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Heavy metals remediation from urban wastes using three species of earthworm (*Eudrilus eugeniae, Eisenia fetida* and *Perionyx excavatus)*

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Remediation of Cadmium (Cd), Lead (Pb), Zinc (Zn), Copper (Cu) and Manganese (Mn) by the three earthworm species - *Eudrilus eugeniae, Eisenia fetida* and *Perionyx excavatus* from three different urban wastes (Municipal Solid Waste (MSW), Market Waste (MW) and Flower Waste (FW)) through the vermicomposting process carried out for sixty days was investigated. The metals concentrations increased gradually from the initial stage till end (P<0.05). Metals concentrations in earthworm tissues was significantly higher in *E. eugeniae* than that of *E. fetida* and *P. excavatus* (P<0.05). The concentrtions of Cd and Cu were higher in earthworms sampled from vermicompost of MSW and of Pb, Zn and Mn were higher in that of MW. Bioaccumulation factor (BAF) of metals (Cd > Zn > Pb > Cu > Mn) implied that Cd accumulation in earthworm tissue was more than that of substrate whereas the reverse was true for other metals.

Key words: Bioremediation, urban waste, heavy metals.

INTRODUCTION

Urban waste is the used and left-over materials in urban systems comprising of household garbage included kitchen waste, green waste (the garden and lawn clippings), street sweepings, sanitation residues, etc. (CPCB, 2000) and is generated in huge quantities now-a-days (Kumar et al., 2009). The disposal of urban waste is of great concern as it poses serious management problems particularly in developing countries like India, where its management is mostly unsystematic and un-scientific (Chattopadhyay et al., 2009; Pattnaik and Reddy, 2010). It causes pollution of land, water and air, which threatens public health with the waste forming the breeding ground for various pathogen-carrying vectors such as mosquitoes, rodents, pigs and others (Forbes, 1996; Hardoy et al., 2001; Kansal, 2002). Moreover, the urban waste is known to contain hazardous persistent pollutants such as heavy metals, being detrimental to the ecosystem including human beings (Smith, 2009; Reddy and Pattnaik, 2009).

The organic component of the urban waste can be recycled and bio-converted into useful end-product called compost and vermicompost when processed by earthworms, which could be utilized as a supplement to chemical fertilizers on farm lands or in potting media. The earthworms not only convert the organic fractions of urban wastes into available nutrients (Pattnaik and Reddy, 2010) but also consequently remediate the per-sistent heavy metals from the wastes by bioaccumulation

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in their bodies during vermicomposting process (Pare et al., 1998; Keener et al., 2001; Gupta et al., 2005; Suthar, 2008; Pattnaik and Reddy, 2010). Studies have shown that the earthworms accumulate heavy metals in their body tissue from contaminated substrates (Edwards and Bohlen, 1992; Gupta et al., 2005; Suthar, 2008). Various environmental factors contribute to the accumulation of As, Cd, Cu, Pb and Zn in earthworms (Vermeulen et al., 2009). Metal concentrations in earth-worm tissue were directly correlated to the level of their contamination in the substrates and soil (Rosciszewska et al., 2003; Hobbelen et al., 2006). Earthworms thus have been considered as important bio-accumulators and bio-indicators of environmental contamination of persistent pollutants like heavy metals (Gish and Christensen, 1973; Udovic et al., 2007; Suthar et al., 2008; Wang et al., 2009).

It is therefore important to know the metals that are accumulative in the earthworm tissue and their fate in the environment. Though several studies revealed the high concentrations of heavy metals pollutants in urban waste and sewage sludge (Smith, 2009; Reddy and Pattnaik, 2009), the problems associated with bioaccumulation and vermiremediation have received very little attention and is poorly understood (Fries, 1982), especially in India. Moreover, adequate information is not available on the vermiremediation as well as bioaccumulation of heavy metals in earthworm body tissue particularly in tropics (Shahmansouri et al., 2005; Suthar, 2008). The present study, therefore, attempted to assess the remediation of heavy metals - Cd, Pb, Zn, Cu and Mn from the organic components of three different urban wastes - the municipal solid waste (MSW), vegetable or market waste (MW) and floral waste (FW) through metal bioaccumulation in the body tissues of three earthworms species - two exotic species *viz, Eudrilus eugeniae* (Kinberg) and *Eisenia fetida* (Savigny) and a local species, *Perionyx excavatus* (Perrier) used for vermicomposting, heavy metal reduction in vermicompost, and also to find out the bio-accumulation factors (BAF). These heavy metals for the present study were chosen based on their toxicity - highly toxic cadmium (Cd), moderately toxic lead (Pb), and less toxic zinc (Zn), copper (Cu) and manganese (Mn) (Wizewardena and Gunaratne, 2004).

MATERIALS AND METHODS

Experimental set up and sampling

Three types of urban waste viz., MSW, MW and FW were chosen for the present study. Municipal solid waste was collected from one of the major garbage dumping site of Puducherry, a small town on the east coast of India and the erstwhile French colony, and the vegetable MW was collected from its main vegetable market, which comprises of different left over vegetables such as cabbage, brinjal, tomato, potato, onion, carrot, turnip and leafy vegetables. The FW was obtained from the *Peltophorum pterocarpum,* belonging to the family Fabaceae and sub-family Caesalpinioideae - a widely-appreciated shade tree and a reclamation plant with dense spreading crown, planted along the road-sides of the Pondicherry Central University campus; it is usually planted on the road sides.

Five samples of each waste were collected randomly and then were mixed to form composite samples before taking smaller sub-samples for analysis. These wastes were characterized and segregated into biodegradable and non-biodegradable components. The MSW, MW and FW were separated and air-dried separately for 48 h and pre-composted for three weeks prior to vermicomposting and composting processes. During pre composting process, the temperature raised up to about 60°C. As such high temperature was lethal to earthworm survival; thermal stabilization was done prior to introducing earthworms into all the three substrates.

