Bioavailability Shift of Heavy Metals in Vermicomposting: Investigating a Potential Approach to Climate Change Mitigation

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ABSTRACT

Organic wastes if properly recycled can mitigate greenhouse gas emissions. Using it as soil amendment through vermicomposting must cover an understanding on the behavior of pollutants, such as heavy metals, in the process. The study aimed to identify the bioavailability shift of Pb, Cr, Ni, Cu and Zn during vermicomposting of combined farm wastes (crop residue + spent mushroom substrate + cow manure) using the Tessier sequential extraction procedure to extract the exchangeable and carbon-bound metal fractions. Concentrations of heavy metals in the substrates before and after vermicomposting, growth performance and heavy metal bioaccumulation of *Eisenia fetida* in three cycles of 45 days each were also studied. Test results revealed an increase in bioavailability for Cu (43.85%), Zn (40.76%) and Ni (16.34%); while bioavailability of Cr and Pb decreased by 12.97% and 1.43% after vermicomposting. Resulting vermicompost assessments showed a significant reduction in the concentration of metals, Zn (40.21 - 49.45%), Ni (20.89 - 54.05%), Cu (27.76 - 53.54%), Cr (9.71 - 22.03%), and Pb (1.97 - 32.93%). Analysis of metal concentrations in earthworms revealed a considerable bioaccumulation of heavy metals in their bodies. Overall, the increase in the bioavailability of some heavy metals was compensated by the reduction of the total heavy metal concentrations in the resulting vermicompost, rendering the concentrations of bioavailable heavy metal fractions lower than their initial values.

INTRODUCTION

Healthy living and production systems that abate carbon dioxide concentrations in the atmosphere are needed much more now than ever. Vermicomposting plays a part in the fight against climate change due to climate-related benefits we get from worms. Compost worms help in minimizing greenhouse gases (GHG) (Chan *et al.*, 2011). Adoption of this technology to produce organic fertilizer keeps organic wastes out of landfills leading to reduction if not totally eliminate emission of landfill gasses. Organic fertilization through use of vermicompost can effectively reduce manufacture and use of synthetic fertilizers which are known to have huge carbon footprints. Further, nitrogen fertilizers applied to soil usually release nitrous oxide (N_2O), an even more potent GHG than methane (Matthews, 1994). Vermicompost increases the level of carbon in soils through a process known as soil-carbon sequestration. More carbon stored in soils increases both nutrient content and beneficial microbial life rendering healthier soils that are more resilient to changing temperature and moisture levels

allowing more plant growth and eventually more stored carbon (Sinha and Heart, 2012). However, global terrestrial ecosystems have become highly polluted due to industrial activities. In effect, most of the organic wastes being utilized are not spared from pollution. There is a strong belief that production and application of vermicompost can potentially contaminate the soil and other organisms with heavy metals specially so if substrates are exposed to these contaminants. The total concentration of contaminants in the soil does not reflect the extent of biological damage (Nahmani *et al.*, 2007), and provides little information on the chemical-specific bioavailability, mobility, or reactivity in the soil (Lu, 2005). Rather, organisms respond only to the bioavailable fraction (Harmsen, 2007). Therefore, a study was conducted to identify the bioavailability shift of heavy metals (Pb, Cr, Ni, Cu and Zn) in combined farm wastes (crop residue + spent mushroom substrate + cow manure) vermicompost under field conditions as well as the heavy metal bioaccumulation in earthworm tissues. Growth performance of *E. fetida* in combined farm wastes system was likewise monitored in this study.

