the normal functions of the endocrine system in animals and humans. Their occurrence in the environment is widespread and increasing in the last years as a consequence of more intense anthropogenic activities and higher amount of wastes released in the environment. Among EDCs, bisphenol A (BPA) is widely used for the production of industrial polymers such as polycarbonates, epoxy resins and flame retardants. BPA is also used as a stabilizer for plastic materials such as polyvinyl chloride, and as a component of food and drink packaging, electrical and electronic devices and numerous goods of daily human utilization. Biodecontamination is a recent technology that utilizes the metabolic potential of microorganisms to decrease the concentration of toxic compounds to undetectable, non-toxic, or acceptable levels. Ligninolytic fungi are known to be able to biodegrade lignin by means of extracellular enzymes with low substrate specificity, which makes them active on a wide range of aromatic compounds, including EDCs. Humic acid (HA) from soil and other natural and anthropogenic matrices can influence the growth and functions of a large number of microorganisms including fungi.

In this work, the potential capacity of the ligninolytic fungi *Trametes versicolor* (*TRA*), *Stereum hirsutum* (*STE*) and *Pleurotus ostreatus* (*PLE*) to remove BPA at a concentration of 4.6 mg L^{-1} from water was evaluated in the absence and in the presence of two HAs from a green compost (GC-HA) and leonardite (L-HA). A new methodology was used that excluded the fungal presence in the medium. At different sampling times and until 23 days, fungal growth was measured and the residual BPA in water solution was quantified by HPLC and UV-visible detection.

In the presence of each fungus and at any sampling time, the residual BPA in water resulted significantly lower than in the control (without fungus). In particular, *TRA* was able to remove about 35% of BPA after 7 days with a subsequent small BPA removal up to 38% after 23 days. In the treatment with *STE*, BPA removal was small until 15 days, but resulted of the same extent of *TRA* after 23 days. An intermediate trend was observed for *PLE* which, similarly to the other two fungi, removed 38% of BPA at the end of experiments. The radial mycelial growth resulted similar for the three fungi along the experiments, and almost doubled after 23 days.

The addition of L-HA in fungal growth medium stimulated *TRA* only in the biodecontamination activity (about 49% of BPA removed in 23 days). The addition of C-HA in fungal medium, stimulated significantly BPA removal from water by *TRA* (up to 65%) and *STE* (up to 55%) and to a lesser extent *PLE* (up to 43%). With respect to treatments without HAs, the presence of any HA, and especially C-HA, in the medium greatly stimulated fungal growth, especially in the case of *TRA*.

Keywords: biodecontamination, bisphenol A, water, ligninolytic fungi, humic acid.

Spectroscopic characterization of digestates obtained by anaerobic digestion of different organic substrates

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Anaerobic digestion (AD) is an efficient waste-treatment technology in which microorganisms degrade organic materials in absence of oxygen to methane and carbon dioxide (biogas). The AD process produces a by-product known as digestate (D) which can be used in land application depending on the degree of digestion attained by the organic matter. An interesting option for improving yields of anaerobic digestion of solid wastes is co-digestion which refers to the combined treatment of several biomasses with complementary characteristics.

In this work, Fourier transform infrared spectroscopy (FTIR) and fluorescence spectroscopy in the excitation, emission and synchronous-scan modes were applied to the study of organic matter transformation during AD of 3 different mixtures of organic substrates: a) organic fraction of domestic solid wastes and sewage sludge (D1); b) pig slurry (D2) and c) energy crop, poultry manure, stomach residues of ruminants and agro-industry residues (D3).

