Bioactivity of Humic Acids from Vermicompost at Increasing Maturity Stages

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1. Introduction

The application of humic substances (HS)-derived products at low concentration and their effects as plant growth promoters have been creating increased interest among farmers. Despite of the technological potential, little information have been accumulated about the mechanism by which HS influences biological activities in plants. It has been widely demonstrated that humic acid (HA) can affect plant growth and metabolism, but scientific efforts linking HA structure to biological activity have so far produced divergent results. The relationship between different levels of bioactivity and variation at chemical structure of HS persists as a challenge for scientific and technological purposes into the direction of improvement of organic fertilizers based on humic matter. It was previously observed that HA induced changes in the developmental program of root growth and in plant development by proliferation of lateral emerging sites and induction of a plasma membrane (PM) H^+-ATPases in different plant species [1]. The aim of this work was to evaluate chemical changes and plant growth-promoting effect of HA from different maturing stages isolated from vermicompost, relating bioactivity and its chemical characteristics.

2. Material and methods

Vermicompost (VC) was prepared using cattle manure putted on concrete cylinder with 150 L of capacity and humidity was maintained at 65–70%. After approximately one month, the earthworm was introduced (*Eisenia foetida*) at a ratio of 5 kg worms per m^3 of organic residue. Two cylinders of each organic residue were used to sample at different time: 0, 30, 60, 90 and 120 days. Before sampling, the content of cylinder was vigorously mixed by manual spade. The VC was chemically characterized (organic carbon, C-N ratio, CEC and HA content). HA were isolated with 0.5M NaOH under N_2 and precipitated with 6M HCl. The diluted HF:HCl was used for 16 hours to decrease the ash content and after that HA was washed with water, dialyzed (membrane cutoff 1000 Da) and freeze dried. The relative Mw

index was obtained by the ratio of low: high and characterized by size exclusion (Polysep-GFC-P 3000 (600 mm per 7.8 mm i.d.) column (Phenomenex) and reverse-phase (Supelco C-18 column) high performance chromatography for hydrophobicity. Maize seedlings with 0.5 cm of root length was treated for 48 h with HA solution (20 mg C L^{-1} of AH and 2 mM $CaCl_2$ at pH 7.0). After this time, the seedlings were transferred to 40 mL of 2 mM $CaCl_2$ at pH 7.0. After 48 h the pH was evaluated using Thermo Orion pHmeter. A preliminary assay was carry-out to verify a putative relationship between H^+ extrusion and PM H^+-ATPase activity [2].

3. Results

Parameters related to organic matter evolution during vermicompost maturation stages. The C-N ratio and lignin content, decreased and CEC and HA content increase (Fig. 1). The relative Mw had shown no change and the hydrophobic content, determined by NMR, showed small changes with the maturation process (Fig. 2). However the hydrophobic content, determined by RP-HPLC showed changes with the maturation process (Fig. 3).