MATERIALS AND METHODS

Preparation of materials

Eisenia fetida were collected from a worm breeding farm in Yueyang, Hunan, China and cultured in the lab for two months. Farm wastes, namely, crop residues (sugarcane leaves and rape), spent mushroom substrates and cow manure, were collected locally from the experimental farms and projects of Hunan Agricultural University. The cow manure was sun dried and crushed. Crop residues were shredded upon collection while spent mushroom substrates were crushed to finer sizes to hasten decomposition. The cow manure, crop residue, and spent mushroom substrate were thoroughly mixed in 1:1:1 (w/w) proportions. The mixture was pre-composted in a covered container for 14 days to initiate thermophilic decomposition, and maintained at 60% moisture content with tap water.

Vermicomposting farm waste mixtures

Vermicomposting was carried out in the field experiment base of Hunan Agricultural University to expose the earthworms to actual field conditions. Two medium-sized rectangular plastic containers (33 cm x 22 cm x 19 cm) served as vermireactors (one as duplicate). The reactors were started with 1.5 kg of feed material. Two hundred healthy adult *E. fetida* were introduced in each reactor. The vermireactors were sprinkled with tap water as needed to maintain 60-80% moisture content. Watering was stopped 5 days before harvesting. After 45 days, the worm castings were harvested. The earthworms and juveniles were manually separated in another container while collected vermicompost was kept in another container. The original earthworms in each treatment were weighed and reintroduced to freshly prepared reactors. All subsequent measurements were taken once in 45 days in the manner described above, resetting the vermireactors each time so that the same sets of worm with which the rectors were started continued to be the principal producers of vermicast. A total of three vermicomposting cycles of 45 days each were conducted during the whole study.

Measurement of operational characteristics

Moisture content was determined by squeeze method on randomly selected areas of each reactor. Temperature was measured daily by piercing a laboratory thermometer in randomly selected areas of each reactor. Similarly, pH was determined using Lei Ci PHS-3C pH meter at 1:5 (w/v) sample: H_2O ratio.

Earthworm biomass and reproductive success

Biomass was determined by removing all earthworms from each container, washed

with tap water and pat dried with paper towels to remove excess water adhering to the worms' body. They were then weighed in a container with 50 ml water using a digital balance. This was done to prevent the worms from desiccation and affect the weight of the earthworms. Reproductive success was determined by counting the number of juveniles and hatchlings collected at the end of every cycle.

Total and bioavailable heavy metal analysis

Heavy metal content, namely, Copper (Cu), Chromium (Cr), Lead (Pb), Nickel (Ni), and Zinc (Zn) in the prepared substrate were determined prior to vermicomposting and at the end of each cycle. The level of heavy metal accumulations in the earthworm tissues was also estimated after vermicomposting. At the end of the third cycle, 50 earthworms were collected from each reactor and placed on wet filter paper in Petri dishes for a period of 48 hours to allow depuration of their gut contents. This was done to prevent misleading results concerning actual heavy metal content in the body tissues as a result of heavy metals present in the gut contents. Afterward, the worms were washed in distilled water, pat dried on paper towels, and killed by freezing. Substrates and vermicompost samples were oven dried at 60ºC until constant weight, ground then sieved through 0.1mm screen. The earthworm, on the other hand, were oven dried at 65ºC for 4 days and crushed. Two tenth (0.2) grams of each sample was placed individually in a plastic crucible with 5ml hydrochloric acid (HCL), heated on an electric heating board (medium heat, about 1 hour) to initiate digestion. When liquid concentrations was less than 2 ml, the crucibles were removed from heat and cooled, 8ml nitric acid (HNO_3) was added then reheated until the liquid solution was almost viscous. The samples were removed from heat, cooled, then 5ml hydrofluoric acid (HF) was added then brought to a low heat until liquid content was about 1ml. Samples were then removed from heat, cooled, and 3ml perchloric acid ($HClO_4$) was added. Samples were then reheated until white fumes were emitted and liquid fraction was already viscous. The residue was allowed to cool then diluted with 10ml 1% HNO3 then transferred to 50ml test tubes. Resulting solutions were brought to 50ml volume with double-distilled water then filtered and analyzed by Flame Atomic Absorption Spectrometry (TAS-990 Super AAS-PGeneral) for the different metals.