Earthen pots were used for vermicomposting and composting; in each pot, five kg of the substrate mixed with cow dung in 3:1 ratio was taken. In the present study, the cow-dung was used as inoculants to accelerate the vermicomposting process (Karthikeyan et al., 2007; Pramanik et al., 2007; Gupta and Garg, 2009), and when mixed with organic waste the mixture served as attractive feeding resource for earthworms (Suthar and Singh, 2009). A total of four sets of earthen pots each set containing six replicates was taken for each substrate material, of which three sets were used for vermicomposting each set using one species of earthworm and the fourth set was used for only composting without using any earthworm.

Similarly, all the three types of substrate materials were used for vermicomposting and composting. When the temperature of the pre composting process retrieved to 25°C, fifty adult earthworms of each species that is, *E. eugeniae, E. fetida*, and *P. excavatus* were introduced on the top of each of the pots of each of the substrates. All these earthworm species chosen for the experiment were collected from the vermiculture unit at Lake Estate (Aurobindo Ashram, Puducherry, India). All the pots were covered on the top by jute cloth cover and wire mesh to prevent and protect the earthworms from the predators - centipedes, moles and shrews. The bottom of each pot was filled with small stones up to a height of 5 cm for air circulation and small holes were drilled at bottom of each pot for good drainage of water. The processes of vermicomposting and composting were carried out for a period of 60 days. The sub-samples of compost and vermicompost from each replicate were collected un-destructively after each interval of 15, 30, 45 and 60 days.

Heavy metal analysis of earthworms' tissue

Earthworms were rinsed and placed in petri dishes on moist filter paper. They were kept in dark for 4 days at 20 to 22°C, during which time all organic material were eliminated from their gut (Ireland, 1975). The filter paper was changed daily. Worms were then frozen at – 10°C to prevent microbial decomposition between collection and analysis, and were oven dried at 80°C for 24 h (Kruse and Barrett, 1985). The earthworm's body tissues were digested using Katz and Jennis (1983) method and Shahmansouri et al. (2005) method for heavy metal analyses**.** The body tissue samples were individually dried, ground and finally burn to ash at

550°C. Afterwards the ash was placed in test tube and 10 ml of 55% nitric acid was added. It was left overnight at room temperature for digestion.

On the following day, the samples were heated at the temperature of 40 to 60° C for 2 h and then at a temperature of 120 to 130°C for one additional hour and then cooled to room temperature. One ml of 70% perchloric acid was added and the mixture was reheated to a temperature of 120 to 130° C for one hour. They were allowed to cool before adding 5 ml of distilled water. Samples were reheated to 120 to 130°C until white fumes emitted. The samples were allowed to cool finally before being filtered. The solutions were filtered through Whatman filter paper No. 41 to 100ml of flasks and heavy metals were estimated using ICP-AES.

Bioaccumulation factor (BAF)

The metal accumulation by earthworms in their body tissue is known as BAF. It is the ratio of the level of metals in earthworms to the substrate. BAF for earthworms were estimated for the metals in earthworm tissues and substrate materials using the method described by Mountouris et al. (2002). The BAF was calculated using the formula:

$BAF = C_{biota}/C_{substrate},$

where C_{biota} and $C_{substrate}$ were the total concentrations of earthworm and substrate used for vermicomposting experiment, respectively. It is always compared to unity. If the metal concentration is high in earthworms' tissue than that of substrate, BAF would be more than a unity. If the concentration of metals in substratre is higher than that in earthworms' tissue, then BAF would be less than a unity.

Statistical analysis

The data in this study was analyzed using the XLStat computer software package (version 2009). ANOVA tests were used to analyze the significance difference between heavy metal contents of earthworm tissues. Relationship between heavy metal concentration in earthworm tissue and their respective vermicompost metal concentration were determined using regression equation.

RESULTS

Metal content of vermicompost

The heavy metal concentrations of the vermicompost produced by the three species of earthworms showed discernible difference and were significantly less compared to that of the respective waste substrates that is, MSW, MW and FW. The removal of Cd, Pb, Zn, Cu and Mn was 89.5, 94.4, 94.0, 89.2, 77.5% from MSW; 88.7, 96.1, 87.9, 95.1, 79.4% from MW and 84.1, 95.6, 94.7, 95.3 and 83.1% from FW through vermicompost processed by *E. eugeniae* whereas it was 85.9, 92.3, 91.0, 86.6, 70.9% from MSW; 87.8, 94.8, 85.2, 87.3, 76.1% from MW and 85.5, 90.4, 80.9, 87.5, 78.3% from FW through the vermicompost produced by *E. fetida,* and 81.8, 90.7, 84.4, 85.4, 68.6% from MSW; 81.5, 92.9, 82.7, 78.1, 75.7% from MW and 78.7, 85.9, 77.6, 80.9, 75.2% from FW through the vermicompost produced by *P. excavatus*, respectively.

The vermicompost produced by *E. eugeniae* possessed low concentration of metals as compared to other two earthworms, that is, *E. fetida* and *P. excavatus;* whereas it was just reverse for the accumulation of metal in earthworms' tissue that is , *E. eugeniae* showed higher accumulation of heavy metal in its tissue than that of *E. fetida* and *P. excavatus*, respectively. The tissue concentrations of the heavy metals – Cd, Pb, Zn, Cu and Mn in three earthworms grown in the waste (substrates) – MSW, MW and FW was negatively and significantly correlated with their respective vermicompost ($P<0.0001$). Thus, the concentrations of metals in earthworms' tissues increased gradually and that in vermicompost consequently decreased gradually as the vermicomposting process progressed from the initial stage of 15 days to final stage of 60 days.

