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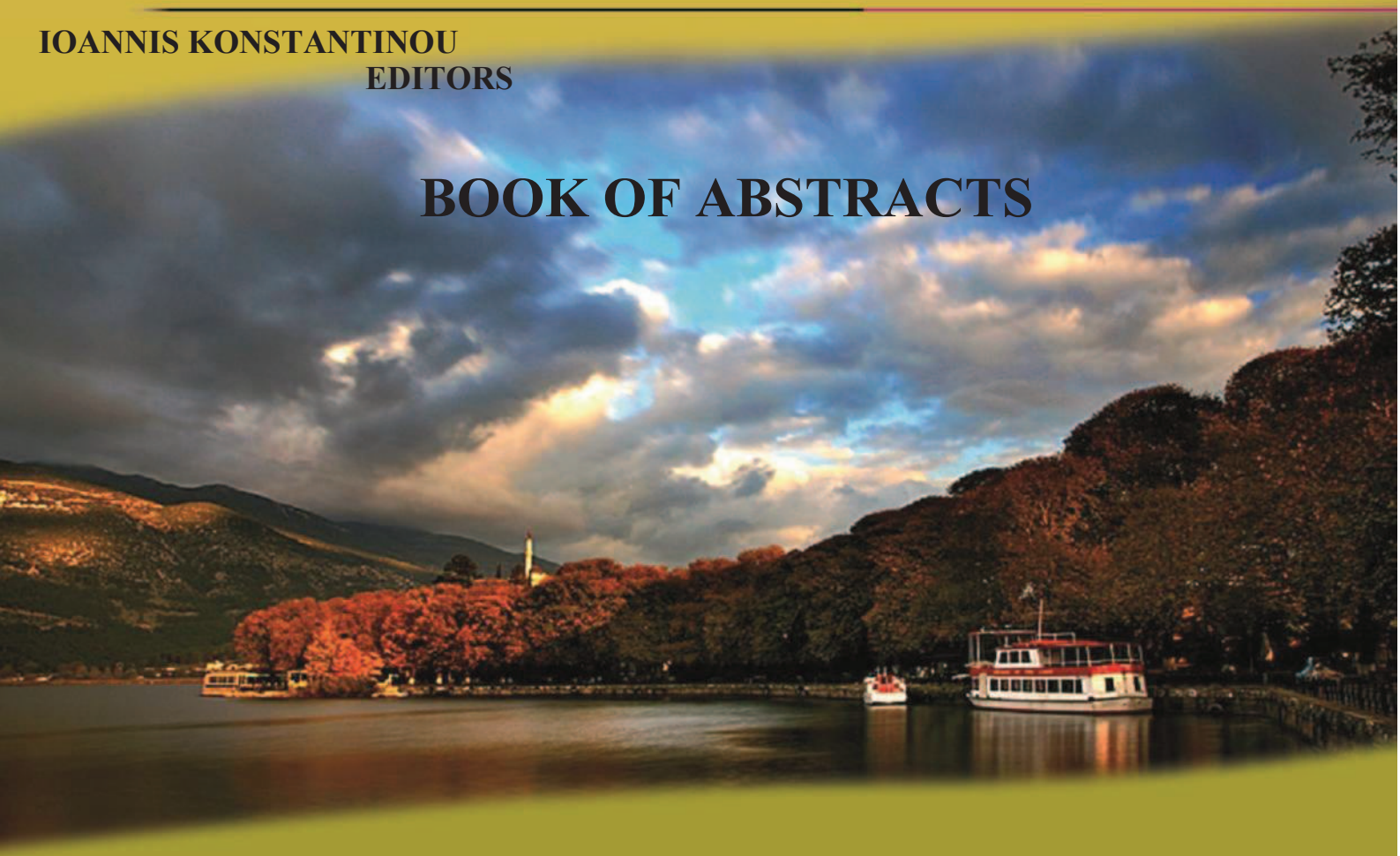

BOOK OF ABSTRACTS

NATURAL ORGANIC MATTER:

