Eurasian Journal of Soil Science 2021, 10(1), 38 - 50

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org

Vermicomposting of anaerobically digested sewage sludge with hazelnut husk and cow manure by earthworm *Eisenia foetida*

Rıdvan Kızılkaya [a,b,*], Zhainagul Yertayeva [c], Sagynbay Kaldybayev [c], Bolat Murzabayev [d], Aigul Zhapparova [c], Zhanybek Nurseitov [c]

[a] Ondokuz Mayıs University, Faculty ofAgriculture, Department of Soil Science and Plant Nutrition, Samsun, Turkey
[b] Agrobigen Research & Development Trade Ltd.Co, Samsun Technopark, Samsun, Turkey
[c] Kazakh National Agrarian University, Almaty, Kazakhstan
[d] M.Auezov South Kazakhstan State University, Shymkent, Kazakhstan

Article Info

Received : 15.04.2019
Accepted : 07.10.2020
Available online : 08.10.2020

Author(s)

R. Kızılkaya *
Z. Yertayeva
S. Kaldybayev
B. Murzabayev
A.Zhapparova
Z. Nurseitov
* Corresponding author

Abstract

Vermicomposting of organic waste has an important part to play in an integrated waste management strategy. The aim of the present study was to investigate the ability of an epigeic earthworm *Eisenia foetida* to transform anaerobically digested sewage sludge (SS) amended with hazelnut husk (HH) and cow manure (CM) in different proportions under laboratory condition (in darkness at 25°C±0,5 ^{0}C). Two approaches investigated in the study were: (1) to find the best medium for growth and reproduction of *E. foetida* in different feed mixtures, (2) to analyze the heavy metal concentrations in different feed mixtures of SS-HH-CM before and after vermicomposting, and (3) to explore heavy metals accumulation of earthworms in sewage sludge with different feed mixtures. Number and biomass of earthworms and heavy metal contents in feed mixtures and earthworms were periodically monitored. The results indicated that maximum earthworm biomass was attained in feed mixture of 20% SS + 40% CM + 40% HH while the earthworm number was highest in feed mixture of 30% SS + 35% CM + 35% HH during the vermicomposting period. Heavy metals concentration (Zn, Cu, Cd, Pb, Ni and Cr) in all feed mixtures decreased associated with the increasing vermicomposting time. The heavy metals' content in the feed mixtures was lower than that of initial mixtures. Metal analysis of earthworms revealed considerable bioaccumulation of heavy metals in their bodies' tissue. Heavy metal analysis of earthworm body showed that increasing proportion of SS in the feed mixtures promoted the heavy metal content of earthworm body.

Keywords: Vermicompost, *Eisenia feotida*, sewage sludge, heavy metal, bioaccumulation.

Introduction

It is well established that a large number of organic wastes can be ingested by earthworms and egested as stabilized humus-like product termed as vermicompost. It is much more fragmented, porous and microbially active than parent material due to humification and increased decomposition (Edwards, 1988; Garg and Kaushik, 2005; Kızılkaya, 2008). Use of earthworms in waste management, organic matter stabilization, soil detoxification and vermicompost production has been reported by Bansal and Kapoor (2000), Kaushik and Garg (2003), Garg and Kaushik (2005), Gupta and Garg (2008). The epigeic forms of earthworms can hasten

doi : https://doi.org/10.18393/ejss.807762
: http://ejss.fesss.org/10.18393/ejss.807762
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

the composting process to a significant extent with production of a better quality of compost as compared with those prepared through traditional composting methods (Ndegwa and Thompson, 2001).

The use of earthworm in sludge management has been termed as vermistabilization (Neuhauser et al., 1988). During this process, important plant nutrients such as N, P, K etc. present in the waste are converted through microbial action into forms that are much more soluble and available to plants than those in the parent substrate (Ndegwa and Thompson, 2001; Kızılkaya and Hepşen 2004, 2007). The end product, namely vermicompost is considered as an excellent product, since it is stable and homogenous, has desirable aesthetics, has reduced levels of contaminants and furthermore, is a valuable, marketable and superior plant growth medium (Aranda et al., 1999).

Sewage sludge, the solid portion that remains after waste water treatment, may be high content of nutrients (N, P, K, etc.) essential trace elements, and organic matter, which improves soil physico-chemical properties and plant nutrient status, thus rendering sewage sludge an effective and cheap alternative to commercial fertilizers. Besides the beneficial effects, potential risks to environmental health associated with either non-essential in living organisms such as Cd, Cr, Ni, Pb and essential in only trace quantities such as Cu, Fe, and Zn have received increasing attention (Mench et al., 1994; McBride, 1995; Kızılkaya and Bayraklı, 2005). For this reason, sewage sludges, including high content of potentially toxic metals, must be stabilized and/or composted with different methods and reduce toxic metal concentrations. Composting and/or stabilizing of sewage sludge is therefore recommended as a method not only to avoid plant growth inhibition but also to facilitate the handling of the dewatered sewage sludge cake where it is mixed with soil.In addition, composting and/or stabilizing offers a minimum risk for the environment or public health, especially in relation to epidemiological aspects and odors (Katayama et al., 1987).