Concentrations of Cd, Pb, Zn, Cu and Mn decreased from 15 to 60 days by 0.10 to 0.05, 0.61 to 0.31, 4.85 to 1.49, 0.74 to 0.47 and 7.98 to 5.16 ppm in vermicompost of *E. eugeniae,* by 0.12 to 0.07, 0.72 to 0.43, 5.85 to 2.24, 0.86 to 0.59, and 8.27 to 6.67 ppm in vermicompost of *E. fetida,* by 0.14 to 0.09, 0.83 to 0.52, 6.51 to 3.86, 0.97 to 0.64, and 9.32 to 7.20 ppm in vermicompost of *P. excavatus* prepared from MSW (Figure 1); the decrease was 0.09 to 0.02, 1.10 to 0.42, 3.89 to 2.19, 0.55 to 0.29, and 10.67 to 8.52 ppm in vermicompost of *E. eugeniae,* 0.11 to 0.04, 1.28 to 0.57, 4.64 to 2.69. 0.68 to 0.31, and 11.27 to 9.84 ppm in vermicompost of *E. fetida,* 0.14 to 0.07, 1.89 to 0.78, 5.23 to 3.15, 0.70 to 0.48, and 12.11 to 10.00 ppm in vermicompost of *P.excavatus* prepared from MW (Figure 2); and it was 0.04 to 0.006, 0.46 to 0.13, 1.60 to 1.04, 0.26 to 0.05, and 3.54 to 2.02 ppm in vermicompost of *E. eugeniae*, 0.05 to 0.02, 0.57 to 0.24, 1.84 to 1.25, 0.31 to 0.14, and 3.99 to 2.57 ppm in vermicompost of *E. fetida*; and 0.06 to 0.03, 0.68 to 0.35, 2.17 to 1.47, 0.40 to 0.20, and 4.53 to 2.95 ppm in vermicompost of *P.excavatus* prepared from FW (Figure 3), respectively.

Concentrations of Cd, Pb, Zn, Cu and Mn in earthworms' tissue were increasing from 15 to 60 days by 0.63 to 0.82, 4.11 to 5.11, 19.47 to 24.34, 2.01 to 3.53, and 15.09 to 20.50 ppm in *E. eugeniae*, 0.60 to 0.80, 3.52 to 4.84, 15.01 to 21.91, 1.61 to 3.13, and 13.77 to 18.46 ppm in *E. fetida* and 0.52 to 0.75, 3.10 to 4.65, 12.68 to 19.10, 1.24 to 2.74, and 11.09 to 16.54 ppm in

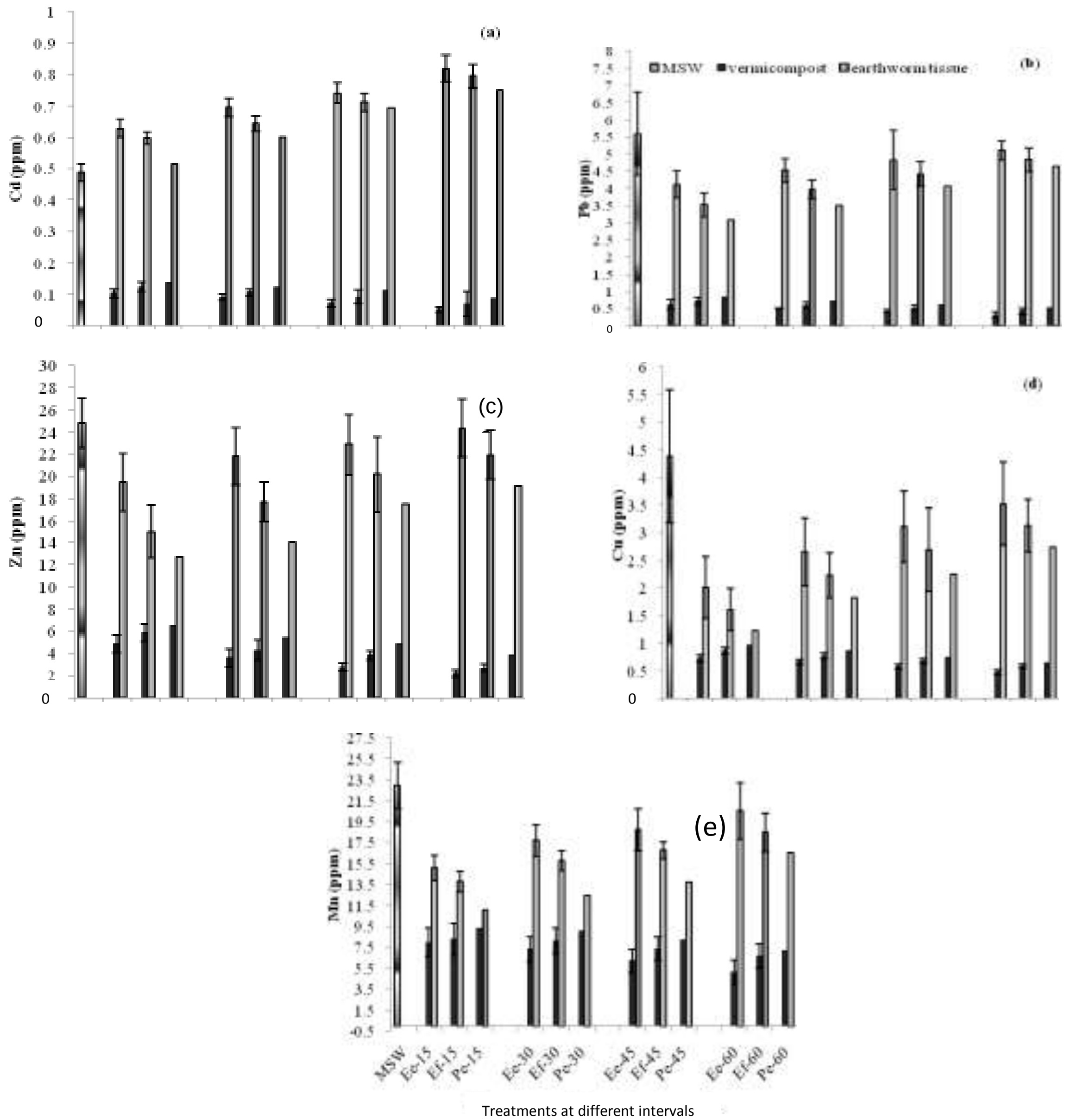

Figure 1. Accumulation of (a) Cd (ppm), (b) Pb (ppm) (c) Zn (ppm), (d) Cu (ppm) and (e) Mn (ppm) in the tissue of three earthworm species – *E. eugeniae* at 15 days (Ee-15), 30 days (Ee-30), 45 days (Ee-45) and 60 days (Ee-60), *E. fetida* at 15 days (Ef-15), 30 days (Ef-30), 45 days (Ef-45) and 60 days (Ef-60), and *P. excavatus* at 15 days (Pe-15), 30 days (Pe-30), 45 days (Pe-45) and 60 days (Pe-60) compared to that present in vermicompost produced from MSW in respect to MSW.