Bioavailable heavy metal fractionation

The exchangeable and carbonate-bound metal fractions were determined following the sequential extraction technique proposed by Tessier *et al.* (1979). One gram of each sample was weighed and placed in 50ml centrifuge tube and analyzed using the following steps:

1. Exchangeable: 8ml 1M $MgCl_2$ solution was added to each sample then agitated at room temperature for 1 hour, centrifuged, decanted, and brought to a 50ml volume with double-distilled water then filtered.
2. Bound to carbonates: After washing the residue obtained from step 1 with double-distilled water, 8ml 1M sodium acetate solution (CH_3COONa) was added, then adjusted to a pH level of 5.0 with acetic acid (CH_3COOH). Samples were agitated periodically at room temperature for 5 hours, centrifuged, decanted, and brought to a 50ml volume with double-distilled water and filtered.

Bioavailability of each heavy metal is defined as the sum of the concentrations of the exchangeable and carbonate-bound fractions compared to the total heavy metal concentration in vermicompost in each cycle.

RESULTS AND DISCUSSION

pH and temperature

pH was measured before the start of cycle 1 (C1) and at the end of each cycle. A significant increase in pH was clearly observed

Table 1: Selected physico-chemical parameters and earthworm performance before and after vermicomposting

Time	pH	Ave. Ambient Temp.(min-max °C)	Earthworm Biomass (g)	No. of hatchlings and Juveniles
Initial	7.95±0.01	-	43.70±4.32[a]	0.00
1st Cycle (C1)	7.91±0.01	17.34 - 24.12	60.92±0.82[b]	874.00±14.14[b]
2nd Cycle (C2)	8.68±0.39	23.91 - 31.56	60.37±1.01[b]	961.50±34.65[c]
3rd Cycle (C3)	8.72±0.28	26.23 - 35.17	60.34±4.04[b]	120.00±28.28[a]
Mean of all Cycles	8.31	26.39	60.54	651.83
ANOVA F	-	-	15.47	583.64
p	-	-	0.011	0.000

Mean value of 2 replicates ± S.D.
a,b Means with the same letter were not significantly different

after vermicomposting except in C1 where it was a bit lower than the initial pH (Table 1). Ambient temperature was observed to be increasing during the vermicomposting cycles.

Suitable conditions for growth of *E. fetida* was 0-35°C, a moisture range of 60-90% moisture, and a pH 5-9 (Edwards and Arancon, 2005). Favorable growth conditions were provided in this study and a 100% survival rate for the earthworms was observed. The significant rise in pH levels in the substrates after vermicomposting could be the effect of the earthworms ability to secrete calcium compounds (Hu *et al.*, 1998) by calciferous glands or by producing alkaline urine (Salmon, 2001) or high activity of gut enzyme alkaline phosphatase (Pramanik *et al.*, 2007).

Growth performance of *E. fetida*

Earthworm biomass significantly increased ($F = 15.47$, $p < 0.05$) from 43.70g to 60.92g after feeding on the substrate in C1, and maintained an almost constant biomass in the succeeding two cycles (Table 1). Overall, the earthworms attained a mean biomass of 60.54 g in three cycles, a 38.54% increment from the initial weight. The number of hatchlings and juveniles were observed to be high in C1 and C2 with an average of 874 and 961 earthworms, respectively and dropped in C3 having an average 120 earthworms.

Increase in earthworm biomass indicated that they readily accepted the new bedding materials. In the case of earthworm fecundity, disparity within cycles can be explained by cocoon production. Cocoons can be produced at any time in the year, but most species of earthworms produce cocoons when the temperature, soil moisture, food supplies and other environmental factors are suitable. Earthworms exhibit fairly complex responses to changes in temperature and most studies conducted to this effect showed that cocoon production was restricted more by temperature. The species studied produced most of the cocoons at 25°C (Dominguez and Edwards, 2004) and can tolerate a temperature range of 0-35ºC (Edwards and Arancon, 2005) Temperature was in increasing trend during the composting period and the high average minimum and maximum recorded ambient temperatures (26.23-¬35.17°C) during C3 already fall in the upper temperature limit for the earthworm thus reducing their cocoon production.