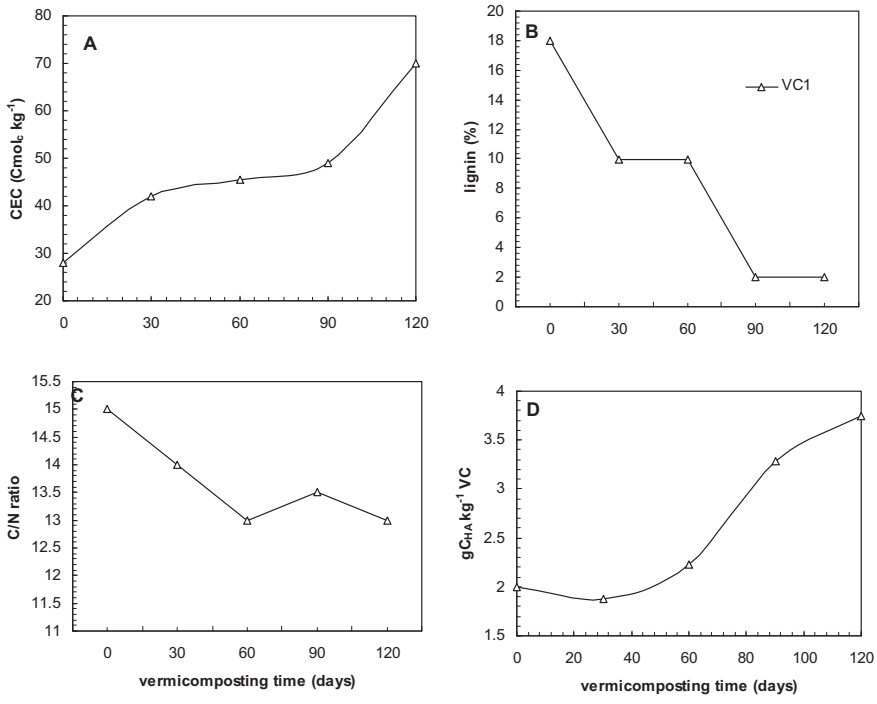

Figure 1: Parameters related to organic matter stabilization according vermicompost maturity time

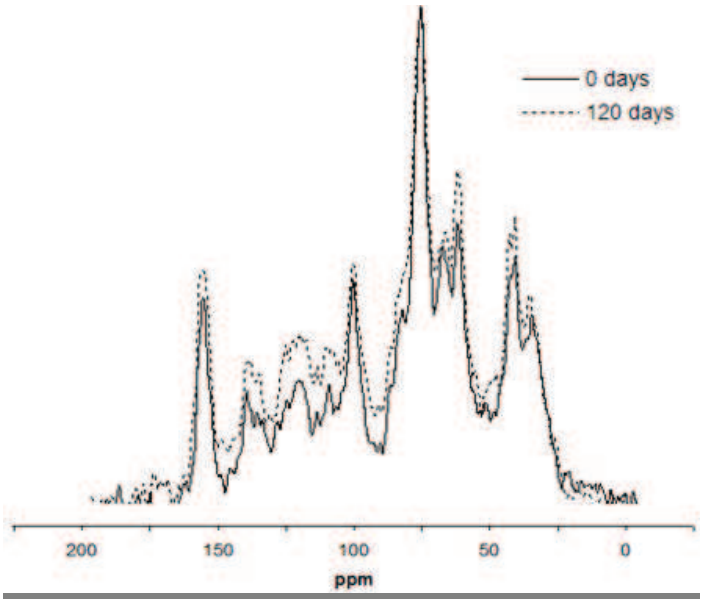

Figure 2: ^{13}C-CPMAS-NMR spectra from vermicompost at different maturing stages