P. excavatus prepared from MSW (Figure 1); 0.51 to 0.72, 7.46 to 9.68, 13.00 to 17.37, 1.24 to 2.12, and 16.96 to 24.02 ppm in *E. eugeniae*, 0.44 to 0.64, 6.06 to 9.25, 10.69 to 15.90, 1.05 to 1.92, and 14.81 to 20.97 ppm in *E. fetida*, 0.40 to 0.59, 5.38 to 8.46, 8.40 to 13.94, 0.80 to 1.71, and 12.88 to 17.91 ppm in *P. excavatus* prepared from MW (Figure 2); 0.37 to 0.60, 1.90 to 2.30, 4.16 to 6.08, 0.56 to 0.84, and 7.57 to 10.09 ppm in *E. eugeniae*, 0.30 to 0.53, 1.63 to 2.21, 3.70 to 5.56, 0.50 to 0.74, and 6.97 to 9.04 ppm in *E. fetida* and 0.21 to 0.46, 1.51 to 2.14,2.88 to 4.81, 0.43 to 0.65, and 5.27 to 7.94 ppm in *P. excavatus* prepared

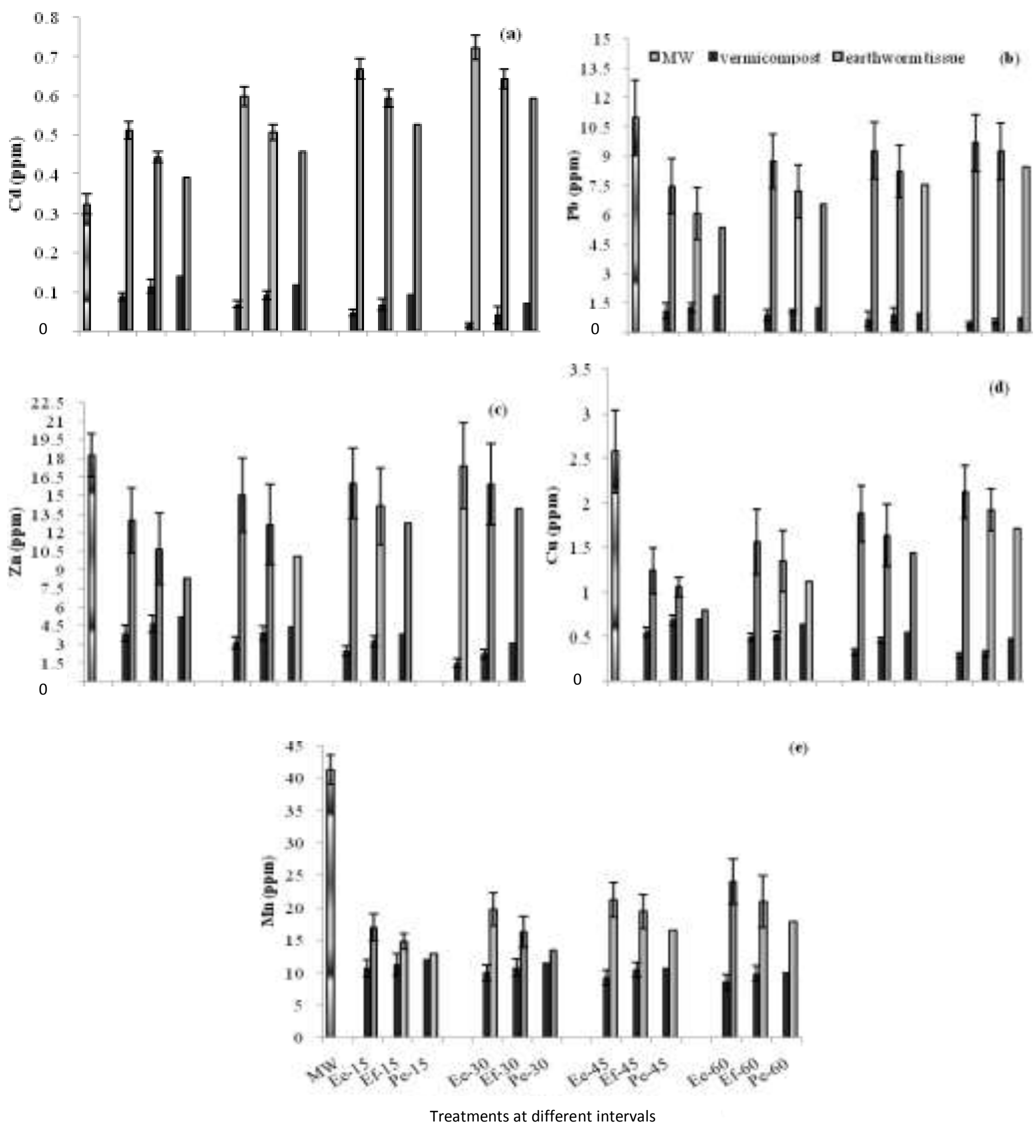

Figure 2. Accumulation of (a) Cd (ppm), (b) Pb (ppm) (c) Zn (ppm), (d) Cu (ppm) and (e) Mn (ppm) in the tissue of three earthworm species – *E. eugeniae* at 15 days (Ee-15), 30 days (Ee-30), 45 days (Ee-45) and 60 days (Ee-60), *E. fetida* at 15 days (Ef-15), 30 days (Ef-30), 45 days (Ef-45) and 60 days (Ef-60), and *P. excavatus* at 15 days (Pe-15), 30 days (Pe-30), 45 days (Pe-45) and 60 days (Pe-60) compared to that present in vermicompost produced from MW in respect to MW.

from FW (Figure 3), respectively.

