Heavy Metals Bioaccumulation by Iranian and Australian Earthworms *(Eisenia fetida)* in the Sewage Sludge Vermicomposting

**M R Shahmansouri, H Pourmoghadas, AR Parvaresh, H Alidadi*

Dept. of Environmental Health, School of Public Health, Isfahan University of Medical Sciences, Iran

ABSTRACT

Vermicomposting of organic waste has an important part to play in an integrated waste management strategy. In this study, the possibility of heavy metals accumulation with two groups of Iranian and Australian earthworms in sewage sludge vermicompost was investigated. *Eisenia fetida* was the species of earthworms used in the vermicomposting process. The bioaccumulation of Cr, Cd, Pb, Cu, and Zn as heavy metals by Iranian and Australian earthworms was studied. The results indicated that heavy metals concentration decreased with increasing vermicomposting time. Comparison of the two groups of earthworms showed that the Iranian earthworms consumed higher quantities of micronutrients such as Cu and Zn comparing with the Australian earthworms, while the bioaccumulation of non-essential elements such as Cr, Cd, and Pb by the Australian group was higher. The significant decrease in heavy metal concentrations in the final vermicompost indicated the capability of both Iranian and Australian *E.fetida* species in accumulating heavy metals in their body tissues.

Keywords: *Heavy metals, Bioaccumulation,* Eisenia fetida, *Vermicompost, Sewage sludge.*

INTRODUCTION

In recent years, earthworms have been widely used in the breakdown of a wide range of organic residues including sewage sludge, animal wastes, crop residues and industrial refuse in producing vermicomposts (Dominguez, 1997; Kale, 1998). Vermicomposting is basically composting with worms. In vermicomposting, worms are fed with decomposed matter and the organic material passes through the earthworm gut whereby a rich end product called worm castings is produced. A worm casting consists of organic matter that has undergone physical and chemical breakdown through the muscular gizzard, which grinds the material to a particle size of 1 to 2 microns. Nutrients present in worm castings are readily soluble in water for uptake by plants (Atiyeh, 2002). The system of decomposition and excretion of organic wastes through the metabolic system of earthworms is vermicomposting. This simple and low-cost technique can be used in the removal of toxic metals and the breakdown of complex chemicals to non-toxic forms (Jain, 2004). The earthworms (*E. fetida*) have the capability to accumulate heavy metals in the sewage sludge vermicompost (Saxena, 1998).

The influence of heavy metals in sewage sludge vermicompost for two groups of Iranian and Australian earthworms (*E.fetida*) and their bioaccumulation were studied in order to de-

***Corresponding author:** Tel: +98 311 7922692, Fax: +98 311 6682509, *E-mail:* shahmansouri@mui.ac.ir

termine whether vermicomposting can be used to reduce metal concentrations in sewage sludge. The objectives of the study included the determination of the relationship between heavy metal concentrations in the sludge (Cr, Cd, Pb, Cu, and Zn) prepared by the two groups of earthworms with their quantities in vermin-composts prepared, and also comparison of the heavy metal bioaccumulation processes by the two groups.

MATERIALS AND METHODS

Preparation of the pilot This study was conducted during the period from June to August, 2004. The sludge used in this study was obtained from the drying beds of South Isfahan wastewater treatment plant. The ratio of sludge used was 7 kg of 80 percent moisture content mixed with 1 kg of sawdust of 5 percent moisture content to provide a suitable C/N ratio of 20.

The vermicompost experiments were performed in two plastic worm-bins of 1.1×0.9×0.5 m^3 (length×width×depth). These plastic bins provided 1 m^2 of exposed top surface. Two groups of Iranian and Australian earthworms were used in this study. Iranian earthworms were collected in Mazandaran province in the north of Iran and Australian earthworms from Australia through Isfahan Water and Wastewater Company. Eight hundred pieces of adult Iranian *E.fetida* and eight hundred pieces of adult Australian *E.fetida* earthworms with approximate weight of 0.4 to 0.5 grams, were placed in two vermicompost plastic bins to provide the optimal sticking density as 0.75 kg feed/kg worm/day. The charactristics of adult Iranian and Australian *E.fetida* were the same. The biomass loading was 1.6 kg. The experiments lasted two months, hence 72 kg of the substrate was needed and each plastic bin contained 72 kg of vermicompost. The moisture content of the vermicompost samples was determined by over drying in 105°C to a constant weight. The ambient temperature and the plastic bins were measured by thermometer. The moisture content of the mixture was maintained at 60 to 70 percent throughout the vermicomposting period and the temperature in plastic bins were kept in the dark at 20-30^{o}C. The composite samples were taken from three different points in each bin. The number of worms was manually checked and recorded on a daily basis. After two months, both groups of earthworms were removed from the vermicompost to be analyzed for heavy metals in their body tissues.