D1 and D3 show similar FTIR spectra being both characterized by prominent peaks at 3400 cm^{-1} assigned to OH stretching and by a band at 1640 cm^{-1} assigned to amide I of protein origin and to aromatic C=C bond; in addition, D1 exhibits intense peaks at 1430 cm^{-1} and 1034 cm^{-1} assigned to inorganic substances such as carbonates and silica deriving from the sewage sludge, whereas D3 shows a number of peaks in the 1550-1400 cm^{-1} range revealing the heterogeneous chemical nature of biomasses from which it is obtained. D2 shows a broad band in the 3400-3000 cm^{-}1 range, a peak of higher relative intensity at 1620 cm^{-}1, a peak of medium relative intensity at 1440 cm^{-1} assigned to aliphatic structures thus revealing the major aliphatic nature of the fresh substrate, and peaks at 1390 cm^{-1} (COO^{-} antisymmetric stretching, C-H and bending of CH_2 and CH_3 groups) and at 1280 cm^{-1} (amide, ethers).

Fluorescence emission spectra of all D samples feature a unique broad band located at 440 nm; excitation spectra show maxima at 330 nm for D1 and D2 and at 350 nm for D3. Synchronous-scan excitation spectra of all D samples present three peaks at 330, 380 and 430 nm; however for D1 and D2 the maximum is located at 330 nm whereas for D3 is located at 380 nm. Generally, peaks at lower wavelength values (<380 nm) are associated to simpler structural components with a low degree of aromatic polycondensation and a low level of conjugated chromophores, whereas humic-like substances are considered responsible for higher wavelength peaks (>380 nm).

Results obtained in this work indicate that the different chemical composition of the fresh organic substrates submitted to the AD process affect the chemical characteristics of the final products.

Keywords: anaerobic digestion, co-digestion, fluorescence spectroscopy. FTIR spectroscopy.

The effect of soil fertilizer Powhumus in *Arnica montana* L. culture

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Arnica montana (from Asteraceae family) is a perennial and an alpine herb, originated from the high mountains of the Alps and the Carpatians. It grows in moist, sandy, acid soils, rich in humus content. It occurs mostly in pinewoods or mixed forests, sometimes in meadows, well exposed to the rays of the sunlight, always colonized by arbuscular mycorrhizal fungi (AMF). The plant can be propagated from root division, cuttings, or seeds, which are of short viability. Arnica is a medicinal plant widely used as a herbal remedy, in cosmetic and liqueur industry. Preparations made from flowering heads have been used in homeopathic medicine for hundreds of years. Its flowers contain essential oils, sesquiterpene lactones and flavonoids with antiseptic, antifungal, antimicrobial and antibiotic activities. Raw material can reduce inflammation and decrease pain, showing also antioxidant and cytotoxic effects. Due to its intensive collection from nature, it is now as a rare and critically endangered species in many European countries. The present work was aimed at finding optimal culture conditions for Arnica plants in order to enable their successful field cultivation.

Plantation of arnica was established in 2009 from nursery transplants on grey-brown podsolic soil with the granulometric composition of heavy loamy sand. Before seedlings transplantation and every spring Powhumus WSG-85□ (produced by Humintech, Düsseldorf, Germany) in a dose 6 kg·ha-1 was applied. Powhumus WSG-85® is organo-mineral fertilizer, gained through alkaline extraction from highly oxidized lignites (known as leonardite). It arises at the chemical and biological humification of plant and organic matter and by the biological activity of micro-organisms especially on sandy and clay soils. It contains 85% humic acids, 12% K2O and 1% iron and has a certificate of qualification for use in organic farming in Poland. In the first year of arnica vegetation plants stayed in a form of rosette, thus yield of flowers was not obtained. Beginning from the second year of vegetation, systematical harvests of inflorescences were gathered. Before the harvests 5 randomly chosen plants from each plot were measured and raw material was dried at 40ºC in a drying chamber. What is more, an enzymatic activity of soil samples were performed. There were used 5 tests: dehydrogenase (the TTC test), urease, protease, acid phosphatase and alkaline phosphatase.

Generally, fertilisation with Powhumus WSG-85□ was the factor that significantly affected yielding and quality parameters of arnica growth on sandy soil as well as increase in activity of all studied enzymes in relation to soil originating from control objects.

Key words: *Arnica montana* L., inflorescences yields, Powhumus, enzymatic activity of soil