Heavy metals in substrates

Concentrations of Cr, Cu, Ni, Pb and Zn in substrates before and after vermicomposting are presented in Table 2. Zn concentration was highest in the initial substrate followed by Cr, Cu, Ni and Pb. The resulting vermicompost in all cycles showed statistically significant reduction in metal contents compared to the

Table 2: Total heavy metal in substrates before and after vermicomposting

Time	Heavy Metals (mg/kg)				
	Cr	Cu	Ni	Pb	Zn
Initial	81.51±0.86[b]	75.63±4.25[c]	67.53±0.92[c]	46.08±2.28[b]	316.71±3.94[c]
1st Cycle (C1)	73.60±9.21[ab]	54.33±1.81[b]	53.43±9.20[b]	30.90±4.53[a]	186.88±6.18[b]
2nd Cycle (C2)	65.38±5.61[ab]	35.14±0.49[a]	37.80±0.33[a]	44.52±3.46[b]	189.37±3.95[b]
3rd Cycle (C3)	63.56±4.07[a]	54.64±3.34[b]	31.03±1.80[a]	45.17±1.73[b]	160.09±9.29[a]
Mean for all Cycles	67.51	48.04	40.75	40.19	178.78
ANOVA F	15.02	66.80	24.07	10.21	253.46
p	0.012	0.001	0.005	0.024	0.000

Mean value of 2 replicates ± S.D.
a,b Means with the same letter were not significantly different

initial substrate except for Pb. Overall reduction rates of heavy metals in three vermicomposting cycles were in the following order: Zn > Ni > Cu > Cr >Pb. Reduction of heavy metals in the vermicompost could be partially due to the metabolic action of earthworms. Earthworms (especially *E. fetida*) can bioaccumulate high concentrations of metals, including heavy metals in their tissues, without affecting their physiology particularly when the metals are mostly non-bioavailable (Sinha *et al.*, 2010). He *et al.* (2009) attributed the loss of heavy metals in sludge compost to leaching of soluble metals during the mesophilic and thermophilic stages of composting. To overcome this effect, excess water from vermireactors were captured and returned back to the vermireactors.

Bioavailable heavy metals in substrate and vermicomposts

Concentrations of exchangeable and carbonate-bound heavy metals in substrates before and after vermicomposting are presented in Table 3 and Table 4, respectively. Analysis of variance (ANOVA) showed significant differences among the cycles especially when compared to the initial concentration before vermicomposting, except for Cr and Cd in the carbon-bound fraction. In the initial substrate, Cr and Pb registered the highest exchangeable fraction concentration followed by Cu, Ni and Zn, while Zn registered the highest carbon-bound concentration followed by Cr, Pb, Ni and Cu. Concentrations of heavy metals in the two fractions at the end of every cycle (except carbon-bound Cu) were lower than the initial concentrations, although this does not indicate that bioavailability is also lower.

Bioavailability of heavy metals in substrate before and after vermicomposting are shown in Figure 1. Bioavailability distribution of the different metals exhibited different patterns during the whole study. Overall, bioavailability of Cu has increased by 43.85%, 40.76% in Zn and 16.34% in Ni. On the other hand, bioavailable Cr was reduced by 12.97% and Pb by 1.43% in three vermicomposting cycles.