In recent years, earthworms have been widely used in the breakdown of sewage sludge and other organic wastes in producing vermicomposts (Jain et al., 2003). Some species of earthworms are known to be potential accumulators of heavy metals and therefore they have been successfully demonstrated in mitigating the toxicity of industrial and municipal waste by vermicomposting technology (Gupta et al., 2005). 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 and Singh 2004). Substantial evidence indicates that earthworms accumulate heavy metals from polluted soils and other media (Ireland, 1983; Goats and Edwards, 1988; Neuhauser et al., 1985; Edwards, 1996; Kızılkaya 2004, 2005). The most common earthworm used for vermicomposting is *Eisenia feotida*, commonly well known as red wigglers. Advantages of *E.feotida* are that it grows rapidly, and uses almost any organic matter as feeds. It has also wide temperate tolerance a high reproductive rate and has the capability of accumulate heavy metal accumulation in the sewage sludge vermicompost (Hartenstein, 1983; Edwards and Bater, 1992).

This study attempted vermicomposting of sewage sludge with hazelnut husk and cow manure attempted. The advantages of this utilization option are (a) it is capable of handling very low to very high quantities of sewage sludge vermicompost, (b) it is simple and cost-effective, thus appropriate for small scale as well as for large scale utilization and (c) the vermicasts have a very popular and ready markets as enrichers of soil (Abbasi and Ramasamy, 1999; Ismail, 1997). Vermicasts are believed to have several components, which improve the soil, where they are applied. Vermicasts are also believed to contain enzymes and hormones that stimulate plant growth and discourage pathogens (Abbasi and Ramasamy, 1999; Szczeck, 1999; Gupta et al., 2005). So, the main objectives of the present study were (i) to find out the appropriate proportion of sewage sludge – hazelnut husk – cow manure for sustainable and the best medium for growth of *E. feotida*, (ii) to analyze the heavy metal content in sewage sludge mixtures before and after vermicomposting, and (iii) to determine the concentration of heavy metals accumulated in earthworm tissues.

Material and Methods

Organic wastes and earthworm

Sewage sludge (pH 7.35, conductivity 1.82 dS m^{-1}, C:N ratio 9) was obtained from the wastewater facility set up by the Ankara Wastewater Treatment Plants, Ankara, Turkey. The sludge was anaerobically digested with a mixture of primary and waste activated sludge typically entering the digester. Hazelnut husk (pH 5.81, conductivity 1.93 dS m^{-1}, C:N ratio 47) collected from hazelnut trees in the Eastern Black Sea Region, Turkey. Hazelnut is one of the major cash crops in Turkey with a yield of 650 000 tons per year; it is basically produced in the Black Sea Region. Cow manure (pH 8.46, conductivity 2.35 dS m^{-1}, C:N ratio 12) mixed with minor amounts of bedding and feed refusals from different cows in Tokat, Turkey mixed within faeces type and dried in the sun. The sewage sludge (SS), hazelnut husk (HH) and cow manure (CM) on an average contained 22.9%, 53.9%, and 20.7% organic C; 2.54%, 1.14%, and 1.70% total N; 2184 $\mu g.g^{-1}$, 379 $\mu g.g^{-1}$, and

189 μg.g^{-1} NH_4^+-N; 1873 μg.g^{-1}, 2490 μg.g^{-1}, and 2294 μg.g^{-1} NO_3-N; 2.43%, 0.34%, and 2.66% total P; 1.14%, 2.19%, and 3.94% total K, respectively. The organic wastes (SS, HH and CM) in this experiment was digested and air dried and sieved to less than 0.5 mm and stored in polyethylene bags at 5 ^{0}C until used. The content of the some heavy metal of interest in the organic wastes are given in Table 1. The *Eisenia feotida* were collected from the same CM. Earthworms were washed with distilled water and kept for 2 weeks before starting the experiment in containers with CM at 25 ± 0.5 0C.

Table 1. Heavy metal concentrations (μg.g^{-1}) in SS, HH and CM used in this study

Heavy metal	SS	HH	CM
Zn	15961,7	120,6	559,1
Cu	392,4	18,9	135,9
Cd	10,34	1,37	3,29
Pb	119,8	8,3	38,3
Ni	111,1	35,7	60,9
Cr	718,1	28,6	105,1

Experimental design

A randomized complete plot design with five replicates per treatment and organic wastes were used. The experiment was performed with the following 11 treatment and given in Table 2.

Table 2. Composition of treatments used for experimentation

Mixture number	Mixture Description	SS		HH		CM	
		(g)	(%)	(g)	(%)	(g)	(%)
1	0% SS + 50% HH + 50% CM	0	0	250	50	250	50
2	10% SS + 45% HH + 45% CM	50	10	225	45	225	45
3	20% SS + 40% HH + 40% CM	100	20	200	40	200	40
4	30% SS + 35% HH + 35% CM	150	30	175	35	175	35
5	40% SS + 30% HH + 30% CM	200	40	150	30	150	30
6	50% SS + 25% HH + 25% CM	250	50	125	25	125	25
7	60% SS + 20% HH + 20% CM	300	60	100	20	100	20
8	70% SS + 15% HH + 15% CM	350	70	75	15	75	15
9	80% SS + 10% HH + 10% CM	400	80	50	10	50	10
10	90% SS + 5% HH + 5% CM	450	90	25	5	25	5
11	100% SS + 0% HH + 0% CM	500	100	0	0	0	0

The organic wastes (SS, HH and CM) were thoroughly mixed (Table 2) on air-dried weight basis by a mixer. These mixtures (500 g dry weight) were placed in a 1-L cylindrical plastic container. Then, three clitellated earthworm Eisenia foetida each weighing between 0.6 and 0.7 g, were placed in the mixed material. Each treatment was replicated three times. The samples were first adjusted to 50% of the soil water holding capacity by adding distilled water and then pre-incubated at 25 ^{0}C for one day (conditioning period). After conditioning, the moisture content of the mixture was maintained at 70% throughout the vermicomposting period and the containers were maintained in darkness at 25°C ± 0,5 ^{0}C. Because, optimal environmental conditions for the growth and reproduction of *E.foetida* fed on wastes are a temperate range of 15-25 °C, moisture content of 43-90% and pH of 5-9 (Kaplan et al., 1980; Edwards, 1988; Neuhauser et al., 1988; Edwards and Bater, 1992). Substrate samples collected every 15 days during vermicomposting period (90 days) to determine the heavy metal distribution, and were stored in plastic vials at 4°C until analysis. Earthworm numbers and biomass gain were recorded for every vermicomposting period.

