



Contents lists available at ScienceDirect

Bioresource Technology

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

Short Communication

Speciation and transformation of heavy metals during vermicomposting of animal manure

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HIGHLIGHTS

- Speciation and mobility of heavy metal in vermicomposting was investigated.
- Earthworm could improve the stability and agriculture values of animal manure.
- Vermicomposting alleviates the mobility and availability of heavy metals.

ARTICLE INFO

Article history:

Received 5 January 2016

Received in revised form 29 February 2016

Accepted 1 March 2016

Available online xxxx

Keywords:

Animal manure

Vermicomposting

Heavy metal

Stabilization

Sequential extraction

ABSTRACT

This work was conducted to evaluate the effects of vermicomposting on the speciation and mobility of heavy metals (Zn, Pb, Cr, and Cu) in cattle dung (CD) and pig manure (PM) using tessier sequential extraction method. Results showed that the pH, total organic carbon and C/N ratio were reduced, while the electric conductivity and humic acid increased after 90 days vermicomposting. Moreover, the addition of earthworm could accelerate organic stabilization in vermicomposting. The total heavy metals in final vermicompost from CD and PM were higher than the initial values and the control without worms. Sequential extraction indicated that vermicomposting decreased the migration and availability of heavy metals, and the earthworm could reduce the mobile fraction, while increase the stable fraction of heavy metals. Furthermore, these results indicated that vermicomposting played a positive role in stabilizing heavy metals in the treatment of animal manure.

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1. Introduction

Land application is one of the most effective methods for handling with animal manure as considerable quantity of organic matter and nutrient in it (Lazcano et al., 2008). Prior to application in agricultural fields, bio-stabilization technique was usually used to convert the animal manure into value-added and biological stabilized product (Hsua and Lob, 2001). Vermicomposting is a novel technique that utilizing the joint action of earthworm and microorganism under aerobic condition. In this method, most of the organic components can be degraded and the residuals are transformed into stabilized vermicompost, which is rich of nitrogen, phosphorus, potassium and humic substance (Song et al., 2014). Besides these positive effects, the heavy metals (e.g. Zn, Cu, Pb, Cr) in vermicompost have gained more and more attention (Li et al., 2009; Suthar et al., 2014). Unlike bio-degradable compounds,

heavy metals can't be decomposed in vermicomposting process (He et al., 2009). Moreover, the heavy metals can accumulate in soil, uptake by plants, and finally affect the health of animals and people through food chain (Liu et al., 2005). Therefore, it is necessary to explore the changes and transformation of heavy metals in vermicomposting.

The total content of heavy metals in final vermicompost and the effect of earthworm on that were reported by several authors (Goswami et al., 2014; Suthar et al., 2014). Song et al. (2014) reported that the heavy metals (As, Pb, Cu, and Zn) in vermicompost were clearly higher than the initial values due to organics degradation by vermicomposting. Conversely, a reduction in heavy metal (Pb, Cd, Cr, and Cu) contents after sludge vermicomposting was found by Suthar et al. (2014), which might be caused by the high accumulation of heavy metals in earthworm tissues. Nevertheless, most of these studies focused on total concentrations and neglected the fractionation of metals. According to previous findings, the mobility and bioavailability of trace metals largely depend on their specific chemical speciation rather than total

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content (He et al., 2009; Wang et al., 2013). Although several reports indicated that vermicomposting could significantly reduce the heavy metals extracted by diethylene triamine pentacetic acid (Song et al., 2014), the speciation and transformation of heavy metals in vermicomposting have not been fully understood.

Sequential extraction can reveal the mobility and potential risk of heavy metals under different environmental conditions (Yuan et al., 2011). He et al. (2009) reported that aerobic composting could decrease the heavy metals belonging to exchangeable and carbonate fraction, while increase the oxidizable and residual forms of heavy metals. Recent study conducted by Singh and Kalamdhad (2013) also indicated the exchangeable and carbonate fractions of Cu, Ni, and Cr were reduced during vermicomposting of water hyacinth. However, limited data are available that use sequential extraction to investigate the composition and transformation of heavy metals in animal manure vermicomposting. Therefore, cattle dung (CD) and pig manure (PM) were selected as the substrates of vermicomposting, and the main objectives of this study were to: (1) study the variations of heavy metal (Zn, Pb, Cr and Cu) speciation in raw substrates and the final vermicompost; (2) evaluate the effect of vermicomposting on mobility and bioavailability of heavy metals.

2. Methods

2.1. Substrates and vermicomposting

CD and PM were procured from a livestock farm in the rural areas of Pudong New District, Shanghai, China. The raw manure was dried under sunlight for one week with periodic stirring before the experiment. Young non-clitellated earthworms *Eisenia fetida* raised in laboratory were picked for further using.

