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BIOCONCENTRATION OF HEAVY METALS IN VERMICOMPOSTING EARTHWORMS (*Eisenia fetida, Perionyx excavatus* and *Lampito mauritii*) IN NEPAL

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ABSTRACT

Vermicomposting of organic waste can play an important part during the waste management process in larger cities such as Kathmandu where 70% of the waste generated is organic. In this study, the possibility of heavy metal (Pb, Cd, Cu and Cr) bioaccumulation by three different species of earthworms *Eisenia fetida, Lampito mauritii* and *Perionyx excavatus* in domestic waste vermicompost was investigated. Quantification of heavy metals by Atomic Absorption Spectroscopy(AAS) in final vermicompost showed a significant reduction in concentration of metals, Pb (11.4-26.0%), Cd (48-61%), Cu (4.9- 29.01%) and Cr (18.90-33.60%) at the end. Bioaccumulation of heavy metal in the composting earthworms was also recorded. Comparison of the three groups of earthworms showed that the bioaccumulation of Pb, Cu and Cr was greater for *P. excavatus* whereas *E. fetida* was the most reluctant. Heavy metal content in the vermicompost was within the limit of USEPA for Biosolids and the compost could be used for the agriculture purpose.

Keywords: Bioconcentration factor, *Eisenia fetida*, heavy metals, *Lampito mauritii, Perionyx excavates,* vermicompost

INTRODUCTION

Vermicomposting is the process by which earthworms are used to convert organic materials (usually wastes) into a humus-like soil conditioning material known as vermicompost. Of the 5,500 species of earthworms found in the world only few species are preferred for use in vermicomposting technology **(Dennis *et al.*, 2010)**. More than 70% of waste generated from the households in Nepal is organic which should be given high priority for its management. The best alternative to filling this waste in landfill sites is to use the household waste as a source for vermicomposting. During 1990s, this technology was introduced and then popularized in Nepal. The most common worms that are used for vermicomposting are *E. fetida, L. rubellus, P. excavatus,* and *L. mauritii*. Use of chemical fertilizers and pesticides was never a reliable method for increasing food productivity. These petrochemical based products are not available indefinitely and above all they have negative impact on the environment and human health. As an alternative, vermicompost is a highly nutritive organic fertilizer and provides high ratio of NPK when applied to the soil **(Lotzof, 1999)**. They can build up soil, restore soil fertility and sustain farm production. Vermicomposting of household waste produces no pollution or unusable residue that makes it very much effective form of recycling.

Wastes in big cities may contain considerable amounts of heavy metals and when processed by earthworms only then can be reduced in metal content (**Jordao *et al.*, 2006**). Earthworms (especially *E. fetida*) can bioaccumulate high concentrations of metals including heavy metals in their tissues without affecting their physiology and this particularly when the metals are mostly non-bioavailable. They can readily bioaccumulate cadmium, mercury, lead, copper, manganese, calcium, iron and zinc and extremely high amounts of Zn, Pb and Cd. Cadmium levels up to 100 mg kg^{-1} dry weight have been found in tissues (**Ireland, 1983**). Also, during the vermicomposting process, the ability of earthworms in removing heavy metals from the final product i.e. vermicompost is of particular significance. **Contreras-Ramos *et al.*** (**2005**) confirmed that the stabilized sludge has metal levels below the limits set by the USEPA in 60 days. **Brahmbhatt (2006)** concluded significant removal of lead and cadmium from vermicomposted sewage sludge.

The availability of heavy metals to the organisms from the substrate is given by Bioconcentration factors (BCF) values. By definition, higher the BCF value, the more chemicals are taken up and higher the potential risk regarding adverse effects on the organism itself and at higher tropic levels **(Janssen *et al.*, 1997; Lock and Janssen, 2001)**. An overview of BCFs in earthworms for inorganic chemicals is given by **Janssen *et al.* (1997)**.

The concentration of heavy metals in vermicompost for three major vermicomposting earthworms (*E. fetida, L. mauritii* and *P. excavatus*) and their bioaccumulation were studied in order to determine whether vermicomposting could be used to reduce metal concentration in the final vermicompost. The objective of this study was to evaluate the level of heavy metals (Pb, Cd, Cu and Cu) in earthworms and vermicompost at the end of experiment. The metal accumulation by composting earthworms was estimated using BCFs. The final objective of the study was to compare heavy metal bioaccumulation process by the three earthworm species.