Changes in the total earthworm mass and the number within each maturity category were determined 15 days. All worms and vermicompost were taken from the core and placed onto a tray. Under red light (to minimise stress) the worms were separated from the vermicompost. Worms separated from the vermicompost were washed thoroughly under slow running water. Most vermicompost separated from the worms freely and was easily removed; however some vermicompost clung to the worms and required further washing. The worms were classified by maturity category into adults, subadults and juveniles. Adults were classified by the presence of a large and clearly visible clitellum. Subadults had no clitellum and tended to be smaller than the adults. Juveniles were very small and transparent. Each category was counted, weighted and immediately stored at -80 ^{o}C to use during the heavy metal analysis.

Total heavy metal contents in vermicompost

The total heavy metal contents of the vermicomposts were determined by atomic absorption spectrophotometry (Perkin Elmer A400) following a digestion with a mixture of Aqua Regia-HNO_3 and HCI.

Heavy metal contents in earthworms body

Earthworms were oven-dried in glass flask at 105^{0}C. The dried earthworms were digested overnight in nitric acid at a rate of 1 ml HNO_3 per mg dry weight of earthworm. After heating at 120^{0}C and evaporation, 1 ml $HNO_3/H_2SO_4/HCl$ (10/2/3; v/v/v) was added. The solution was heated at 180^{0}C; after cooling, samples were diluted with deionized water up to 25 ml. The concentrations of copper and zinc in earthworms were determined by flame atomic absorption spectrophotometry using and air-acetylene-flame device (Perkin Elmer A400) (Scaps et al., 1997).

Bioaccumulation Factors (BAF)

BAF for earthworm E.feotida were estimated based on the heavy metals in earthworm tissues and substrate materials using the method described by Pearson et al. (2000). The BAF is defined as follows: BAF=Cbiota/Csubstrate, where Cbiota and Csubstrate were the total heavy metal concentrations (in μg.g^{-1}) in taxa (earthworm) and substrate (used for vermicomposting experiment), respectively. It was possible to obtain BAF estimates for heavy metals since the earthworm concentrations for these metals reached steady state levels during the testing period.

Statistical analysis

All data were analyzed using SPSS 11.0 (Statistical Package for Social Science) statistical software. Analysis of variance (two-way ANOVA) was carried out using two factors randomized plot design (mixture ratio and vermicomposting period). The means were compared using by the LSD (Least Significant Difference) test, with a significance level of $P< 0.01$. All the figures presented include standard deviation of the data. The asterisks, *, ** and *** indicate significance level at $P<0.05$, 0.01 and 0.001 respectively.

Results and Discussion

Earthworm production and reproduction

Figure 1 and 2 show the values obtained from the experiments for production and reproduction in *Eisenia foetida* in different feed mixtures. Increasing proportion of SS in the feed mixtures caused the decrease in survival and growth of *E.foetida*. Mortality was recorded in ≥ 60% SS feed mixtures (mixture no 7, 8, 9, 10, 11) at all vermicomposting period. This indicated that a greater percentage of SS in the feed mixture was significantly toxic for the production and reproduction of *E.foetida*. This situation may be related high NH_4-N and Zn concentrations of anaerobically digested SS. Similarly, Elvira et al. (1997) showed worms were unable to survive in paper-pulp mill sludge; however, feed mixtures of paper-pulp mill sludge with pig and poultry slurry were suitable materials for vermicomposting. They attributed this mortality to degradation processes, resulting in changes of the environmental characteristics. Masciandaro et al. (2002) have also reported the some results for vermicomposting of anaerobic and aerobic sludges using *E.foetida*. In this study, feeds having higher percentage of anaerobic sludge were not accepted by the worms. The other study conducted by Harstenstein and Mitchell (1978) determined that anaerobically digested sludge was found to be acutely toxic to *E.feotida* but this toxicity a disappeared when the sludge was allowed to age for 2 months as thin layers exposed to air. On the other hand, Edwards (1988) indicated that organic wastes containing much ammonia (> 500 μg.g^{-1}) or large amounts of inorganic salts are toxic to *E.feotida*. In addition, Harstenstein and Mitchell (1978) suggested that various sludge treatments with salts of heavy metal at these concentrations were not toxic to *E.feotida* over a six week period: Cd at 100 μg.g^{-1}, Ni at 1000 μg.g^{-1}, and Pb at 5000 μg.g^{-1}. The same sludge treated with Cu at 2500 μg.g^{-1} or with Zn at 10000 μg.g^{-1} was toxic.

