NATURAL ORGANIC MATTER RESEARCH

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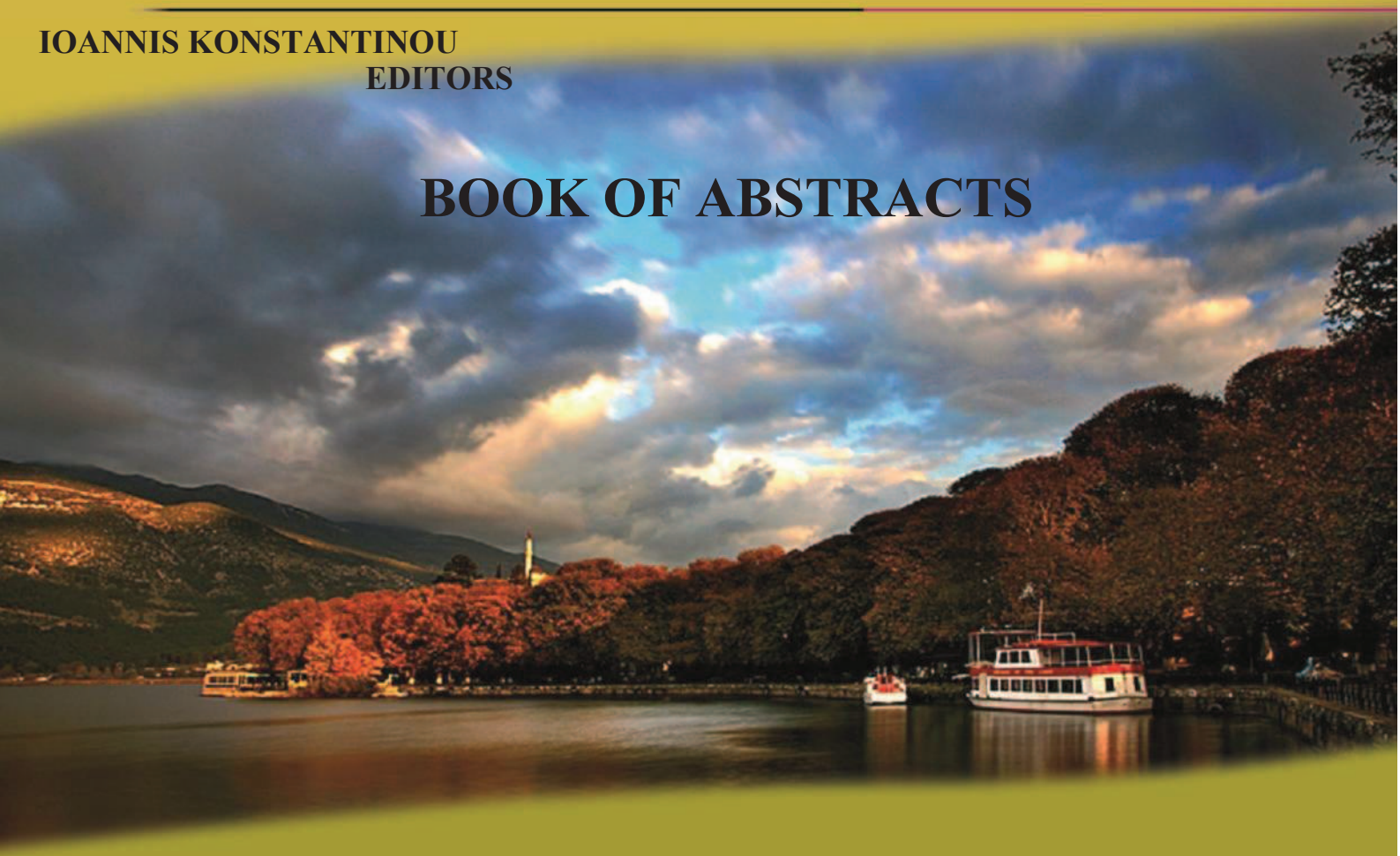

BOOK OF ABSTRACTS

NATURAL ORGANIC MATTER:

Structure-Dynamics

Innovative Applications

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Ecotoxicity assessment of functionalized activated charcoal on the activity of the enzyme acetylcholinesterase and its effect in the presence of pesticides

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Abstract The study of soil organic matter of the "*Terras Pretas de Índios*" showed an efficient compost model for improving the physical chemistry properties of the soil. The charred materials have the condensed aromatic structures but it does not provide carboxylic groups, that are important for its reactivity and its contribution to soil CEC. The objective of this study is to evaluate the ecotoxicity of functionalized charcoal compounds (humic acids like material – HALM) through its sub-lethal kinetic behaviour of the enzyme AChE even in the presence of pesticides. Activated charcoal was chemically oxidized using sodium hypochlorite. Brains of rats were used for ecotoxicity assay. The IC_{50} (half maximal inhibitory concentration) for AChE was 65 mg L^{-1} of HALM. The presence of HALM (10 mg L^{-1}) reduced the AChE inhibition by the tested pesticides (methomyl; methyl parathion; and atrazine) probably due a decrease of pesticide availability to form stable complexes with AChE.