Metal accumulations in earthworm tissues

Comparison of earthworms

The comparison of heavy metal accumulation in tissues of three earthworm species used in vermicomposting of three waste substrates, i.e., vermicompost of MSW (Figure 1), MW (Figure 2) and FW (Figure 3) showed that the concentration of Cd, Pb, Zn, Cu and Mn in the tissue of *E. eugeniae* was 1.03 to 1.18, 1.04 to 1.23, 1.09 to 1.30, 1.11 to 1.24 and 1.09 to 1.21 fold higher than that of *E. fetida* and was 1.07 to 1.76, 1.07 to 1.39, 1.25 to 1.56, 1.24 to 1.62 and 1.24 to 1.46 fold higher than that of *P. excavatus*; whereas that of *E. fetida* was higher by 1.03 to1.43, 1.03 to 1.14, 1.11 to 1.28, 1.11 to 1.32 and 1.12

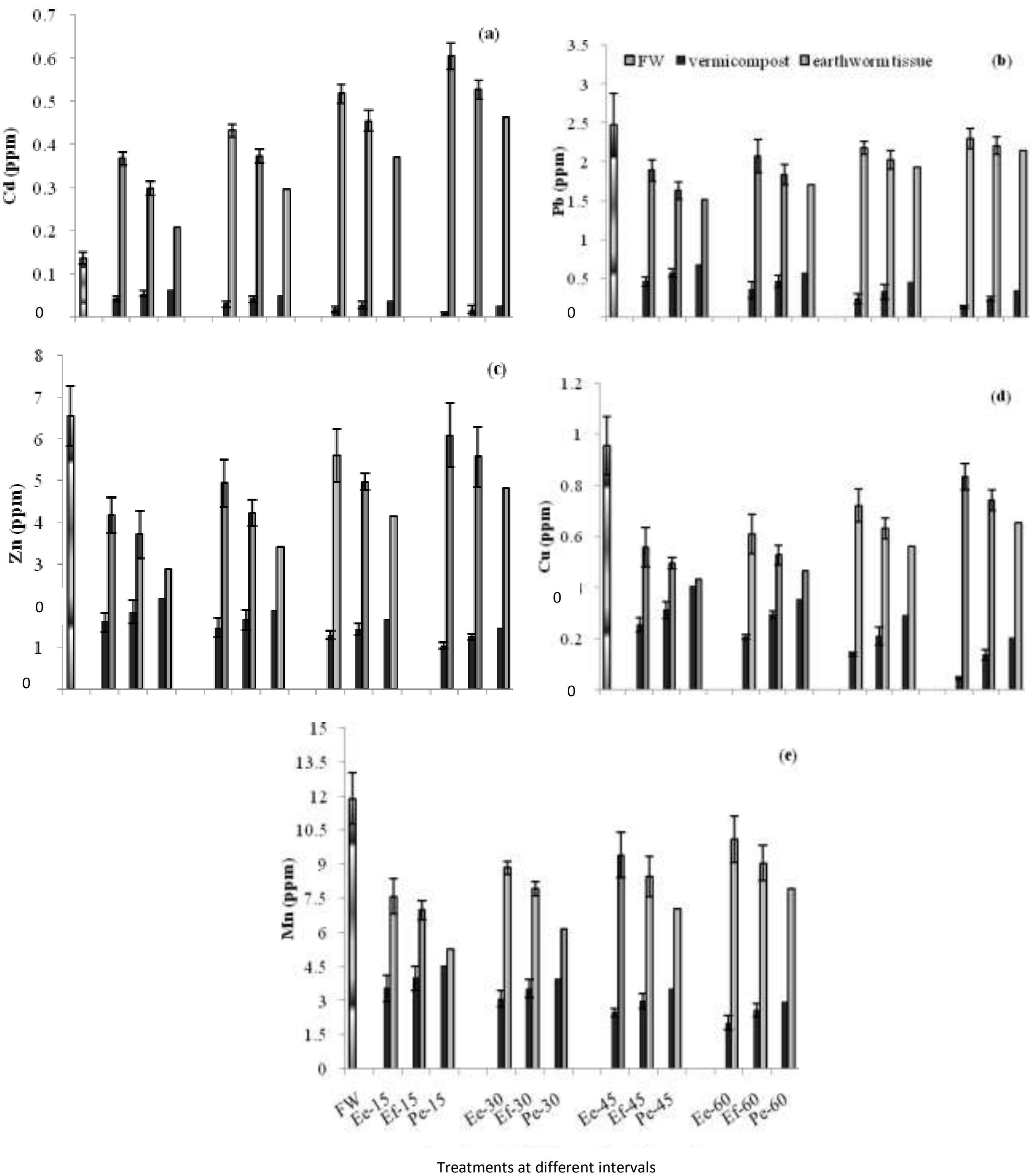

Figure 3. Accumulation of (a) Cd (ppm), (b) Pb (ppm) (c) Zn (ppm), (d) Cu (ppm) and (e) Mn (ppm) in the tissue of three earthworm species – *E. eugeniae* at 15 days (Ee-15), 30 days (Ee-30), 45 days (Ee-45) and 60 days (Ee-60), *E. fetida* at 15 days (Ef-15), 30 days (Ef-30), 45 days (Ef-45) and 60 days (Ef-60), and *P. excavatus* at 15 days (Pe-15), 30 days (Pe-30), 45 days (Pe-45) and 60 days (Pe-60) compared to that present in vermicompost produced from FW in respect to FW.

to 1.29 fold than that of *P. excavatus* at different intervals, respectively.