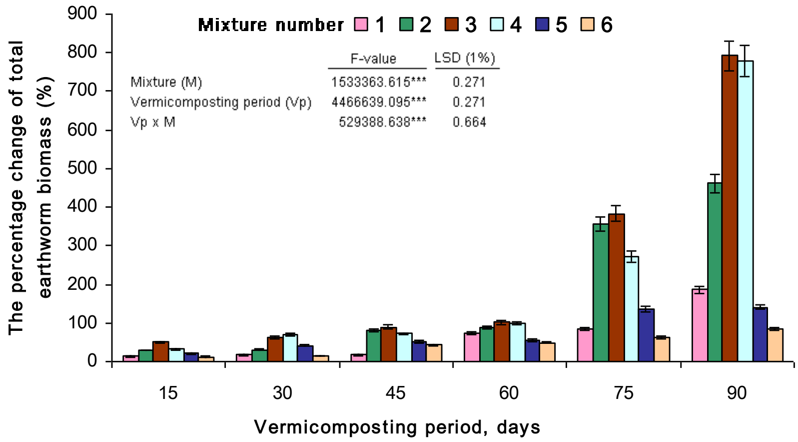

Figure 1. The percentage change of total earthworm biomass in experimental units obtaining different feed mixtures treatments (n=5).

During the vermicomposting period, the percentage changes of total earthworm biomass production by E. foetida in different feed mixtures were given in Figure 1. The numbers of *E. foetida* in the studied feed mixtures associated with observation period were given in Figure 2. In all feed mixtures, significant differences in the total earthworm biomass were recorded. The percentage change of total earthworm biomass was similar in all vermicomposting periods. Feed mixtures no.3 (20% SS + 40% CM + 40% HH) had the highest worm masses while the lowest was observed in the 50% SS + 25% CM + 25 HH feed mixture (no.6) at $P<0.001$.

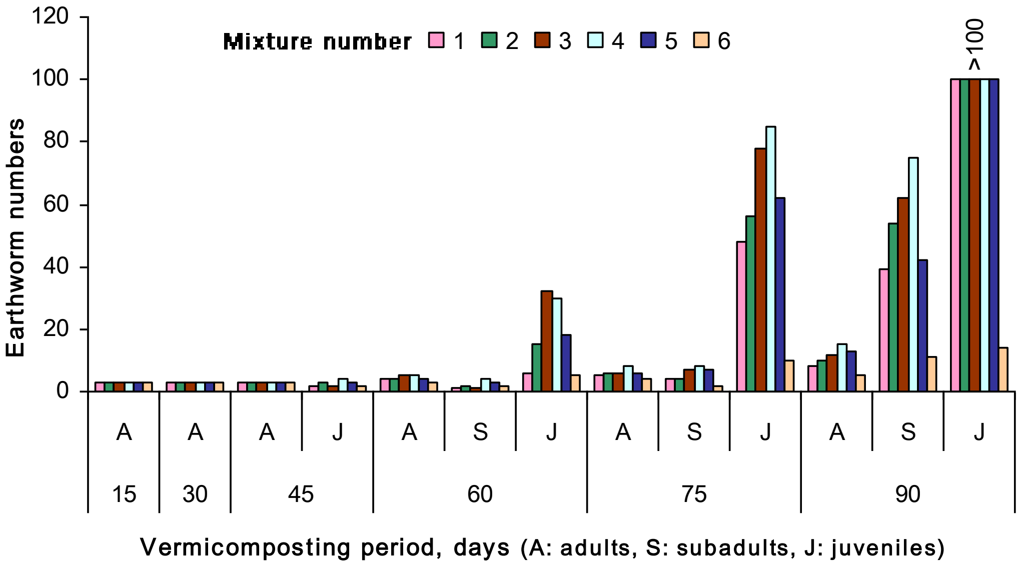

Figure 2. Population dynamics of earthworm *E.feotida* in experimental units obtaining different feed mixtures treatments (n=5).

Increasing percentage of SS in the feed mixtures led to decrease in the number of earthworm *E.feotida*. The net number gain by *E.foetida* was higher in feed mixtures no. 3 and 4 compared to other feed mixtures. The maximum earthworm biomass and number were observed in the 75th or 90th day in all the feed mixtures. Based on the results of six sampling dates for each class of worms, there was interaction between sampling time and feed mixture. However, the population dynamics within mixture feeds tended to be cyclical; for example, mixture feeds had high numbers of adults at three sampling time and low adult numbers, then at the next sampling time, the reverse was recorded. To account for this cyclical shift in populations were depicted in Figure 2.

Total heavy metal contents in vermicompost

Heavy metals appear in the sewage sludge from a variety of sources like batteries, consumer electronics, ceramics, light bulbs, plastics, house dust and paint chips, etc. So, the vermicompost made from sewage sludge may have higher heavy metal concentrations (Gupta and Garg, 2008). In small amounts, many of these elements may be essential for plant growth, however, in higher concentrations they are likely to have detrimental effects upon plant growth (Whittle and Dyson, 2002). So, prior to vermicompost application to the soils, there is in need of determining the heavy metal concentrations in final vermicomposts. The results indicated that initial heavy metal contents of SS were higher than HH and CM (Table 1), resulting in higher heavy metal concentrations in SS containing initial feed mixtures. Table 3 presents the heavy metal status in different mixtures of SS, HH and CM before vermicomposting.