MATERIAL AND METHODS

Earthworms and substrate preparation

The study was conducted from March to May, 2012. Domestic wastes used for the vermicomposting was obtained from local community. Organic waste was collected and then sorted into three groups as kitchen waste, litter waste and paper waste. About 10g of each sorted substrate was collected and subsequently stored at room temperature for the heavy metal analysis. Before being fed to earthworms, the substrates were moistened and allowed to self-decompose for a week. The vermicomposting experiment was carried out in 3 pots each in triplicate. Worm bins of size 75 cm x 15 cm, each of which provided $1m^2$ of exposed top surface were used for the purpose. Three types of earthworms were used for vermicomposting of the three different organic wastes based on the their dietary habits i.e. *E. fetida* was fed with litter waste, *L. mauritii* was fed with kitchen vegetable waste and *P. excavatus* was fed with paper waste. Each plastic bin contained 5 Kg organic substrate and about 200 healthy adult earthworms. Vermicomposting was then carried out for 45 days. During this period, the moisture content of bin was maintained at 60-70 percent and the plastic bins were kept in dark at 20-30^{0}C. After 45 days, 20 worms were removed from the vermicompost and placed in sterilized glass Petri dishes with moistened Whatman no. 1 filter paper. They were kept in dark for 3 days to remove the ingested material and subsequently washed with deionized water and processed for heavy metal analysis.

Heavy metal analysis

The heavy metal contents of the vermicompost were measured according to the process of **Maboeta (2003)**. For sample preparation, about 10g each of vermicompost and the substrate was separately weighed and dried in hot air oven overnight at 60^{O}C. It was crushed in a dry, clean mortar and pestle to make powder form and sieved at 1mm to remove small grits. 1g of the powder was used for the acid digestion purpose. Earthworm samples were prepared at the end of experiment. Gut contents of the worms were removed by placing the worms in a Petri dish with moistened filter paper for 24 hours. Then the worms were washed with deionized water and dried in oven at 60^{0}C for 72 h till constant dry weight, tissues were grinded to powder form using mortar and pestle. About one gram of the powder was used for the acid digestion purpose. Open acid digestion method using conc. nitric acid was carried out for the sample preparation purpose according to **Katz and Jennes (1983)**. 1g of dry sample each of substrate, vermicompost and earthworms was digested in concentrated nitric acid at 200^{0}C up to continuous heating for 4-5 h till the dark reddish brown fumes started to clear out. The digest was then filtered through Whatman no. 40 (Grade 589) filter paper and final volume was made 25ml using deionized water. The final filtrate solution was used for the metals (Pb, Cd, Cu and Cr) determination by Flame Atomic Absorption Spectrometry (FAAS) with Perkin Elmer VMAA240FS instrument using APHA 21st Edition 3111B test method **(APHA, 2005)**. For each case triplicate samples were analyzed.

Bioconcentration factor

BCFs (bioconcentration factors) for earthworm's *E. fetida*, *P. excavatus* and *L. mauritii* were estimated for heavy metals in earthworm tissues and substrate materials using the method described by **Mountouris *et al.* (2002)**. The BCF is defined as follows: $BCF= C_{earthworm}/C_{soil}$, where $C_{earthworm}$ and C_{soil} are the total concentrations in mgkg^{-1} in earthworm and soil respectively.

Statistical Analysis

All the reported results were expressed as means of three replicates and all data were analyzed using the SPSS (Statistical Analysis for Social Sciences) statistical analysis. One way ANOVA was used to analyze the significant difference between the groups. Duncan t- test was also performed to test the homogeneity of the results.

RESULTS AND DISCUSSION

The ambient temperature and that of plastic bins as measured daily varied between 20 to 28^{0}C and 22 to 25^{0}C respectively. The moisture content and the pH ranged from 65 to 75 percent and 6.4 to 7.6 respectively. According to **Edwards (2000)**, moisture content of 60 to 90 percent, temperature range of 0 to 35^{0}C, and a pH range of 5-9 are most suitable for the vermicomposting worms. Hence, suitable growth conditions were provided during the study.