Structure-Dynamics

Innovative Applications

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Molecular characteristics and bioactivity of humic acids isolated from vermicomposts

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Abstract Vermitechnology is an effective composting method which transforms different residual biomasses into nutrient rich organic fertilizer. In fact, the mature vermicompost is a renewed organic source suitable to provide humic substances with high biological activity. The chemical characteristics of humic acids isolated from different vermicomposts, produced with cattle manure, sugarcane bagasse, sunflower cake from seed oil extraction and filter cake from sugarcane factory, were accessed by thermochemolysis and 13C solid state NMR spectroscopy. More than 200 different molecules were found and were possible to identify chemical markers on humic acids according the nature of organic source. The large hydrophobic character of humic extracts and the preservation of altered lignins derivatives confer to HAs the ability to induce lateral root emergence in maize seedlings.

Introduction

Vermicomposting is the post-thermophilic biodegradation of agricultural biomasses through the interaction between earthworms and microorganisms. The positive effects produced by the application of mature vermicompost on plant development, it is related to, both, the large content and availability of biologically-active plant promoters represented by the hormone-like humic acids (HA) produced during vermicomposting (1; 2). In fact, the humic like organic matter isolated from vermicomposts showed an high biological activity (3) thereby acting as an effective plant growth promoter (4). HAs isolated from vermicompost effectively induced the synthesis of plasma membrane (PM) H+-ATPase in a typical auxin-like response, thereby significantly enhancing lateral root emergence (1; 2).

However, the effectiveness of HA from vermicompost as bio-factors, strongly depend on the quality of raw organic biomasses and on the final molecular composition of humified organic compounds. Non-destructive spectroscopic methods such as the 13C Cross-Polarization Magic-Angle-Spinning Nuclear Magnetic Resonance (13C-NMR) is extensively used to identify content, distribution and biochemical modification of molecular components in natural organic substrates and compost materials. The plant physiological response, following the application of HS from different vermicomposts, has been previously related to the hydrophobic molecular characteristic of humic components, as measured by solid state NMR analysis (5; 6).

Additional complementary molecular characterization of complex matrices may be obtained by the combination of NMR spectra with the structural information provided by thermally assisted hydrolysis and methylation reaction (thermochemolysis) followed by gas chromatography-mass spectrometry. The thermochemolysis allow a useful qualitative and quantitative measurement of structural components and their relationship with biological activity (7).

The aim of this study was to evaluate, by 13C- NMR and thermochemolysis, the molecular characteristics of humic acids isolated from five mature vermicomposts of different composition, as related to the bioactivity response in the lateral root emergence of maize seedlings.

Experimental

The HS were isolated from five different vermicomposts prepared from the following substrates: cattle manure (A), cattle manure+sugar cane bagasse (1:1 w/w as dry mass) (B), cattle manure+sunflower cake (1:1 w/w as dry mass) (C), cattle manure+sugarcane bagasse+sunflower cake (1:1:1 w/w/w/ as dry mass) (D) and filter cake from sugarcane factory (E). The 13C CPMAS NMR spectra, thermochemolysis and bioactivity assay of HS were conducted as reported elsewhere (5; 7)

Results and Discussion.

Thermochemolysis of HS released more than 200 different molecules, which were identified as methyl ethers and esters of natural compounds. Most of humic components were from plant and microbial origin and were mainly represented by lignin derivatives, followed by alkyl biopolymers, nitrogenous compounds, terpenes and sterols products (Table 1).

The 13C-NMR spectra of HS showed an overall composition dominated by lignin and lipid components and minor amount of polysaccharides and carbohydrates (Fig. 1).