Heavy metals analysis

The heavy metal contents of the vermicompost were measured by Maboeta method (2003). About one gram of vermicompost from different stages of the vermicomposting cycle was obtained to prepare the required samples. These samples were subjected to digestion by adding 25 ml of nitric acid (1+1) and then were placed on a hot plate and heated for 4 h at 90 to 95^{0}C. During digestion, care was taken to ensure that the samples did not dry out. After digestion, the samples were poured into 100-ml flasks through filter paper and injected into the Flame Atomic Absorption, Perkins Elmer 2380 to determine heavy metal concentration.

The heavy metals in the earthworm's body tissues were digested using Katz and Jennies method (1983). Samples were individually dried, ground and finally burned to ash at 5500ºC. Afterwards, the obtained ash was placed in test tubes and 10 ml of 55% nitric acid was added. It was left overnight at room temperature for the digestion to start. On the following day, the samples were heated at a temperature of 40–60°C for 2 h and then at a temperature of 120–130°C for one additional hour, then cooled to room temperature. One milliliter of 70% perchloric acid was added and the mixture was reheated to a temperature of 120–130°C for one hour. They were allowed to cooling before adding 5 ml of distilled water. Samples were reheated to 120-130° C until white fumes emitted. The samples were allowed to be cooled finally before being micro-filtered. The solutions were filtered through Whatman No. 41 filter paper into 100-ml flasks and measured using Flame Atomic Absorption Perkins Elmer 2380.

Statistical analysis

All the reported results were expressed as mean of three replicates and all data were analyzed using SPSS statistical analysis. Comparisons of the means were made using the least significant difference test calculated at *P*-values.

RESULTS

The ambient temperature and that of the plastic bins as measured daily varied from 17 to 25°C and 19 to 23°C, respectively. The moisture content in the two plastic bins of vermicompost varied between 63 to 75 percent, and the pH ranged from 5.5 to 7.2, in that order. A temperature range of 0-35°C, a moisture range of 60-90 percent, and a pH range of 5-9 were utilized as suitable conditions for the growth of *E.fetida* (Edwards, 2000). Thus, favorable growth conditions were provided in this study. At no stage during this study any mortalities were observed in the two groups of earthworms. The results of heavy metals (Cr, Cd, Pb, Cu and Zn) contents for the two groups of earthworms, pre- and post-vermicompost samples are summarized in Table 1. The results from heavy metal measurements in the body tissues of Iranian and Australian earthworms are summarized in Table 2. Results from Bioconcentration Factors (BCFs) of the body tissues of Iranian and Australian groups of earthworms after vermicomposting for 60 d are summarized in Table 3. The heavy metal contents in Iranian and Australian vermicomposts and EPA limits are showed in Fig. 1.

Table 1: Concentrations of Cr, Cd, Pb, Cu and Zn in vermicompost produced by Iranian (I) and Australian (A) earthworms (mg/kg)

Time (day)	Cr		Cd		Pb		Cu		Zn	
	I	A	I	A	I	A	I	A	I	A
0	108	108	17.4	17.4	184.4	184.4	380.1	380.1	390.7	390.7
10	100	102	16.3	15.6	154.8	130.4	348.8	356.4	387.1	379.5
25	74	78	12.1	9.3	132.1	125.7	336.8	321.3	310.1	354.1
40	43	37	10.0	7.4	84.2	91.8	241.6	281.6	240.8	289.6
60	38	35	8.6	7.1	75.6	70.4	215.2	225.8	206.2	219.9
Removal %	64.81	67.59	50.57	59.20	59.00	61.82	43.38	40.59	47.22	43.72

Table 2: Concentrations of Cr, Cd, Pb, Cu and Zn in Iranian (I) and Australian (A) earthworm body tissues (mg/kg)

Time (day)	Cr		Cd		Pb		Cu		Zn	
	I	A	I	A	I	A	I	A	I	A
0	4	7	2.1	1.7	15.3	15.9	25.31	28.31	58.31	56.02
10	17	32	3.4	3.5	21.8	28.1	35.50	39.85	89.70	82.68
25	44	45	4.1	4.3	29.1	39.8	48.08	54.82	111.25	97.45
40	58	52	4.8	4.6	34.4	47.2	84.19	102.91	141.25	132.67
60	66	73	6.8	7.1	59.1	64.8	132.51	132.71	178.41	169.31
Total increase	62	66	4.7	5.4	43.8	48.9	107.2	104.4	120.1	113.29

