Science of the Total Environment 562 (2016) 289–295

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Investigating organic molecules responsible of auxin-like activity of humic acid fraction extracted from vermicompost

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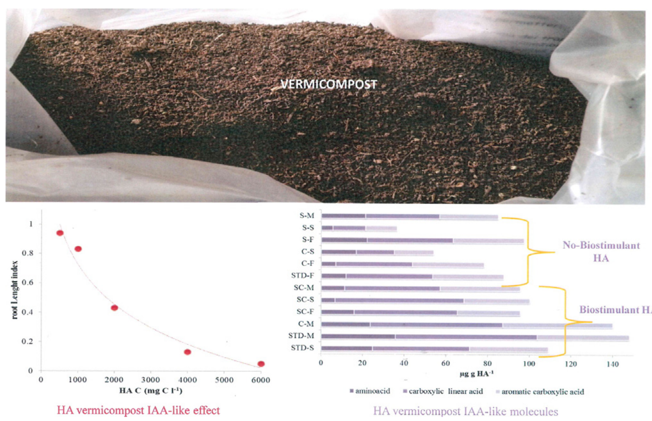
HIGHLIGHTS

- Vermicomposting converts waste into organic fertilizer.
- Vermicomposts can have biostimulating effect for the presence of hormone-like molecules.
- Auxine-like activity was associated to the vermicompost humic acid fraction (HA).
- HA carboxylic acids and amino acids, were reported to act as auxin-like molecules.
- A linear regression was found between molecules and auxin-like activity.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 January 2016
Received in revised form 29 March 2016
Accepted 29 March 2016
Available online xxxx

Editor: D. Barcelo

Keywords:
Auxin-like property
Humic acid
Organic compounds
Tannery effluent
Vermicomposting

ABSTRACT

This work studied the auxin-like activity of humic acids (HA) obtained from vermicomposts produced using leather wastes plus cattle dung at different maturation stages (fresh, stable and mature). Bioassays were performed by testing HA concentrations in the range of 100–6000 mg carbon L^{-1}. ^{13}C CPMAS-NMR and GC–MS instrumental methods were used to assess the effect of biological processes and starting organic mixtures on HA composition. Not all HAs showed IAA-like activity and in general, IAA-like activity increased with the length of the vermicomposting process. The presence of leather wastes was not necessary to produce the auxin-like activity of HA, since HA extracted from a mix of cattle manure and sawdust, where no leather waste was added, showed IAA-like activity as well. CPMAS ^{13}CNMR revealed that HAs were similar independently of the mix used and that the humification process involved the increasing concentration of pre-existing alkali soluble fractions in the biomass.

GC/MS allowed the identification of the molecules involved in IAA-like effects: carboxylic acids and amino acids. The concentration of active molecules, rather than their simple presence in HA, determined the bio-stimulating effect, and a good linear regression between auxin-like activity and active stimulating molecules concentration was found ($R^2 = -0.85$; $p < 0.01$, $n = 6$).

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http://dx.doi.org/10.1016/j.scitotenv.2016.03.212
0048-9697/

1. Introduction

Leather manufacture is a water-intensive process that generates a significant volume of liquid and solid waste (CETESB, 2005). In a typical tanning process, for each 1 mg of salt skin treated by tanning, 0.5 mg of chemicals and 15–40 m^3 of water are used, producing only 200 kg of tanned leather (20% yield) and a disproportionate amount of solid and liquid residues rich in chromium, sulphur and organic matter (CETESB, 2005). Untreated wastewater and solid waste generated by the tanning industry can cause serious environment damage, particularly to surface and ground water. In order to avoid pollution, specific chemical, physical and biological treatments have been adopted to reduce waste toxicity, enhancing waste tannery use/recycling systems.

In this perspective, vermicomposting is reported to be an effective treatment able to reduce the toxic effect of the tannery effluent, improving nutrient availability of organic matter (OM) by heartworm activities, *Eisenia fetida* L. (Selladurai et al., 2010).

Earthworms are able to modify organic waste both physically and biochemically by mixing and grinding the mass, while microbes present in the worms' guts are responsible for biochemical degradation and modification of organic matter (Aira et al., 2008). Moreover, earthworms are able to accumulate heavy metals in their choragogen cells, resulting in detoxification of polluted biomasses (Huges, 1980).

Several types of industrial wastes (i.e. textile mill sludge, sugar industry wastes and paper waste) have been successful vermicomposted to obtain useful and safe composts (Ravindran et al., 2008; Vig et al., 2011). Vermicompost applications have been reported to increase both yield and growth of several food and ornamental crops, since they are composts rich in plant-available nutrients such as nitrates, phosphates and exchangeable calcium and soluble potassium (Arancon et al., 2006). In other cases, vermicomposts have been shown to stimulate plant growth through "hormone induced activity" mechanisms (Arancon et al., 2006; Sinha, 2009).

The biostimulant capacity of vermicomposts has been tested, principally, by using the humic acid (HA) fractions i.e., the most representative fraction of compost (HA concentrations range from 39% dm to 80% dry matter – d.m.) (Sinha, 2009). HAs are defined operationally as representing the alkali-soluble acid-insoluble fraction of organic matter (IHSS, 2015). From a chemical point of view HAs consist of a mix of small organic molecules chemically linked into hydrophilic/hydrophobic supramolecular domains (Piccolo, 2002; Canellas et al., 2002).

