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Application of a set of complementary techniques to understand how varying the proportion of two wastes affects humic acids produced by vermicomposting

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ABSTRACT

A better understanding of how varying the proportion of different organic wastes affects humic acid (HA) formation during vermicomposting would be useful in producing vermicomposts enriched in HAs. With the aim of improving the knowledge about this issue, a variety of analytical techniques [UV–visible spectroscopic, Fourier transform infrared, fluorescence spectra, solid-state cross-polarization magic-angle spinning (CPMAS) ^{13}C nuclear magnetic resonance (NMR) spectra, and thermal analysis] was used in the present study to characterize HAs isolated from two mixtures at two different ratios (2:1 and 1:1) of tomato-plant debris (TD) and paper-mill sludge (PS) before and after vermicomposting. The results suggest that vermicomposting increased the HA content in the TD/PS 2:1 and 1:1 mixtures (15.9% and 16.2%, respectively), but the vermicompost produced from the mixture with a higher amount of TD had a greater proportion (24%) of HAs. Both vermicomposting processes caused equal modifications in the humic precursors contained in the different mixtures of TD and PS, and consequently, the HAs in the vermicomposts produced from different waste mixtures exhibited analogous characteristics. Only the set of analytical techniques used in this research was able to detect differences between the HAs isolated from each type of vermicompost. In conclusion, varying the proportion of different wastes may have a stronger influence on the amount of HAs in vermicomposts than on the properties of HAs.

1. Introduction

Vermicomposting is a recycling technology that allows the conversion of diverse organic wastes into stable organic products through an aerobic biodegradation of organic matter by earthworms and microorganisms under mesophilic temperatures. The end products produced by vermicomposting, called vermicomposts, are excellent organic products for agriculture because they are stable organic material rich in plant nutrient and humic acids (HAs). In particular, the amount and quality of HAs contained in vermicomposts can be used to evaluate the safe impact and successful application of these organic products on soils because HAs are considered as important indicators of their degree of maturity and stability (Senesi et al., 2007). Therefore, vermicomposts richer in quality HAs are more valuable as biofertilizers or organic soil amendments for agronomic practices.

In spite of the importance of HA content in vermicomposts, humification processes occurred in vermicomposting are not well known yet. Hence, to improve the knowledge about the humification of organic wastes through vermicomposting, a set of complementary analytical techniques [UV–visible spectroscopic, Fourier transform infrared, fluorescence spectra, solid-state cross-polarization magic-angle spinning (CPMAS) ^{13}C nuclear magnetic resonance (NMR) spectra, and thermal analysis] was used in the present study to characterize the HAs isolated from different mixtures of two complementary organic wastes before and after vermicomposting. These modern analytical techniques have been used by previous studies to determine functional and structural properties of HAs produced by vermicomposting (Campitelli et al., 2012; Campitelli and Ceppi, 2008a; Li et al., 2011; Plaza et al., 2008; Romero et al., 2007; Sen and Chandra, 2007), but none of these techniques provides enough information by itself to characterize HAs. Therefore, the combination of them can overcome the limitations of each one to distinguish among diverse kind of HAs isolated from different vermicomposts.

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The present study specifically investigated how the quantity and properties of HAs produced by vermicomposting of a mixture of two complementary organic wastes might change as a result of varying the proportions of each waste. This topic is relevant since the mixing of two complementary wastes is required for vermicomposting wastes with characteristics that impede an optimal earthworm development. Moreover, a better understanding of how varying the proportion of different wastes affects the formation of HAs during vermicomposting would be useful to produce vermicomposts richer in HAs. To provide insight into this subject, the present study conducted a pilot-scale experiment in which two complementary wastes (viz. tomato-plant debris and paper-mill sludge) were mixed at two different ratios and vermicomposted. Tomato-plant debris and paper-mill sludge were chosen because they are a noteworthy instance of two complementary wastes which could not be vermicomposted unless they were mixed. Tomato-plant debris (TP) from greenhouse crops cannot be processed by earthworms because contain lethal levels of salts and ammonia (Fernández-Gómez et al., 2010b). In the case of the paper-mill sludge (S), which is generated by paper-production industry, previous studies have also reported that this waste needs to be mixed with another complementary waste because its N content is too low for allowing optimum earthworm growth (Elvira et al., 1996). A recent laboratory-scale study demonstrated that mixing TP and S at 2:1 or 1:1 ratio is the most suitable way to improve the earthworm development during vermicomposting and thus producing stable and mature vermicomposts (Fernández-Gómez et al., 2013). Therefore, the present study characterized by using a wide set of techniques the HAs contained in mixtures of TP with S made at those two different ratios (2:1 and 1:1), before and after being vermicomposted at pilot-scale.