Table 3: Bioconcentration factors (BCFs) of body tissues of Iranian and Australian earthworms after

vermicomposting for 60 days (mg/kg)

	Cr	Cd	Pb	Cu	Zn
Iranian vermicompost	0.611	0.391	0.320	0.348	0.456
Australian vermicompost	0.676	0.408	0.351	0.349	0.433

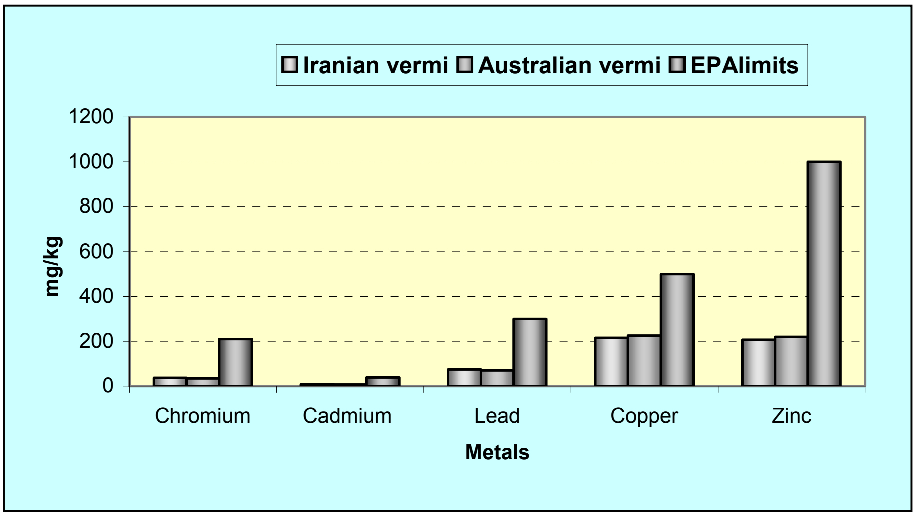

Fig. 1: Heavy metal contents of the final vermicomposts and EPA limits (mg/kg)

DISCUSSION

According to Table 1, heavy metal concentrations in the vermicompost decreased with increasing time. Reduction Cr, Cd and Pb concentration values in the vermicompost produced by the Australian group were higher than those of Iranian group, while the reduced concentration levels of copper and zinc, as micronutrients, in the vermicompost produced by the Iranian group were higher than those of Australian group ($P<0.05$). Thus, the Iranian groups of earthworms consumed higher levels of micronutrients while the Australian group removed higher quantities of non-essential elements such as Cr, Cd and Pb. According to Table 2, the heavy metal concentrations in earthworm body tissues increased with time. The concentration levels of Cr, Cd and Pb in the body tissues of the Australian group increased more as compared to the Iranian group, while Cu and Zn concentrations in the body tissues of the Iranian group increased more than in the other group ($P<0.05$). Thus, the Iranian group of earthworms consumed higher amounts of the micronutrients but the bioaccumulation of non-essential elements was higher in the Australian group.

According to Table 3, the BCFs of the five heavy metals in vermicomposting for 60 d by the Iranian group were ranked as: Cr>Zn> Cd> Cu>Pb, while the BCFs of the five heavy metals in vermicomposting for 60 d by the Australian group were ranked as: Cr>Zn>Cd> Pb>Cu. Our results confirmed the results of Dai, 2004.

The bioconcentration factors of the Iranian group were higher for Cu and Zn, while bioconcentration factors of the Australian group were higher for Cr, Cd, and Pb. Our results confirmed the results of Beyer, 1987 and Morgan, 1999.

Dewatered sludge and sawdust as amendment materials can be made into a vermicompost. The heavy metal contents at the end of the process decreased to lower values than in the initial materials. All heavy metal contents in the final products of vermicompost did not exceed the US EPA standards limits (Fig. 1). There-

fore, Iranian and Australian vermicomposts may be safe in terms of heavy metals and could be used as soil conditioners.
The decrease in heavy metal concentrations in the final vermicompost indicates the capability of Iranian and Australian *E. fetida* in accumulating heavy metals in their body tissues. The present results contradict those of Barrera, 2000, but agree well with the results reported by Leonard, 2001.

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