Metal content in vermicompost

In all the three groups, the final vermicompost showed considerable reduction in metal contents than the initial substrates (Table 1). The reduction of total heavy metal content as compared to the initial contents was in the following ranges: 11.4% (litter waste) to 26% (paper waste) in Pb, 48% (kitchen waste) to 61% (litter waste) in Cd, 4.9% (paper waste) to 29.01% (kitchen waste) in Cu, and 18.9% (kitchen waste) to 33.6% (paper waste) in Cr.

Table 1 Total metal content (mgkg^{-1}) in initial substrate material and final vermicompost

Substrate	Pb		Cd		Cu		Cr	
	S	V	S	V	S	V	S	V
Litter waste	37.09 ± 1.88	32.87 ± 0.53	4.50 ± 0.35	1.74 ± 0.09	60.76 ± 2.00	43.5 ± 1.06	25.30 ± 0.78	17.50 ± 1.06
Kitchen waste	47.63 ± 1.21	40.75 ± 1.06	5.12 ± 0.53	2.66 ± 0.69	65.65 ± 1.16	46.60 ± 1.20	24.02 ± 0.65	19.47 ± 1.45
Paper waste	45.09 ± 1.03	33.37 ± 0.18	4.06 ± 0.73	1.75 ± 0.07	323.36 ± 2.86	307.50 ± 8.13	26.95 ± 0.24	17.88 ± 1.60

All values represent mean ± SD n=3. S= substrate, V= vermicompost.

There was a pronounced reduction in total heavy metal content in the final vermicompost after 45 days. It is clear that reduction of total heavy metal content was directly related to the earthworm activity in the vermicomposting system. The observed difference in metal contents of final vermicompost for different substrates (Table 1), however, could be related to the different metabolic physiology of metals as well as the type of earthworm species.

Metal contents in earthworm tissues

The results of heavy metal (Pb, Cd, Cu and Cr) contents in the three groups of earthworms after the completion of vermicomposting process are summarized in Table 2. Metal contents in tissues of vermicomposted worms was in the range of 8.75±2.12 (*E. fetida)* to 9.62±1.23 (*P. excavatus)* for Pb, 1.08±0.18 (*E. fetida) to* 1.33±0.10 (*L. mauritii*) for Cd, 13.5±1.77 (*E. fetida*) to 18.75±0.71 (*P. excavatus*) for Cu and 10.5±1.06 (*E. fetida*) to 12.13±0.53 (*P. excavatus)* for Cr. Many studies have revealed that earthworms can accumulate heavy metals within their tissues during the process of vermicomposting **(Gupta *et al.* 2005; Suthar *et al.* 2008)**. The present data suggested that vermicomposting could be the most appropriate technology for heavy metal remediation from domestic organic waste.

Table 2 Total metal contents (mgkg^{-1}) in earthworms

Earthworm species	Pb	Cd	Cu	Cr
E. fetida	8.75±2.12	1.08±0.18	13.5±1.77	10.5±1.06
L. mauritii	9.38±0.53	1.33±0.07	14.25±0.35	11.38±0.18
P. excavatus	9.62±1.23	1.13±0.04	18.75±0.71	12.13±0.53

All values represent mean ± SD; n=3.

Heavy metal content in earthworm tissue was most significant in P. excavatus for Cu accumulation. The three groups of earthworms did not show any significant difference for bioaccumulation for Pb, Cd and Cr (Duncan: P = 0.05). However Cu content did show significant difference between *P. excavatus* and the *E. fetida* and *L. mauritii* (Duncan: P=0.55). The greater metal concentration in composting worms clearly indicates the accumulation of heavy metals from the vermicomposting substrate. In general, the content of metals in earthworms depends on inhabiting substrate metal content **(Lukkari *et al.*, 2006)**. This study confirms and further extends the earlier studies that earthworms can accumulate a considerable amount of metals in their tissues during vermicomposting.