Removal of heavy metals from the waste substrates by earthworms

The concentration of Cd, Pb, Zn, Cu and Mn in the tissues of the three species of earthworms showed significant difference (P< 0.05) from that of their waste substrates, that is, MSW (Figure 1), MW (Figure 2) and FW (Figure 3). The removal of Pb, Zn, Cu and Mn was 9 to 26, 2 to 22, 20 to 54, and 11 to 34% by *E. eugeniae,* 13 to 37, 12 to 40, 29 to 63, and 19 to 40% by *E. fetida* and 17 to 45, 23 to 49, 38 to 72, and 28 to 52% by *P. excavatus* from MSW; it was 12 to 32, 5 to 29%, 18 to 52, and 42 to 60% by *E. eugeniae,* 16 to 45, 13 to 41 ,25 to 59, and 49 to 64% by *E. fetida* and 23 to 51, 24 to 54, 33 to 69, and 57 to 69% by *P. excavatus* from MW; and 7 to 24, 7 to 37, 13 to 41, and 15 to 36% by *E. eugeniae,* 11 to 34, 15 to 44, 22 to 48, and 24 to 41% by *E. fetida* and 13 to 39, 26 to 56, 31 to 55, and 33 to 56% by *P. excavatus* from FW at different intervals, respectively; whereas the Cd concentration was more in tissue of *E. eugeniae, E. fetida, and P. excavatus* by 29 to 68, 22 to 63, and 6 to 54% than in MSW; 58 to 123, 37 to 98, and 21 to 83% than in MW, and 170 to 344, 119 to 288 and 54 to 240% than in FW at different intervals, respectively.

The tissue concentrations of the heavy metals in earthworm species used in vermicomposting of FW were lower than those used in vermicomposting of other two substrate wastes (P <0.05). The concentration of Cd, Zn and Cu in the tissue of earthworms used in vermincomposting of MSW was higher by 1.11 to 1.35, 1.37 to 1.52 and 1.53 to 1.70 fold than that of MW and 1.36 to 2.47, 3.94 to 4.68 and 2.86 to 4.35 fold than that of FW, respectively; whereas their concentration in the tissue of earthworms used in vermicompost of MW was higher by 1.20 to 1.88, 2.85 to 3.13 and 1.83 to 2.62 fold than that of FW, respectively. The concentration of Pb and Mn in the tissue of earthworms used in vermicompost of MW was higher by 1.72 to 1.93 and 1.03 to 1.20 fold than that of MSW and by 3.56 to 4.25 and 2.06 to 2.44 fold than that of FW, respectively; whereas the increase was 2.04 to 2.22 and 1.96 to 2.08 fold in earthworms used in vermicompost of MSW compared to that in FW, respectively.

Bioaccumulation factor

The values of BAF, in the present study, showed significant variation among three earthworm species across different wastes and time intervals (P<0.05) (Table 1). The BAF for Cd was more than unity and it was 2.3 ± 0.4 in tissue of *E. eugeniae*, 2.04 ± 0.39 in that of *E. fetida*, and 1.76 ± 0.42 in that of *P. excavatus* used in vermincomposting process of urban waste (Table 1 and Figure 4). However, the ranges of BAFs were less than unity in other four metals. BAFs of Pb, Zn, Cu and Mn were 0.83 ± 0.08, 0.83 ± 0.11, 0.67 ± 0.14, and 0.68 ± 0.08 in tissue of *E. eugeniae;* 0.74 ± 0.11, 0.73 ± 0.12, 0.58 ± 0.14, and 0.61 ± 0.07 in that of *E. fetida*, and 0.68 ± 0.12, 0.62 ± 0.13, 0.50 ± 0.14, and 0.50 ± 0.08 in *P. excavatus* used in vermicomposting process of urban waste, respectively.

DISCUSSION

The present findings showed that earthworms can accumulate higher concentration of the heavy metals in their body tissues with consequent remediation in the concentration of metals in the waste substrate and consequent considerable reduction in concentration in vermicompost (Hartensein and Hartensein, 1981; Gupta et al., 2005; Suthar, 2008; Suthar and Singh, 2009). According to Edwards and Bohlen (1996) the waste substrate ingested by earthworm undergone chemical and microbial changes while passing through the alimentary canal and a great proportion of the organic fraction is converted into soluble forms that are more available to organisms. Part of the organic matter is digested, with the increase in pH and microbial activities in the gut. As a result, the possibility for metals to be bound to ions and carbonates (that is in more soluble fractions) increases in ingested material (Morgan and Morgan, 1999).

These soluble fractions can be accumulated in earthworm tissues during transit of waste through worm's gut, which results in reduction of metals in worms' excreta coming out as vermicompost and in turn, increased in the tissue of earthworm (Gupta et al., 2005; Suthar et al., 2008). Dia et al. (2004) suggested that bioaccumulation of metals in earthworms is their ability to eliminate the excess of metals. The concentrations for all the metals were found higher in the tissue of *E. eugeniae* than that of *E. fetida* and *P. excavatus*. These three species showed a considerable difference in metal concentrations in their tissues which was probably due to variation in their metabolism (Morgan and Morgan, 1992); it could be probably a species-specific feature. The variation in dietary intake of the metals could be an important factor contributing to differences in the availability of metals in their body tissues as well as vermicompost.

Hopkin (1989) stated that the earthworms have a specific capacity to regulate metals, particularly trace