Introduction

The study of soil organic matter (SOM) of the "*Terras Pretas de Índios*" (TPI) (archaeological extremely fertile soil found in the Amazon) showed an efficient compost model for improving the physical chemistry properties of the soil and provides carbon sequestration. The charred materials have the condensed aromatic structures that ensure their recalcitrance in the soil, and hence is an efficient material for carbon sequestration (half-life ranging from decades to millennia), but it does not provide carboxylic groups, that are important for its reactivity and its contribution to soil CEC, as found in the SOM of TPI. Studies indicate that it is possible to functionalize these materials chemically (oxidation) (1)

However, it is important to evaluate the risks that the use of biochar, and its products, as a soil amendment can represent to the environment. Ecotoxicological tests reveal whether and in what quantity chemical substances, isolated or in mixtures, are harmful to the environment, and how and where their effects are expected to be shown in living matter. The effects can be acute or chronic and result in death or changes in morphology, physiology and histology, manifesting altered growth, reproduction, metabolism, and behavior of test organisms (bioindicators). These tests provide information about and identify possible risks and negative physical and chemical alterations that the environment can suffer through the introduction of a specific substance. They provide the concentration at which the tested chemical substance has a toxic potential, serving as a preventive system of protection and warning. Besides it is necessary to access its possible interactions with other agricultural inputs, such as pesticides. Therefore the toxicity tests based on a selective and sensitive biomarker for possible sub-lethal effects are needed.

The choice of the enzyme acetylcholinesterase (AChE) as an indicator of ecotoxicity was based on the following criteria: (1) high sensitivity to the presence of different classes of pesticides (including being used as a sensor for quantification); (2) use of a protein fraction capable to metabolize organophosphate pesticides in their anticholinesterase way; (3) molecule widely used as bioindicator in ecotoxicity tests with the soil fauna.

The objective of this study is to evaluate the ecotoxicity of functionalized charcoal compounds (humic acids like material – HALM) through its sub-lethal kinetic behaviour of the enzyme AChE. Additionally, has been described kinetics of AChE in the presence of HA with different concentrations of commercial formulations of known pesticides with anticholinesterase activity and acute toxicity in humans (methomyl; methyl parathion; and atrazine).

Despite the common mechanism of anticholinesterase action for methomyl and methyl parathion, the latter acts as an indirect inhibitor. However, the metabolite of parathion (paraoxon) is a potent anticholinesterase agent. Atrazine, although a herbicide (AChE is not the target molecule), presents a significant effect on cholinesterase metabolism and it has described ecotoxicological tests based on in AChE (2).

Experimental

Activated charcoal was chemically oxidized using sodium hypochlorite. Afterward, the HALM was obtained according the method suggested by IHSS (alkali solubilization; precipitation at acid pH; dialysis and freeze drying of the acid insoluble fraction).

A unpurified brains extract of rats Wistar (*Rattus norvegicus*) was used for ecotoxicity assay. The brains were removed and washed with distilled water (4 °C); processed using a tissue homogenizer; subjected to saturation with ammonium sulphate; and centrifuged at 300 g at 4 °C. The catalytic activity of AChE of the was measured under experimental conditions obtained by experimental design (2^3 center composite). Measurements of enzyme activity of AChE was performed by spectrophotometric method, measuring the absorbance (412 nm) of the formed product from acetylthiocholine (ATCh) metabolism (3). This measuring was performed by adding the substrate ATCh (3.2 10^{-4} mol L^{-1}) and DTNB (3.2 10^{-4} mol L^{-1}). The incubation time of the assays was 2 h at 37 °C.

Results and Discussion

Kinetic characterization of AChE: The protein fraction used in ecotoxicity tests shows Michaelis constant (K*m*) of 0.4 mmol L^{-1} and maximum reaction rate (V*m*) of 1.0 mmol min^{-1} mg^{-1} of protein. These values show the affinity of AChE for the substrate used in the tests, expressed by K*m* and efficiency in its hydrolysis, represented by V*m*. The protein material obtained from the rat brain, although a simple fractionation procedure, reached a satisfactory kinetic profile. It can be considered that the substrate used in the kinetic assays has affinity for the AChE molecule enough to compose essays with rapid and sensitive kinetic response fraction.