Vermicomposting was carried out in bench scale vermireactor (30 cm diameter × 20 cm depth, plastic container) with each fed with 500 g CD and PM (dry weight) as the substrate, respectively. 50 non-clitellated earthworms (*E. fetida*) with 200 mg average individual live weight were inoculated into the reactors. Three replicates were established for each reactor and the container without adding earthworms was conducted as control. Moisture content was kept at 70–90% by sprinkling distilled water. Vermireactors were put in dark with temperature maintaining at 23 ± 1 °C.

The samples collected at initial (0 d) and the end of experiment (90 d) from vermicomposting and control were air-dried, ground and sieved through a 0.15 mm nylon mesh for further analysis. 5 g worms were randomly selected and placed in dark for 24 h to clear their gut, cleaned by distilled water, and freeze-dried prior to heavy metals analysis.

2.2. Physico-chemical analysis

The pH and electrical conductivity (EC) were determined by a pH and conductivity meter in water extracts (1:20, w/v), respectively. An elemental analyzer Vario EL III (German) was used to measure the C and N contents in samples. The humic acid-like C (HAC) and fulvic acid-like C (FAC) were measured according to the report of Romero et al. (2007).

2.3. Heavy metal analysis

Sequential extraction recommended by Tessier et al. (1979) was used to determine the speciation of Zn, Pb, Cr and Cu in samples. The heavy metal was divided into five fractions: exchangeable (F1), bound to carbonates (F2), bound to Fe and Mn oxides (F3), bound to organic matter (F4), and residual (F5). The specific extraction procedure was given in Table S1.

The plastic containers and glassware were soaked in nitric acid over-night and cleaned with deionized water before use. 1.0 g dried sample was used to extract heavy metals in 50 mL polypropylene centrifuge tubes. After each extraction, the supernatant was collected by centrifugation at 8000g for 5 min, filtered through a 0.45 µm membrane and diluted to volume. The residue was washed with 10 mL of Milli-Q water by shaking, then centrifuged at 8000g for 5 min to recover the solids without loss. Heavy metal concentrations in all extracts were determined by inductively coupled plasma optical emission spectrometry (ICP) (Optima 2100 DV, PerkinElmer). Heavy metal contents in earthworm tissue were digested using HNO₃–HF–HClO₄ and determined by ICP as well.

2.4. Statistical analysis

All results were presented as the average of three replicates. The nutrient and heavy metal contents were expressed on dry weight basis. One-way ANOVA following Tukey's test was used to identify the significant differences between raw material and final products with or without earthworms by SPSS 17.0. The probability level used for statistical significance was $p < 0.05$ for all tests.

3. Results and discussion

3.1. Performance of vermicomposting

Vermicomposting significantly modified the physical and chemical properties of raw substrates (Table S2). The pH was obviously lower in final vermicompost and control as compared to the initial manure. This might be ascribed to the generation of organic acids from microbial metabolism during decomposition of raw substrates (Lazcano et al., 2008; Yadav and Garg, 2011). Moreover, the nitrification could decrease the pH as well. On the contrary to pH, both vermicomposting and the control clearly increased the value of EC in CD and PM. The release of soluble salts, such as phosphate, ammonium and nitrate could contribute to the increase of EC (Lazcano et al., 2008). It should be noted that vermicompost had higher EC than the control, which indicating that more available mineral salts are accumulated in vermicompost over the control by organic matter degradation.

As shown in Table S2, vermicomposting led to a significant stabilization in organic matter as reflected by the decline of total organic carbon (TOC). Moreover, the loss of TOC was more in vermicompost (23.6% and 22.6% for CD and PM, respectively) than the control (11.2% and 11.6% for CD and PM, respectively) in comparison with initial materials. The combined action of earthworms and microorganisms was the main reason for more TOC loss from raw manure as relative to the control (Yadav and Garg, 2011). C/N ratio is an important indicator to assess the maturity of compost or vermicompost. In this study, the reduction of C/N ratio (43.6% and 28.9% for CD and PM, respectively) during vermicomposting was much more significantly as compared to the control (30.2% and 16.0% for CD and PM, respectively) after three months experiment. Therefore, it can be concluded that the addition of earthworms could accelerate the decomposition of organic matter and improve the stability of CD and PM.

A great increase of HAC was observed in the final vermicompost (Table S2), which was consistent with previous studies in vermicomposting of livestock residues or other organic wastes (Romero et al., 2007). These results suggested that vermicomposting could stabilize raw organic wastes by increasing the humus contents in substrates. Moreover, larger HAC was found in vermicompost than that in the control, which might indicate vermicompost characterized with higher humification degree. The

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