Bioconcentration factors for different metals

Total mean BCF values for different heavy metals was as follows: 0.217± 0.03 (for Pb), 0.260 ± 0.03 (Cd), 0.166 ± 0.08 (Cu), and 0.446 ± 0.04 (Cr). BCF value for Cr was highest and statistically greater than other three heavy metals (Pb, Cd and Cu). A lowest BCF value was obtained for Cu in paper waste composted by *P. excavatus* due to a very high concentration of Cu in waste. BCF value for Cr in kitchen waste composted by *L. mauritii* was highest in this study. According to Table 3, the BCFs of the four heavy metals in vermicomposting for 45 days by the three earthworms were ranked as: Cr>Cd>Pb>Cu. The results confirmed the results of **Dei (2004)** and **Shahmansouri *et al.* (2005)**.

Table 3 Bioconcentration factors (BCFs) derived for different metals

Earthworm species	Pb	Cd	Cu	Cr
E. fetida	0.241 ± 0.06	0.241 ± 0.06	0.223 ± 0.03	0.416 ± 0.05
L. mauritii	0.196 ± 0.006	0.260 ± 0.01	0.218 ± 0.009	0.474 ± 0.02
P. excavatus	0.213 ± 0.02	0.280 ±0.04	0.058 ± 0.08	0.450 ± 0.02
Total[a]	0.217± 0.03	0.260 ± 0.03	0.166 ± 0.08	0.446 ± 0.04

All values represent mean ± SD; n=3.
[a]Mean of total replicates (n = 9).

The most commonly used composting worm, *E. fetida*, showed relatively greater values of BCF for total Pb (0.241 ± 0.06) and Cu (0.223 ± 0.03). The difference among different heavy metals for BCFs could be related to the difference in physiology of metal and the earthworm species. BCF values calculated in this study, however, were lower than those reported by earlier workers **(Suthar and Singh 2008; Shahmansouri *et al.*, 2005; Ireland, 1979; Pizl and Josens, 1995; Efroymson *et al.*, 1996)**. Different species can show a considerable difference for tissue metal content mainly because of the difference in their food/substrate and metabolic physiology **(Morgan and Morgan, 1992)**. Also it has been suggested that the accumulation of a heavy metal and its regulation mechanism could be species specific **(Hopkin, 1989)**.

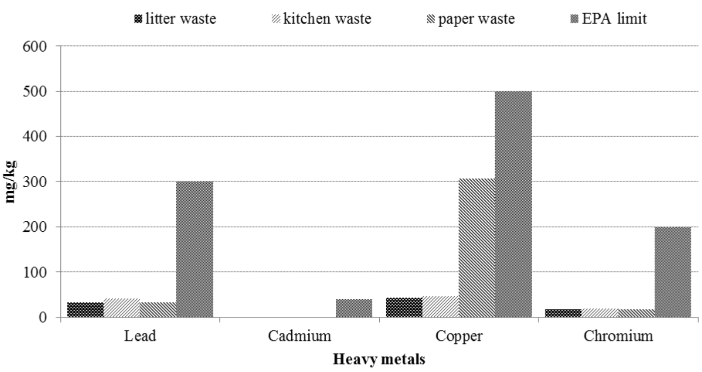

Figure 1 Heavy metal contents of the final vermicompost and the EPA limits (mgkg^{-1}).

All heavy metal contents in the final vermicompost did not exceed the US EPA standard limits **(US EPA, 1993)** (Fig. 1). The vermicompost derived from composting of kitchen waste, litter waste and paper waste are of Grade A quality that are suitable for use in soil for agriculture purposes. Thus the obtained vermicompost were of good quality in terms of heavy metal concentration **(US EPA, 1993)**.

CONCLUSION

Our study confirmed that vermicomposting of different types of domestic wastes by earthworms is safe with regards to heavy metal levels in the final vermicompost. Also, to be noted is that paper waste contains high concentration of copper (yet lower than the US EPA limit) so it use as a substrate should be accompanied with other substrate so as to dilute the copper concentration in the final vermicompost as well. Vermicompost is a really potent organic fertilizer which can be used in sustainable agriculture practice. It is a low energy requiring, self-regulated, zero waste technology that has the potential to reduce the organic waste management problems in urban cities of Nepal.

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