2. Materials and methods

2.1. Waste mixtures and vermicomposting processes

Tomato-plant debris (TD) was composed of leaves, stems, and roots from tomato plants discarded from greenhouses after harvest (23% moisture, 7.2 pH, 10 dS m^{-1} electrical conductivity, 386 g kg^{-1} total organic C, 17 g kg^{-1} total N, 87 g kg^{-1} lignin, 116 g kg^{-1} hemicellulose, 287 g kg^{-1} cellulose, 32.4 g kg^{-1} humic acids). This organic waste was collected from a treatment plant for greenhouse wastes located in Motril (Granada, southern Spain), a region where tomato is the predominant greenhouse crop. Paper-mill sludge (PS) was a secondary sludge (64% moisture, 8.2 pH, 0.6 dS m^{-1} electrical conductivity, 175 g kg^{-1} total organic C, 7.3 g kg^{-1} total N, 194 g kg^{-1} lignin, 94 g kg^{-1} hemicellulose, 111 g kg^{-1} cellulose, 17.3 g kg^{-1} humic acids) collected from the wastewater-treatment plant of Torraspapel S.A., a paper-mill company also located in Motril. Earthworms of the species *Eisenia fetida* were collected from a culture bank at Zaidín Experimental Centre (Granada, southern Spain).

Two mixtures of TD and PS were made: one at a ratio of two to one (TD/PS 2:1) and another at a ratio of one to one (TD/PS 1:1), as described in Fernández-Gómez et al. (2013). A total of 18 kg (dw) were separately made for each type of waste mixture. After a 2-week period, in which the mixtures were air dried (20 °C) to eliminate the volatile substances potentially toxic to earthworms (Elvira et al., 1996), each mixture was remoistened and placed in a wooden rectangular vermireactor (1 m^2). Two similar populations of 1000 earthworms (250 ± 1 g total worm biomass) were inoculated in both vermireactors as described in Benítez et al. (2002). Each mixture of TD and PS was vermicomposted separately for 6 months inside a dark room with controlled temperature (24 ± 1 °C). The moisture content of both mixtures was kept at 80% by periodical watering until the end of vermicomposting. The vermireactors were not replicated because previous studies have demonstrated that pilot-scale vermicomposting systems are highly reproducible if the wastes are vermicomposted under controlled conditions (Sen and Chandra, 2007; Suthar, 2010).

At the end of the vermicomposting processes the earthworms were removed by hand. Three independent samples were taken before earthworm inoculation and at the end of vermicomposting from each type of waste mixture. The samples were air-dried and crushed to pass through a 0.5 mm sieve for analysis. Composite samples, which were obtained by mixing equal amounts of the three independent samples taken from each type of waste mixture, were used for humic acid analyses. Thus, the heterogeneity of the waste mixtures was minimised.

2.2. Chemical analyses of initial waste mixtures and produced vermicomposts

The pH and electrical conductivity (EC) were measured in a 1:10 sample:water (dw:v) ratio by using a pH meter and a conductivity meter, respectively. Total organic carbon (TOC) and total nitrogen (N) were determined with a LECO TruSpec CN analyzer (LECO Corporation, St. Joseph, USA). Total P were measured by the nitrovanadomolybdate method and total K by photometry (Williams, 1984). Hemicellulose, cellulose, and lignin were analyzed with the aid of the Ankom 200 Fibre Analyzer following the method described in Goering and Van Soest (1970).

2.3. Isolation of humic acids

Isolation of HAs from the individual TD and PS wastes as well as from the two different waste mixtures: TD/PS 2:1 and TD/PS 1:1, before and after vermicomposting, was carried out following the conventional method described in Schnitzer (1982). Briefly, a solution of 0.1 M $Na_4P_2O_7$ and 0.5 M NaOH was added to the sample ground to 0.5-mm at a weight:volume ratio of 1:3. After shaking mechanically and centrifugation, the supernatant was acidified with 6 M HCl to pH 1.0 and was stood for 24 h to allow the coagulation of the HA fraction, then centrifuged. The HAs precipitated were recovered by dissolving in distilled water. Right after HA solution was dialysed, freeze-dried, and weighed to calculate the HAs content as the extraction yield.

2.4. Chemical characterization of isolated humic acids

The C, H, N, and S contents of isolated HAs were determined using a LECO CHNS-932 elemental analyzer (LECO Corporation, St. Joseph, USA). The O content was calculated by the difference. Carboxyl group content was estimated by direct potentiometric titration as the value of charge at pH 8.0. Likewise, the phenolic hydroxyl-group content as two times the change between pH 8.0 and pH 10.0. The sum of the content in carboxyl group plus phenolic hydroxyl group was used as a measure of total acidity (Ritchie and Perdue, 2003).

2.5. Ultraviolet–visible spectroscopic analysis of isolated humic acids

The spectroscopic characteristics of isolated HAs were determined using a Shimadzu UV-1800 spectrophotometer (Duisburg, Germany). The specific absorptivity (ε) values of isolated HAs were determined by recording absorbance at 280 nm on solutions of 10 mg L^{-1} of HA in 0.1 M KNO_3 at pH 7 (Chin et al., 1994). Absorbance of isolated HAs at 465 and 665 nm were recorded on solutions of 300 mg L^{-1} of HA in 0.05 M $NaHCO_3$ to determine E_4:E_6 ratios (Chen et al., 1977).