Hormone-like activity of vermicompost HA has been attributed to the presence of hormones derived either from raw materials used to produce compost (Arthur et al., 2007) or produced ex-novo during biomass composting (Pathma and Sakthivel, 2012).

Vermicomposting parameters have been reported to affect the microbiota producing plant growth regulators (PGRs). In particular, the maintenance of low temperatures allowed the production of vermicompost with high auxin, gibberellin and cytokine concentrations, compared with compost obtained under higher temperature conditions (Pathma and Sakthivel, 2012).

Among the hormone-like effects of HA, the auxin-like is that most commonly described (Muscolo et al., 1998; Canellas et al., 2002; Zandonadi et al., 2010), being associated with the HA capability to affect root development through vegetal cellular pump activation (Canellas et al., 2002; Zandonadi et al., 2013). In general, the presence of auxin was most likely agent for producing the HA effect on plants (Muscolo et al., 1998; Canellas et al., 2002; Schiavon et al., 2010). Nevertheless, some authors have reported an auxin-like effect independently of the presence of the auxin, ascribing this effect to the presence of specific organic molecules, named hormone-like compounds (e.g., Nardi et al., 2002; Trevisan et al., 2010; Scaglia et al., 2015).

Carboxylic organic acids (oxalic, tartaric, and phenolic acids) and amino acids were reported to have auxin-like effects when they were tested as pure molecules or when they were present as constituents of the organic fraction (i.e. dissolved organic matter fractions) (Pizzeghello et al., 2006; Colla et al., 2014; Singh et al., 2014).

The chemical complexity of HA renders difficult the analytical identification of auxin-like molecules promoting the bio-stimulation effect, and this has led to attempts to identify which of the organic fractions composing HA have auxin-like properties (Dell'Agnola and Nardi, 1987; Canellas et al., 2002; Quaggiotti et al., 2004). Humic acid fractionation into low molecular (HALMW) and high molecular weight (HAHMW), and water-soluble fractions (WHA) (Zandonadi et al., 2013), indicated that molecular weight did not affect the auxin-like activity (Dell'agnola and Nardi, 1987; Muscolo et al., 1998). A possible explanation of this result consists in the fact that hormone-plant receptors interact with small auxin-like molecules present in HA rather than with the total HA (Trevisan et al., 2010; Zandonadi et al., 2013; Canellas et al., 2011). The vermicomposting process affects the auxin-like properties of HA; Dobbss et al. (2010) found that biodegradation processes promoted biostimulant activity, leading to the mature vermicompost having a higher auxin-like activity than the parental materials.

The aim of this work was to study the auxin-like effect of HAs extracted from vermicomposts produced with various mixes incorporating tannery wastes. In particular, by using different analytical approaches, i.e. ^{13}CPMAS NMR and GC/MS, the organic molecules responsible for biostimulant effects were investigated.

2. Materials and methods

2.1. Vermicompost production

This study was conducted using HAs extracted from vermicomposts prepared with tannery wastes in the form of both sludge and tanned chips mixed with cattle manure and sawdust.

The vermicomposters were set up in 25 L plastic barrels containing different proportions of fresh wastes (based on dry volume). The proportion of the substrates was determined by a combination of their C:N ratio. Each barrel received 20 L of the mixture of wastes as follows: i. Sludge mix (S): 14.5 L of cattle manure, 2.5 L of sawdust, and 3 L of sludge; ii. Tanned Chips mix (C): 14 L of cattle manure, 2 L of sawdust, and 4 L of tanned chips (fresh volume); iii. Sludge + Tanned Chips mix (SC): 14 L of cattle manure, 2 L of sawdust, 1.5 L of sludge, and 2.5 L of tanned chips. For comparison, a vermicomposter with no leather wastes was also prepared (standard sample - STD) by mixing 17 L of cattle manure, and 3 L of sawdust by fresh volume.

After the mixtures were made, the material rested for 1 week; then, all of the contents were turned manually once a week.

For vermicompost production, 500 newborn earthworms (*Eisenia fetida* L.) (Sobloco – Santa Candida Farm, São Carlos, Brazil) were added to each vermicomposter. Each vermicomposting was performed in triplicate.

The experiments were conducted during the months of April–August 2014 (135 days) in months characterized by maximum temperatures of: 29 °C (Apr), 27 °C (May), 24 °C (Jun), 21 °C (Jul) and 26 °C (Aug); and minima of 18 °C (Apr), 13 °C (May), 14 °C (Jun), 11 °C (Jul) and 13 °C (Aug). During this time, the rainfall was of 57 mm (Apr), 49 mm (May), 31 mm (Jun), 15 mm (Jul) and 24 mm (Aug). The vermicompostings were carried out at the Laboratório de Química Ambiental (LQA), Instituto de Química de São Carlos (IQSC), Universidade de São Paulo (USP), Brazil (Nunes et al., 2016).

2.2. Vermicompost characterization

In order to characterize the vermicomposts, 1 L samples of organic substrate were collected from each vermicomposter. Then a single combined sample was prepared from the triplicated samples. The material was air-dried, ground and sieved to 0.5 mm.

Samples were characterized for the organic matter (OM) (NEN, 1994), total organic carbon (TOC) (ISO, 1995), pH (ISO, 1994a), cation