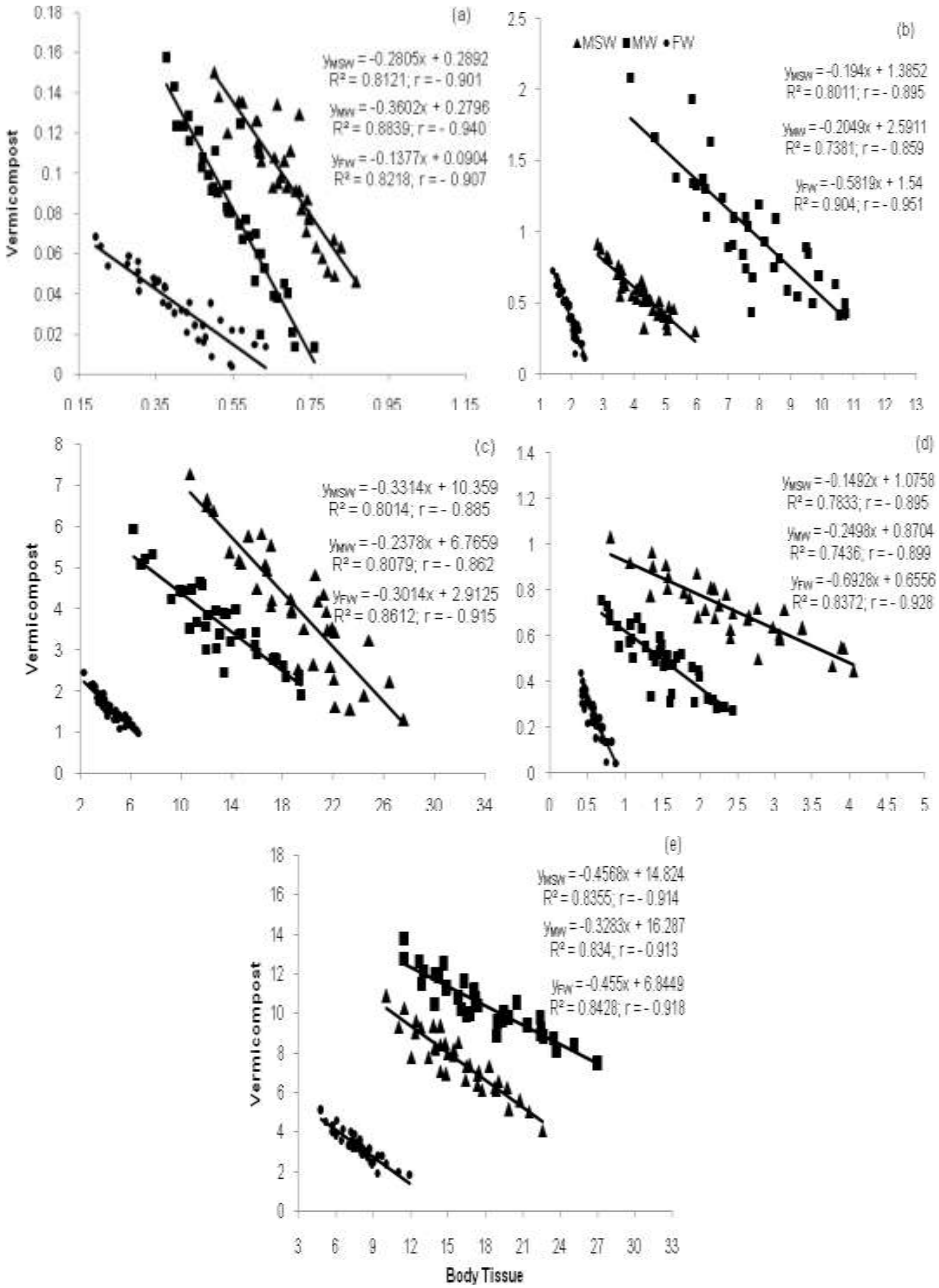

Figure 4. Regression analysis showing relationship between accumulation of (a) Cd (ppm), (b) Pb (ppm) (c) Zn (ppm), (d) Cu (ppm) and (e) Mn (ppm) in the tissue of three earthworm species – *E. eugeniae, E. fetida* and *P. excavatus* at 15, 30, 45 and 60 days with that present in vermicompost produced from MSW, MW and FW.

Table 1. Heavy metals' Bio- Accumulation Factor (BAF) of *Eudrilus eugeniae (Ee), Eisenia fetida (Ef)* and *Perionyx excavatus (Pe)* during vermicomposting of MSW, MW and FW at different time intervals.

	Vermicomposting process								
	MSW			MW			FW		
	Ee	***Ef***	***Pe***	***Ee***	***Ef***	***Pe***	***Ee***	***Ef***	***Pe***
Cd									
15 Days	1.28	1.22	1.05	1.58	1.37	1.21	2.69	2.19	1.54
30 Days	1.42	1.32	1.23	1.84	1.56	1.41	3.17	2.73	2.17
45 Days	1.52	1.46	1.42	2.06	1.83	1.62	3.81	3.34	2.73
60 Days	1.67	1.62	1.54	2.23	1.98	1.83	4.44	3.87	3.39
Pb									
15 Days	0.73	0.63	0.55	0.68	0.55	0.49	0.76	0.66	0.61
30 Days	0.81	0.71	0.62	0.79	0.66	0.59	0.84	0.74	0.69
45 Days	0.86	0.79	0.73	0.84	0.75	0.69	0.88	0.82	0.78
60 Days	0.91	0.86	0.83	0.88	0.84	0.77	0.93	0.89	0.86
Zn									
15 Days	0.74	0.6	0.51	0.71	0.59	0.46	0.64	0.56	0.44
30 Days	0.84	0.71	0.57	0.82	0.69	0.55	0.75	0.64	0.52
45 Days	0.92	0.81	0.71	0.88	0.78	0.7	0.86	0.76	0.63
60 Days	0.97	0.88	0.77	0.95	0.87	0.78	0.93	0.85	0.74
Cu									
15 Days	0.46	0.36	0.28	0.48	0.41	0.31	0.59	0.52	0.45
30 Days	0.6	0.5	0.41	0.61	0.52	0.43	0.64	0.55	0.49
45 Days	0.71	0.61	0.55	0.73	0.63	0.56	0.76	0.66	0.59
60 Days	0.8	0.71	0.62	0.82	0.74	0.66	0.88	0.78	0.69
Mn									
15 Days	0.66	0.6	0.48	0.41	0.36	0.31	0.64	0.59	0.44
30 Days	0.77	0.69	0.54	0.48	0.39	0.33	0.74	0.67	0.52
45 Days	0.82	0.73	0.6	0.51	0.47	0.4	0.79	0.71	0.59
60 Days	0.89	0.81	0.72	0.58	0.51	0.43	0.85	0.76	0.67

metals such as Cu and Zn in their bodies, and the bioaccumulation and regulation mechanisms could be species-specific. He also suggested that exposure duration could be main determinant for observed differences in metal concentration in the tissue which is in corroboration with the present findings.