Table 3. Heavy metal concentrations ($\mu g.g^{-1}$) in mixture feeds before vermicomposting

Heavy Metal	Mixture number					
	1	2	3	4	5	6
Zn	339,89	1902,07	3464,26	5026,44	6588,62	8150,81
Cu	77,40	108,90	140,40	171,90	203,40	234,90
Cd	2,33	3,13	3,93	4,73	5,53	6,34
Pb	66,87	132,00	197,12	262,25	327,37	392,50
Ni	23,28	32,92	42,57	52,22	61,87	71,52
Cr	48,32	54,60	60,88	67,17	73,45	79,73

The results of comparisons revealed that heavy metal concentrations in final vermicompost in the feed mixtures no. 1–6 were lower than that of the initial feed mixtures (Figure 3) and these heavy metal concentrations increased from the feed mixtures no 1 to 6. Feed mixtures no 4–6 contained more SS than that of feed mixtures no 1–3. Heavy metal concentrations in the vermicompost decreased associated with time increasing (Figure 3).

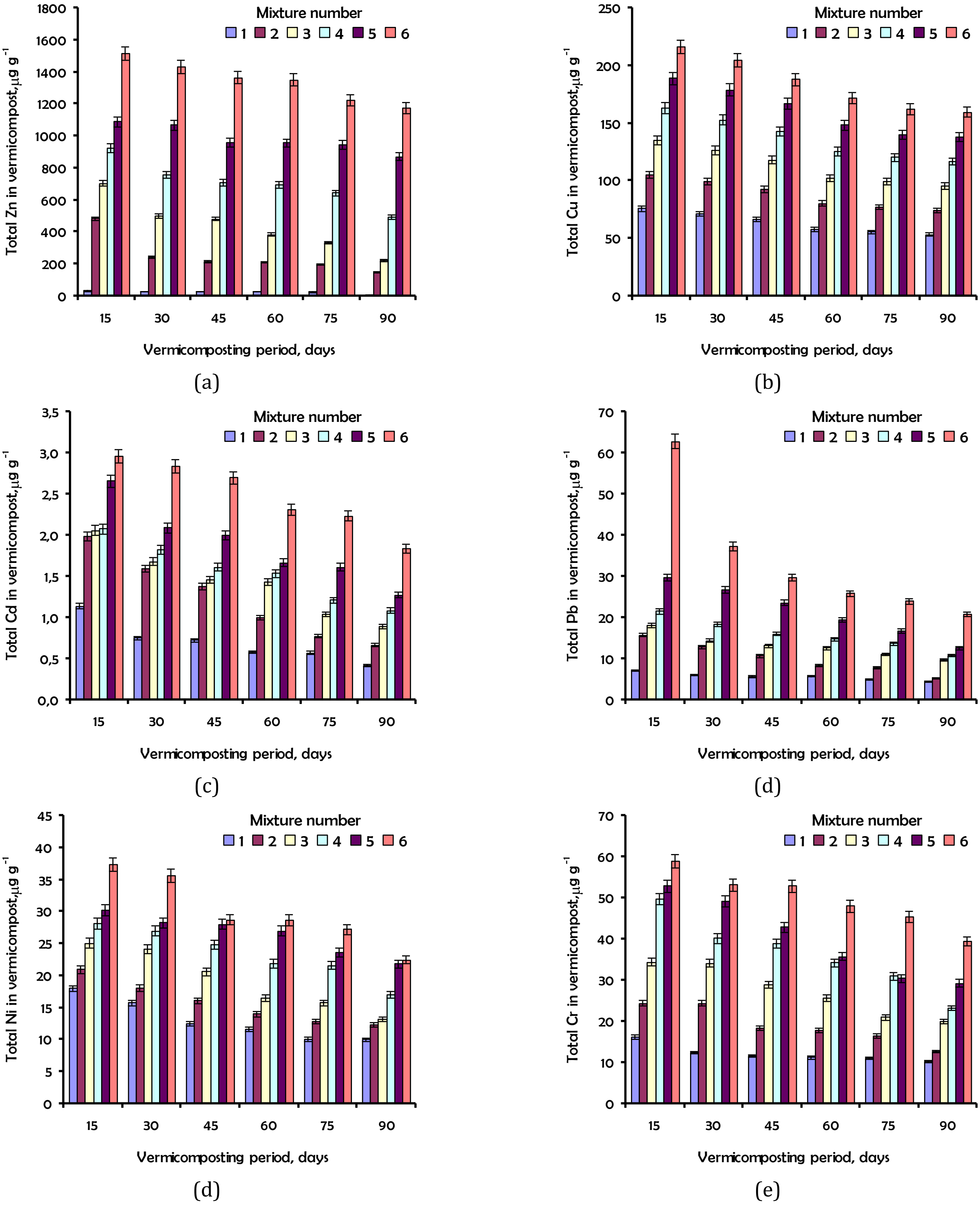

Figure 3. Changes in heavy metal concentrations (μg.g-1) in vermicomposting of different feed mixtures associated with time during vermicomposting process. Vertical bars indicate standard error of mean of three replicates at 95% confidence level. a) Zn   b) Cu   c) Cd   d) Pb   e) Ni   f) Cr

Zn was the highest concentrations in all mixture feeds and followed by Cu, Cr, Pb, Ni and Cd. The analysis of postvermicomposted samples revealed considerable decline in metal concentrations in all mixture feeds. The results of ANOVA (Table 4) indicated that there were significant differences among feed mixtures for the contents of Zn, Cu, Cd, Pb, Ni and Cr at different vermicomposting period.