The increase and decrease of the bioavilability of heavy metals in vermicompost could be partially explained by the following factors: 1) increase in vermicompost pH might enhance affinity for heavy metals due to pH-dependent surface-charge density of colloids present thus leading to lower concentrations in the vermicompost (Shan *et al.*, 2002); and 2) microbial communities indigenous to the earthworm species and earthworm activities increase microbial population and immobilize metals in the vermicompost by several processes. Bacterial cells attain extremely high ratio of surface area to volume, which provide a strong ability of adsorbing and immobilizing heavy metal ions from soil solution. As a result,

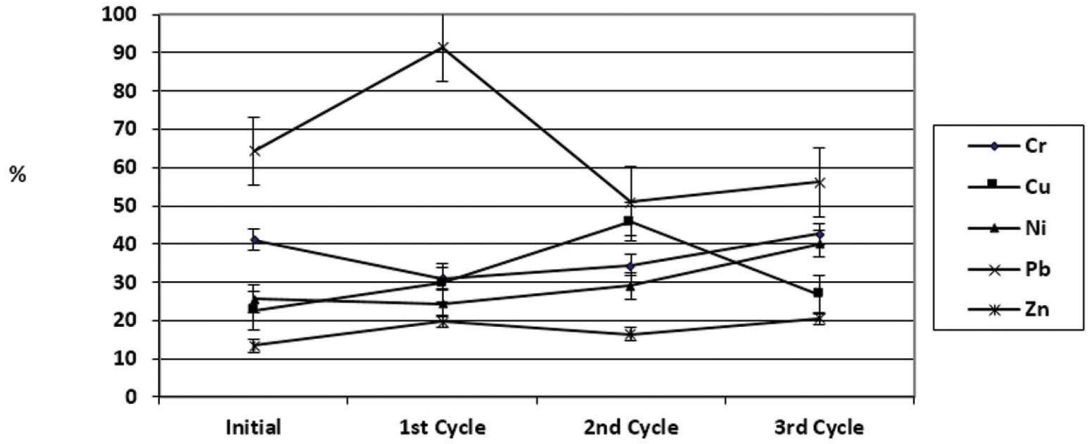

Figure 1: % Bioavailability of heavy metals in substrate before and after vermicomposting

Table 3: Exchangeable heavy metal in substrates before and after vermicomposting

Time	Heavy Metals (mg/kg)				
	Cr	Cu	Ni	Pb	Zn
Initial	20.15±0.43^{d}	8.76±0.04^{c}	8.04±1.07^{c}	20.02±0.24^{c}	6.14±0.08^{c}
1st Cycle (C1)	10.65±0.15^{a}	7.48±0.13^{b}	6.34±0.28^{ab}	18.82±0.57^{c}	3.68±0.13^{b}
2nd Cycle (C2)	12.23±0.12^{b}	7.60±0.37^{b}	4.84±0.25^{a}	14.71±0.93^{a}	2.48±0.26^{a}
3rd Cycle (C2)	16.30±3.96^{c}	6.77±0.00^{a}	7.27±0.16^{bc}	16.66±0.03^{b}	3.80±0.18^{b}
Mean for all Cycles	13.05	7.28	6.15	16.73	3.32
ANOVA F	390.20	35.41	11.59	35.11	148.32
p	0.000	0.002	0.019	0.002	0.000

Mean value of 2 replicates ± S.D.
a,b Means with the same letter were not significantly different

Table 4: Carbonate-bound heavy metal in substrates before and after vermicomposting

Time	Heavy Metals (mg/kg)				
	Cr	Cu	Ni	Pb	Zn
Initial	13.36±3.35^{a}	8.36±0.05^{b}	9.28±0.18^{d}	9.59±0.31^{b}	36.19±0.06^{b}
1st Cycle (C1)	12.20±0.58^{a}	8.68±0.11^{c}	6.73±0.28^{c}	9.43±0.62^{b}	33.44±0.49^{b}
2nd Cycle (C2)	10.28±1.75^{a}	8.49±0.17^{bc}	6.15±0.11^{b}	8.07±0.28^{a}	28.68±2.47^{a}
3rd Cycle (C2)	10.81±1.71^{a}	7.88±0.06^{a}	5.15±0.18^{a}	8.70±0.23^{ab}	29.04±1.20^{a}
Mean for all Cycles	11.10	8.35	6.01	8.73	30.38
ANOVA F	0.89	19.63	162.44	6.47	13.5
p	0.520	0.007	0.000	0.051	0.015