Table 1 Total yield (μg g^{-1} d.w.) of main thermochemolysis products released from HAs

	A	B	C	D	E
Lignin	950	4271	2976	65	870
G6/G4	2,6	1,6	1,9	nd	2,1
S6/S4	4,7	2,7	2,2	4,7	2,7
ΓG	2,8	0,6	2,3	0,8	0,4
Alkyl	291	1274	942	228	239
Sterol	40	130	305	212	118
N-deriv	148	1618	818	49	409

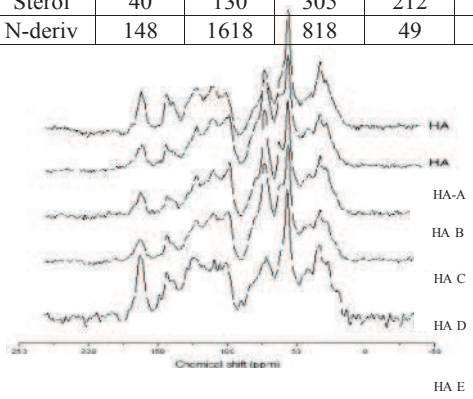

Figure 1 13C CPMAS NMR spectra of vermicompost HAs

All the HS from mature vermicomposts showed an improvement from 36 to 135%, in the number of lateral roots, in respect to control plants (Fig. 2). The most hydrophobic humic acids isolated from cattle manure and filter cake biomasses, provided the best stimulation on lateral roots emergence, while the effect decreased in HA from sugarcane bagasse, sunflower cake and mixed materials, characterized by the progressive lowering of hydrophobic and aromatic molecular components (Table 2).

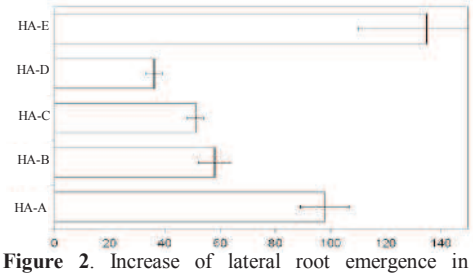

Figure 2. Increase of lateral root emergence in maize seedling treated with vermicompost HAs compared to control

These results are consistent with previous findings (3; 5; 7) where it was observed the humic fractions with larger hydrophobicity were able to provide a steady high bioactivity, the presence of aromatic lignins compounds being closely related with the ability of HA to induce lateral root emergence.

Table 2 Hidrophobicity and aromaticity of vermicompost HAs as calculated from NMR chemical shift regions (2; 5)

	A	B	C	D	E
HB	1.79	0.79	0.82	0.76	0.99
Ar	27.4	23.5	22.4	24.0	27.9

An apparent inconsistency with this hypothesis is represented by the results of thermochemolysis, which showed the largest lignin content in the HS from sugarcane bagasse (Tab 1). However the most abundant aromatic monomers found in the pyrograms of HS-B were the 3-(4-methoxyphenyl)-2-propenoic and 3-(4,5-dimethoxyphenyl)-2-propenoic derivatives, whose accounted for about the 40% of total lignin content. These molecules originate from either the side chain oxidation of lignin units but also from the aromatic domains of plant biopolymers, whose important relative contribution in herbaceous plant may had overrated the total content of lignin in the humic extracts from sugarcane.

Moreover the higher values of lignin structural index found in the pyrograms of HS-A and E (Tab. 1), suggested the occurrence of wider an intense decomposition process of lignin biopolymers in humic fraction from cattle manure and filter cake. The most advanced humification may hence have promoted a preferential accumulation of small active aromatic fragments which should allow a more prompt physiological response as compared to partial undecomposed lignin residues (8). In addition to the content of specific molecules, also the ratio of hydrophobic to hydrophilic moieties is considered an important characteristic for the bioactivity of humic extracts. The role of humic hydrophobicity may be explained by the selective preservation, of active biofragments, which may be successively released by conformational changes of humic associations in solution (2; 5). In fact, plants treated with humic substances enhance exudation of organic acids, thereby leading to a modification of structural arrangement and to a release of active molecules in rhizosphere. Although the role of hydrophobic humic components needs further evaluation in order to gain a deeper insight of the structure-activity relationship, the association of detailed molecular characterization and bioactivity essays are unavoidable requirements for a more accurate and valuable utilization of humic material as plant-growth promoters and for a comprehensive understanding of the plant-SOM interaction**.**

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