Table 4. Results of ANOVA

		Mixture (M)		Vermicomposting period (VP)		M x VP	
		F-value	$LSD_{\alpha=0.01}$	*F*-value	$LSD_{\alpha=0.01}$	*F*-value	$LSD_{\alpha=0.01}$
Metals in vermicompost	Zn	16540,184***	14,583	751,316***	14,583	46,145***	35,721
	Cu	15211,053***	1,045	2132,948***	1,045	36,015***	3,440
	Cd	11854,068***	0,021	5271,295***	0,021	86,898***	0,052
	Pb	11285,000***	0,355	3434,383***	0,355	561,634***	0,870
	Ni	12515,521***	0,224	4535,933***	0,224	95,949***	0,548
	Cr	14032,726***	0,452	2844,023***	0,452	128,698***	1,108
Metals in Earthworm body	Zn	16650,914***	68,047	648,531***	68,047	44,540***	166,681
	Cu	6058,989***	0,501	4714,220***	0,501	1348,415***	1,227
	Cd	14797,186***	0,036	2502,279***	0,036	34,193***	0,088
	Pb	15248,899***	0,430	2097,935***	0,430	36,192***	1,053
	Ni	10417,604***	0,321	7020,564***	0,321	18,785***	0,786
	Cr	16215,307***	2,852	1100,241***	2,852	41,510***	6,987
Bioaccumulation factor	Zn	6273,274***	0,188	2916,186***	0,188	1667,778***	0,461
	Cu	2516,827***	0,002	7206,373***	0,002	1641,486***	0,004
	Cd	5323,754***	0,015	9396,826***	0,015	591,053***	0,038
	Pb	9617,526***	0,020	5594,875***	0,020	482,634***	0,049
	Ni	12995,577***	0,013	2334,413***	0,013	402,773***	0,033
	Cr	9122,221***	0,028	4789,747***	0,028	724,567***	0,069

*** $P<0.001$

Based on the chemical analysis of vermicomposted samples, considerable reduction in heavy metals concentrations was observed for all feed mixtures (Figure 4). Vermicomposted material had reduced heavy metal content at the end of the experiment. The reductions ranged between 74.6 and 98.3% for Zn, 2.1 and 32.0% for Cu, 36.6 and 81.8% for Cd, 12.2 and 84.3% for Pb, 53.2 and 79.2% for Ni and between 75.8 and 91.1% for Cr (Figure 4).

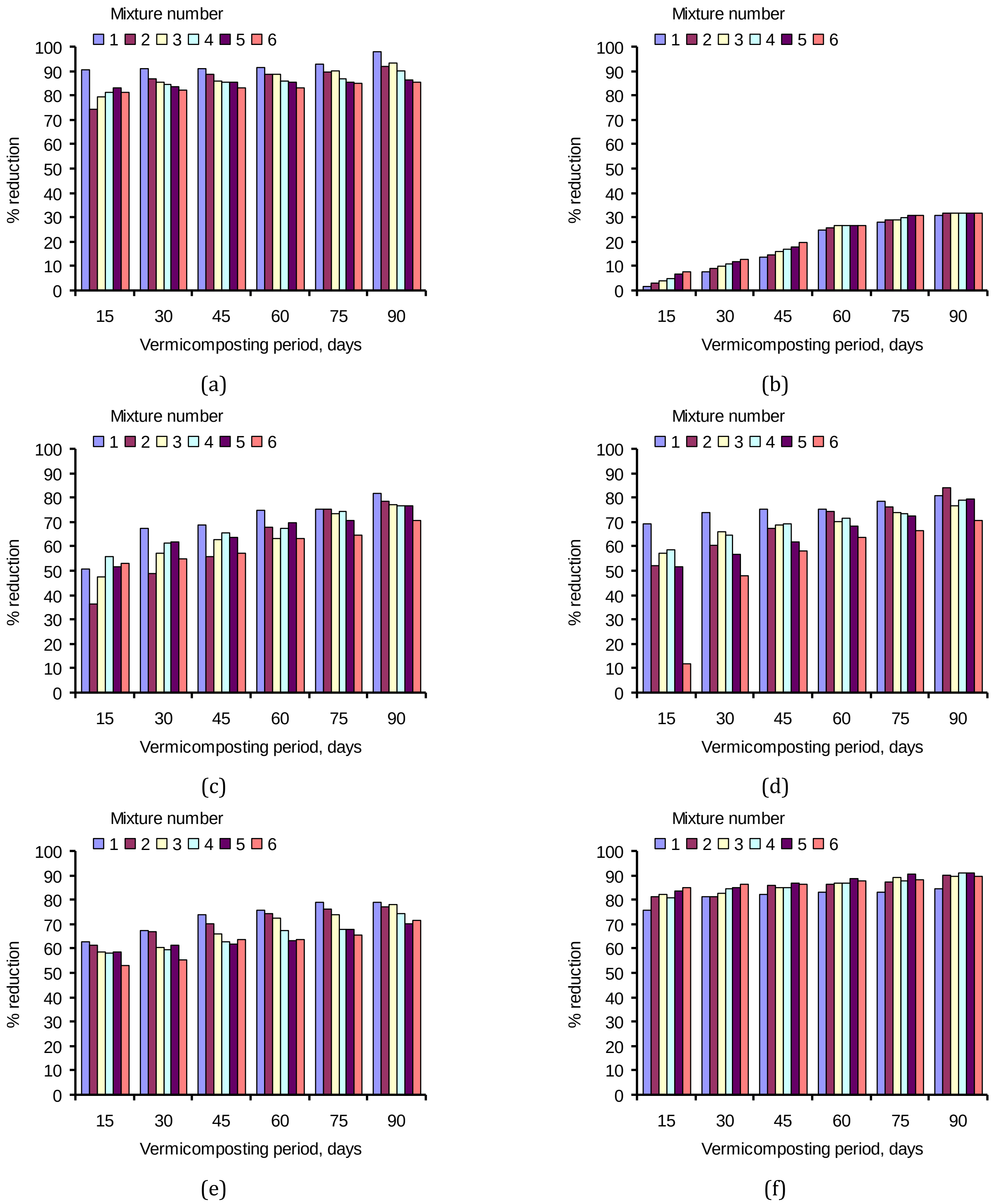

Figure 4. % reduction of heavy metals of different feed mixtures with time during vermicomposting process
a) Zn b) Cu c) Cd d) Pb e) Ni f) Cr