Suthar et al. (2008) reported that species-specific metal physiology in earthworms may alter the concentration of metals in their tissues. The amount of organic fractions in ingesting material denotes the availability of soluble forms of metals in a worm's gut. Lukkari et al. (2006) stated that binding of metals to organic matter particularly more tightly bound fractions partly reduced the availability of metals to earthworms. The earthworm gut could modify the mobility of metals and favor their assimilation. Holmstrup et al. (2010) demonstrated in their study that cadmium, lead and copper accumulated to high concentrations in *Dendrobaena octaedra.*

The present findings clearly indicated that concentrations of Pb, Zn and Mn in tissue was higher in earthworms used for vermicomposting of MW, while that of Cu and Cd were higher in the worms used for composting of MSW; on the other hand, earthworms used in composting of FW showed the lower concentrations of all the metals. The concentrations of metals in earthworms were directly dependent on the metal concentrations of substrate waste in which they were used for composting. These findings are supported by earlier workers (Heikens et al., 2001; Lukkari et al., 2006; Kamitani and Kaneko, 2007). Gupta et al. (2005) reported that earthworm tissue metal level was directly related to their proportion in fly ash in vermibeds. Similar pattern of metal bioaccumulation was observed by Suthar et al. (2008), which further supported the hypothesis that tissue-metal level reflects the metal availability in the substrates.

The earthworms showed the greater concentrations of Cd in their tissues, than that of waste substrates; whereas the concentrations of Pb, Zn, Cu and Mn were more in waste than that in their tissue at the end of the vermicomposting process. The present findings clearly showed the earthworms accumulating and remediating metals from the waste substrates. In consistence to the present findings, Graff (1982) reported that earthworms (*E. fetida* and *E. eugeniae)* accumulated the heavy metals from the municipal garbage compost and concentrated them in their body tissues. The earthworm in general used three ways to handle the metals: (1) immobilization in fatty (chloragogen) cells of gut wall, (2) storage in waste nodules (or 'brown bodies') formed within the body cavity, and (3) excretion through the calciferous glands (Andersen and Laursen, 1982). The gut-related processes in earthworm may also increase metal availability. The earthworm chloragosomes consisting of modified epithelial cells, the eleocytes of the gut containing constituents of ion exchange compounds - phosphoric acid, carboxyl, phenolic hydroxyl and sulphonic acid groups acted as a cation exchange system capable of taking up and accumulating heavy metals (Ireland, 1978; Morgan and Morgan, 1988; Cooper, 1996). The accumulation of metals, especially Cd in earthworms is most probably due to the binding of metals by metallothioneins (Kagi and Kojima, 1987).

The low concentration of heavy metals in earthworm tissue at initial stage of composting and gradual increase with the progress of vermicomposting reaching higher concentrations at 60 days of composting was a consistent trend of higher metals accumulation in earthworm tissue. It is probably due to the metal levels in earthworm tissue being directly related to the availability of metals over different time intervals. The lower tissues metal concentration in tissues of earthworms with shorter time intervals, that is, at 15 and 30 days further supports the hypothesis. As the time passed, earthworms consume a great amount of organic waste to acquire required nutrition, and during the process metals are liberated in free forms due to the enzymatic actions in their gut resulting in accumulation in their body tissue (Suthar, 2008). When the composting process progressed from 15 to 60 days, more metals in available forms were absorbed by the epithelial layer of gut and incorporated into the body tissue while the wastes transited through it.

The amount bioavailability of metals during the vermincomposting process can be calculated effectively using BAF or bioconcentration factors (BCFs) (Suthar and Singh, 2009). The BAFs have been used widely to quantify the bioaccumulation of pollutants in aquatic and terrestrial biota with assumption that organisms achieve a chemical equilibrium with respect to a particular medium or route of exposure (Mountouris et al., 2002; Hsu et al., 2006). The degree of BAFs mainly depended upon level of contamination and characteristics of waste, and the earthworm species used. Suthar and Singh (2009) suggested that BAFs could be an important indicator of metal bioconcentartion during the process of vermicomposting. BAF range increased as the time of vermicomposting process progressed from 15 to 60 days. The BAF was low in initial stage of 15 days and was higher at 60 days in the tissue of earthworms used in the vermicomposting of the waste substrates. The BAF was higher for *E. eugeniae* than that of *E. fetida* and *P. excavatus*. The BAF was higher for Cd and accordingly that of the other metals ranked in the order of Cd >Zn>Pb>Cu>Mn. The BAFs for all the heavy metals showed positive correlation with each other in all the three wastes, i.e., MSW (range of R^2 - 0.906 to 0.980, $P<0.001$), MW (R^2 - 0.956 to 0.989, $P<0.001$), FW(R^2 - 0.924 to 0.993, $P<0.001$), which clearly indicated that the heavy metals present in the wastes are accumulated in the body tissue of earthworms on which they devour. Higher BAF for Cd in all the samples clearly implied that the Cd concentrations in the tissues of earthworms exceeded many times the concentrations in the waste substrate.

Earthworms easily accumulate Cd and retain it in their body tissue. These results confirmed the findings of earlier studies (Brewer and Barret, 1995; Rebanova et al., 1995; Lapinski et al., 2002; Li et al., 2010). The BAFs of earthworms were higher in wastes that showed relatively better mineralization. Thus, the BAF values were directly related to the amount of waste assimilated by the worms (Suthar et al., 2008). The observed differences for BAFs could be related to the difference in specific metabolism and regulating mechanism of organic matter fractions of organo-metal compounds in the earthworm body (Lukkari et al., 2006; Suthar and Singh, 2009).

Suthar et al. (2008) have demonstrated higher ranges of BAFs for earthworms collected from contaminated substrates; while some earlier studies reported considerable ranges of BCFs for metals in earthworms (Dia et al., 2004; Hsu et al., 2006).

Conclusions

The present findings suggested that vermicomposting could be an appropriate technology for remediation of metals from obnoxious wastes. It was found that the vermicompost of urban wastes was not only rich in plant nutrient but also have minimum risk of environmental contamination due to lower metal concentrations availability in it. Internal metal concentrations in all the three earthworms were significantly and negatively correlated to heavy metal concentrations in their respective vermicompost.

The present study depicted that the earthworm especially, *E. eugeniae* can be utilized effectively for *ex situ* remediation of metals from urban waste. The higher BAF ranges for metals implied higher metal accumulation in earthworms' tissue, which may affect the food chain through biomagnification. It further revealed that the accumulation of metals in worm's tissue, especially uptake of metals, not only remediated the metals from the urban wastes and their vermicomposts but also improve vermicompost quality reducing the metal concentration.

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