AChE inhibition curve in the presence of different concentrations of HALM: [Figure 1] The catalytic activity of the enzyme AChE decreased by 50% in the presence of 65 mg L^{-1} of HALM (IC_{50}).

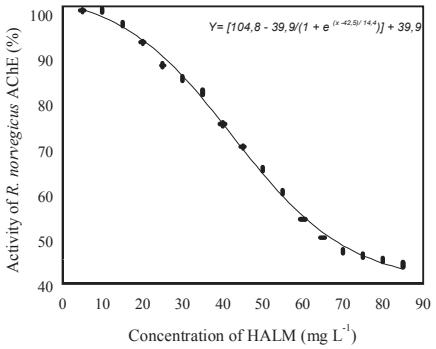

Figure 1. Acetylcholinesterase (AChE) inhibition curve in the presence of different concentrations of humic acids like materials obtained from functionalized charcoal (HALM). Catalytic conditions: sodium phosphate buffer 0.04 mol L^{-1} (pH 7.4). All assays were performed in quadruplicate and the standard deviation ≤ 0.006.

Effect of the presence of HALM in the kinetics of inhibition of AChE by pesticides: [Figure 2] For the initial tests the concentration of 10 mL^{-1} was chosen for HALM to avoid the its inhibitory effect on AChE activity. So the inhibitory action on AChE would be exclusively due the presence of pesticides.

The presence of HALM (10 mg L^{-1}) reduced the AChE inhibition by the tested pesticides (methomyl; methyl parathion; and atrazine), probably due a decrease of pesticide availability to form stable complexes with AChE. This indicates a potential for environmental protection (inhibition of pesticides toxic effect), however could also to require a dose adjustment when HALM is used as soil amendment. On the other hand, the HALM and pesticides showed no synergism on their effects on AChE.

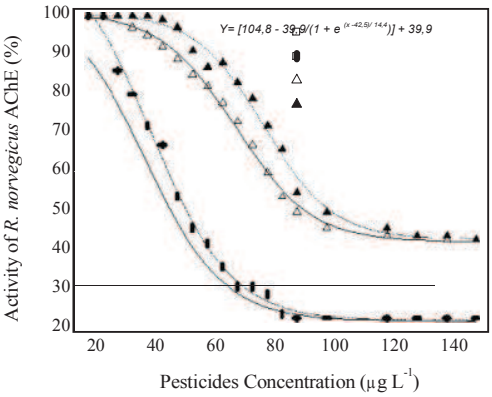

Figure 2. Effect of the HALM presence in the inhibition kinetics of AChE by pesticides: All assays were performed in quadruplicate and the standard deviation ≤ 0.02./ $Y = [A_1 - A_2/(1 + e^{(x - X0)/dx}] + A_2$.

Ecotoxicological tests assess the effects on AChE in experiments "*in vivo*" and "*in vitro*" generally consider their lethal effect on test organisms or the in vitro effect on AChE extracted these organisms (4).In this work the study of sub-lethal toxicity of functionalized charcoal and its effect in the presence of pesticides using enzymatic assays was employed. The effect on the toxicity of atrazine AChE has already been described tests Earthworms species (2) showing the sensitivity of the ecotoxicity biondicador to agricultural environments. The HALM had an inhibitory effect on AChE (IC_{50} of 65 mg L^{-1}), on the other hand, at low concentration (10 mg L^{-1}) it reduced the inhibitory effect of the tested (methomyl; methyl parathion; and atrazine) pesticides, indicating a need of dose adjustment, on the other hand this indicates a potential for environmental protection by HALM.

REFERENCES

(1) Linhares, C. R. ; Lemke, J. ; Auccaise, R. ; Duó, D. A. ; Ziolli, R. L. ; Kwapinski, W. ; Novotny, E. H. *Pesquisa Agropecuária Brasileir*a, 2012, 47, 693-698.

(2) Stenersen, J; Brekke, E.;Engelstad, F. *Soil Biol. Biochem*. 1992, 21, 1761-1764.

(3) Ellman, G.L. et al. *Biochemical Pharmacology*. 1961. 7, 88-95.

(4) Caselli F., Gastaldi L., Gambi N., Fabbri E. 2006. *Comparative Biochemistry and Physiology*, Part C 143, 416–421

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