The heavy metal reduction increased associated with vermicomposting period depending upon earthworm growth activities. Therefore, it was attributed to the earthworm activity and/or vermicomposting time in the waste decomposition system. Some of the metals were accumulated in body tissues. Similarly, previous studies have revealed that earthworms can accumulate heavy metals in their tissues during the process of vermicomposting (Hartenstein and Hartenstein 1981; Graff 1982; Garg and Kaushik 2005; Gupta et al.

2005). Garg and Kaushik (2005) reported a considerable loss in heavy metal contents from solid textile mill sludge mixed with poultry droppings. They attributed the heavy metal loss from substrate to accumulation by earthworm body tissues. Also, Gupta et al. (2005) studied the vermicomposting of fly ash by mixing it with cow dung in different ratios and reported 30–50% loss in heavy metal content in different combination, at the end. They reported that heavy metals bioaccumulated in earthworm tissues. This study confirmed that earthworms could efficiently reduce the metal content in substrate.

Heavy metals in Earthworm body

The earthworm *E.feotida* collected at the end from different feed mixtures showed considerable concentrations of metals in their bodies (Figure 5). The difference among feed mixtures in terms of contents of metals in earthworms was statistically significant (Table 4).

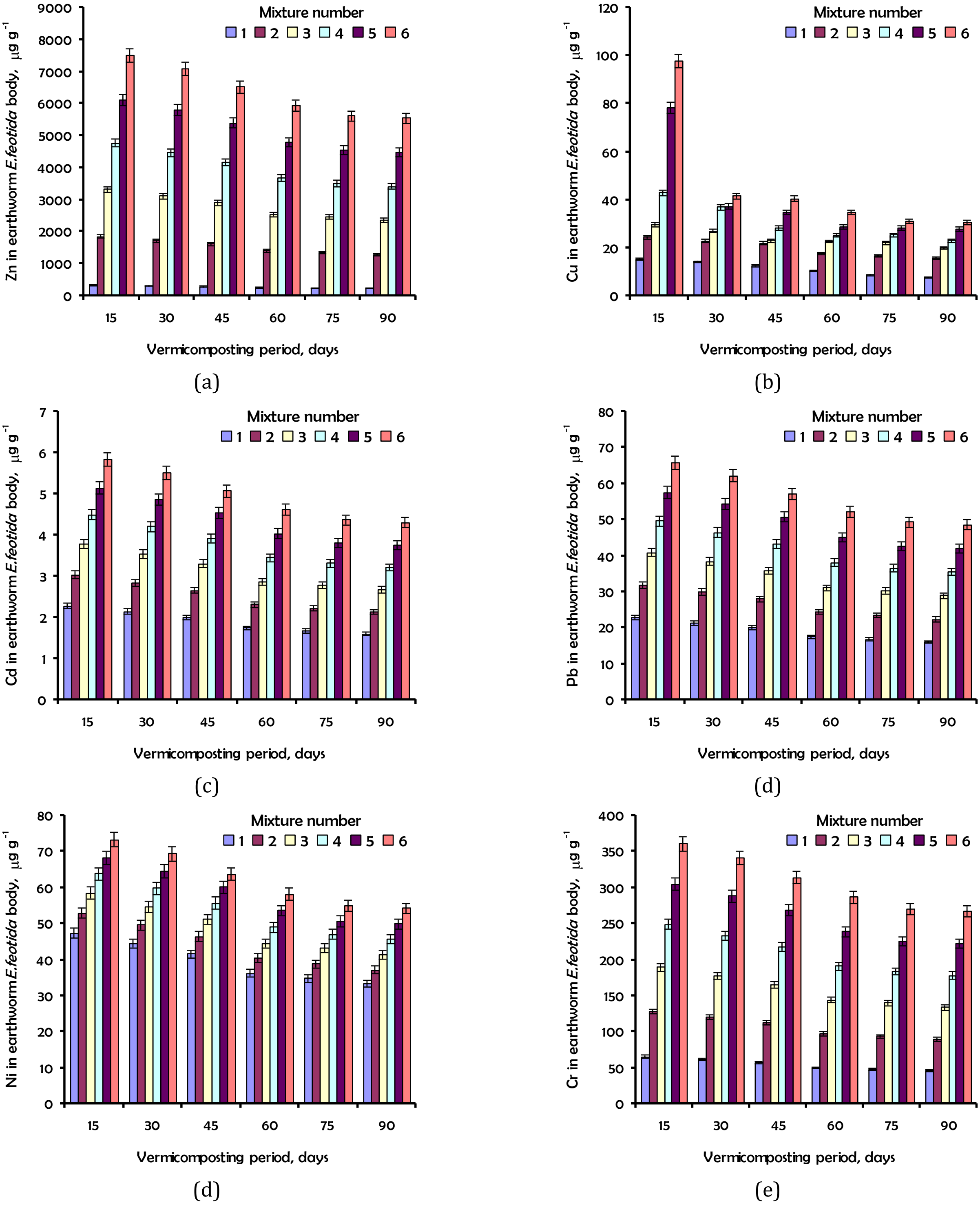

Figure 5. Changes in heavy metal content in earthworm *E.feotida* body with time during vermicomposting process. Vertical bars indicate standard error of mean of three replicates at 95% confidence level.
a) Zn b) Cu c) Cd d) Pb e) Ni f) Cr