Mean value of 2 replicates ± S.D.
a,b Means with the same letter were not significantly different

Table 5: Heavy metals accumulated by earthworms reared in combined farm wastes

Metals	Concentration (mg/kg)
Cr	10.6±0.24
Cu	37.13±0.14
Ni	16.39±0.32
Pb	5.73±0.37
Zn	119.71±6.96

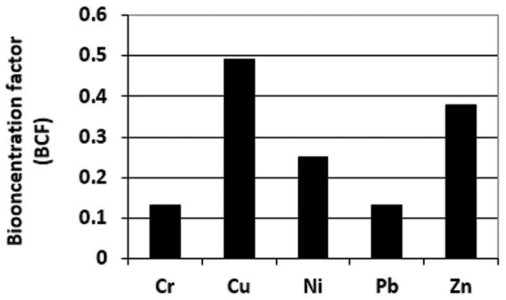

Figure 2: Bioconcentration factor of different heavy metals accumulated in earthworms in relation to initial substrate metal concentration

a shift from bioavailable to less bioavailable binding sites can be expected for heavy metals (Beveridge and Schultze-Lam, 1995), and this could be true to Cr and Pb in this study. In contrast, the increase in bioavailability of Cu, Zn, and Ni could be associated with the activities of earthworms and soil microorganisms as influenced by the presence of earthworms as well. There must be some heavy-metal-chelating metallophores produced by earthworms (Wen *et al.*, 2004). Similarly, these metallophores can also be produced by microbes such as strains of *Pseudomonas* and *Enterobacter*, which often exist in soils (Neilands and Leong, 1986).

Heavy metals in earthworms

Concentrations of heavy metals accumulated in earthworm tissues are presented in Table 5. Chemical analysis of earthworms revealed a considerable accumulation of heavy metals in their body. The bioconcentration factors (BCFs) or the concentration of heavy metal in earthworms relative to the concentration of same heavy metal in the substrate (Fig. 2) are perfectly consistent with the increase and decrease of percent bioavailability of heavy metals after vermicomposting. This could mean that the bioaccumulation factor of heavy metals in earthworm tissues could be directly proportional to the change of bioavailability of heavy metals after vermicomposting. The difference among different metals for BCFs could be related to the difference in specific metal regulating mechanism in earthworms.

CONCLUSION

Based on the findings of the study, the following conclusions are drawn: Substrate total heavy metal, exchangeable and carbonate-bound heavy metal fraction concentrations and heavy metal bioavailability were affected by the vermicomposting process. Although bioavailability of some heavy metals increased, total exchangeable and carbon-bound heavy metal concentrations in the vermicomposts did not increase. Decrease in total heavy metals can be attributed to the activity of earthworms, influence of earthworms to soil microorganisms, bioaccumulation in earthworm tissues and possible leaching through drainage of excess water. Although heavy metal contents in the vermicompost produced were below the limits set by Chinese, US and European standards, care on sourcing of substrate materials as to knowledge to its exposure to heavy metals must be adhered to attain maximum efficiency in terms of protection from possible pollution of bioavailable heavy metals in agriculture and climate change mitigation.

Although results have shown a high variability in the bioavailability shift and accumulation of different heavy metals using the chosen substrates and earthworm species, the study provided a wider lens on the behavior of the specific pollutants in vermicomposting.

It was indicated that vermicomposting using *E. fetida* can redistribute heavy metal mobility in different directions, thus, further studies are needed using different substrates and earthworm species, expanding the scale to bigger vermicompost production and trying other sequential extraction procedures to further elucidate the findings.

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