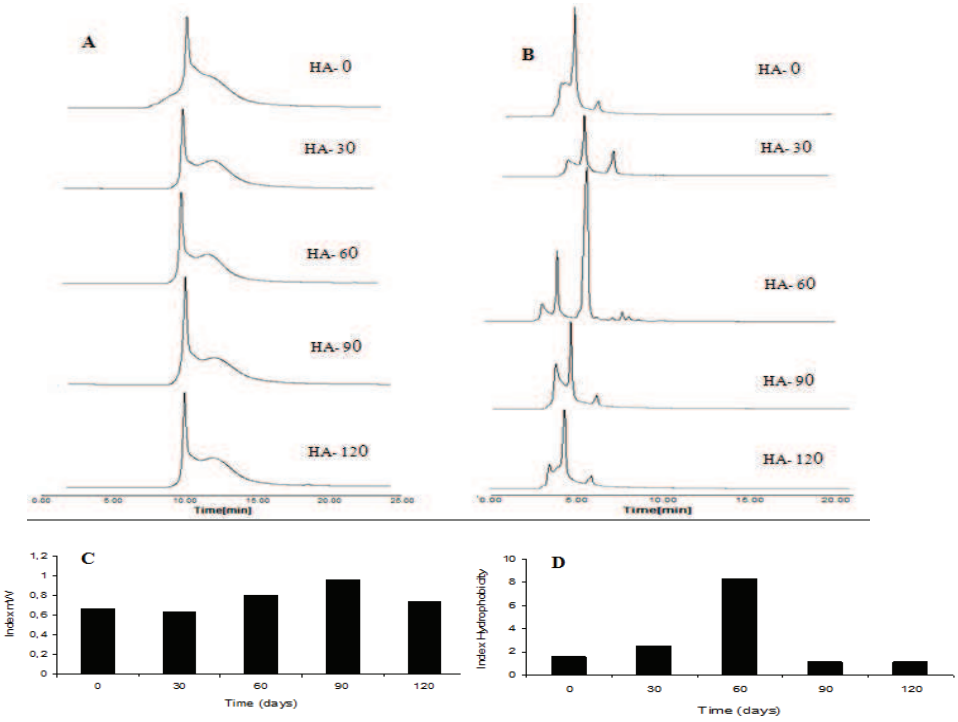

Figure 3: HPSEC chromatograms - Mw distribution (A), RP-HPLC Hydrophobicity (B) index Mw (C) and index hydrophobicity (D) of HA from vermicompost at different maturing stages (0, 30, 60, 90 and 120 days)

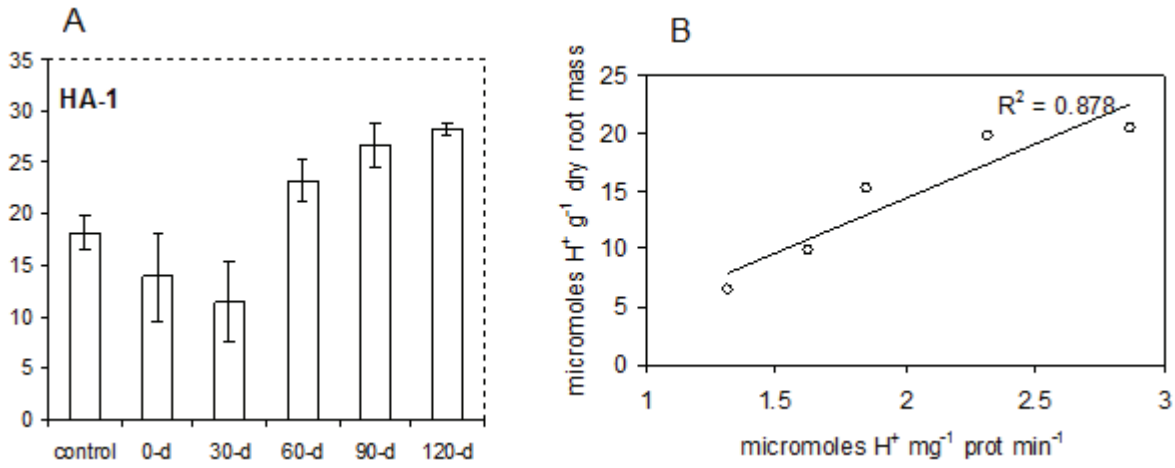

Figure 4: A: Proton extrusion (mM H^+ g^{-1} dry root) for 2 mM $CaCl_2$ solution of maize root seedlings treated with 48 hours of HA isolated from cattle manure vermicompost at different maturing stages (0, 30, 60, 90 and 120 days). B: relationship between H^+ extrusion and PM H^+-ATPase activity

At the end of vermicompost maturation it was observed a selective increase of C-aryl, *O*-aryl and C-carboxyl species as a consequence of carbohydrates decrease (Fig. 2). The relative molecular size/weight (Mw) revealed no changed with HA maturation (Fig. 3A and C), but the hydrophobicity evaluated by RP-HPLC increase at 60-d and after this time decrease (Fig, 3 B and D). After 60-d of vermicomposting process the HA displayed bioactive effect compared with control manifested by increaser of maize root seedlings H^+ extrusion (Fig. 4A), which gave a good correlation with PM H^+-ATPase activity (Fig. 4B).

We concluded that at the initial stages of vermicompost maturing process the HA had diminished plant growth promoting effect. Increased bioactivity of HA evaluated by H^+ extrusion at the solution and PM H^+-ATPase activity by HA was achieved with enhanced vermicompost maturing coupled with increase of hydrophobic domains on its structure, mainly until 60 days. However the form of protection of bioactive molecules in the structure of AH is not even well understood and needs to be more study. Furthermore the process of humification by itself is not a sufficient to explain the promotion of plant growth promoted.

References.

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