The heavy metal concentrations in earthworm body tissues decreeased associated with time, and at all vermicomposting period of the experiment, heavy metal content of earthworm body in the feed mixture no. 6 (50% SS + 25% HH + 25% CM) was significantly higher (P<0.01) than that of other feed mixtures. The observed difference in heavy metal contents in vermicomposted material for different feed mixtures may be related to the different rates of SS for heavy metals. It meant that the heavy metal level in earthworm was directly related to the contents of metals in feed mixtures. Our finding, first two treatments supported the above statement (Figure 5).

Bioaccumulation of high concentration of metals is well documented (Hsu et al., 2006). According to Kızılkaya (2004, 2005) and Suthar (2008), earthworms accumulate a considerable content of metal in their tissues and can serve as useful biological indicator of contamination due to the fairly consistent relationships among the concentrations of certain contaminants in earthworms. In this study, SS proportion in feed mixtures seemed to be of primary importance. Finding on concentration of heavy metals in earthworm *E. feotida* collected from vermibeds with higher proportion of SS e.g., feed mixture no 5 and 6, further supported the above hypothesis. Zn and Cu concentration was greater in tissues of inhabiting earthworms than other studied heavy metals. It may be attributed that these metals could be a part of different metabolic requirements of earthworms for Zn and Cu. Ireland (1983) stated that Cd did not appear in earthworm tissues indefinetely and the BAF ratio decreases with increasing Cd concentration, unlike Pb that appears in tissues continously. Carter et al. (1983) found some regulation of Zn and Cu, but not of Cd which reached a maximum in earthworm tissue of about 34 $\mu g.g^{-1}$. Graff (1982) examined the accumulation of heavy metals in *E.feotida* before and after feeding on compost made from municipal garbage. The heavy metal contents ($\mu g.g^{-1}$) before and after feeding were: Cu 4 to 29, Zn 140 to 640, Pb 3 to 14, Cd 2 to 9. These data indicated that earthworm *E.feotida* was extracting the heavy metals from the compost and accumulating them in their tissues. Previous reports on the metal accumulation ability of earthworms stated that the metals like Cu and Ni are not bioaccumulated by the earthworms (Barerra, 2001), but results of the present study explored considerable bioaccumulation of these metals in earthworms. This study confirmed and extended the earlier studies that earthworms can accumulate a considerable amount of metals in their tissues when inoculated in SS. In general, the content of metals in earthworms depends on inhabiting substrate metal contents (Lukkari et al., 2006).

Bioaccumulation factors (BAF)

The bioacumulation factors (BAF) for the different heavy metals in the earthworm *E.feotida* body tissues during the 90 day vermicomposting period are depicted in Figure 5. BAF varied associated with the different feed mixtures in this study (Table 4). When the BAF were calculated in relation to the total metal concentrations in different mixture feeds, the highest value came from Zn. The BAF were higher than 3 for Zn, Cr and Pb but lower than 3 for Cd, Ni and Cu in mixture feeds. In addition, the results showed that elevation of BAF increased with in SS proportions for all metals except Cu and Cr (Figure 6). BAF of the six heavy metals in vermicomposting for 90 d by the earthworm *E.feotida* was ranked as: Zn> Cr> Pb> Cd>Ni>Cu.

Composting earthworm *E.feotida* showed relatively greater values of BAF for Zn and Cu than compared to heavy metals. The difference among different metals for BAF may be related to the difference in specific metal regulating mechanism in earthworms. Recent studies have revealed that accumulation of metals, especially Cd, Cu and Zn, in earthworms is mainly due to the binding of metals by metallothioneins (Kagi and Kojima, 1987). The BAF ranges calculated in this study, however, were higher than those of reported by earlier researchers (Dia et al., 2004; Hsu et al., 2006; Suthar and Singh 2008; Kızılkaya 2004, 2005). The observed difference for BAF in present and past studies could be related with the level of metals contamination and exposure duration or earthworm species type (Suthar and Singh, 2008). According to Morgan and Morgan (1992), difference species can show a considerable difference for tissue's metal contents mainly due to difference in their food selectivity and metabolic physiology. Similarly, Hopkin (1989) suggested that earthworms have specific capacity to regulate metals, particularly trace metals, such as Cu and Zn, in their bodies, and accumulation and regulation mechanisms could be species-specific. It was also suggested here that exposure duration could be main determinant for observed differences in BAF; although the exposure duration was relatively longer in this study (i.e., 90 days) compared to previous studies (Kizilkaya 2004, 2005). Few past studies reported considerable ranges of BAF for metals in earthworms (Dia et al., 2004; Hsu et al., 2006). The higher BAF ranges for metals suggested that vermicomposting could be a risky technology if applied to stabilize SS. There is great possibility of entering of toxicants via earthworms to organisms occupying different trophic levels, if proper management of inoculated worms is not made (Suthar and Singh, 2008).

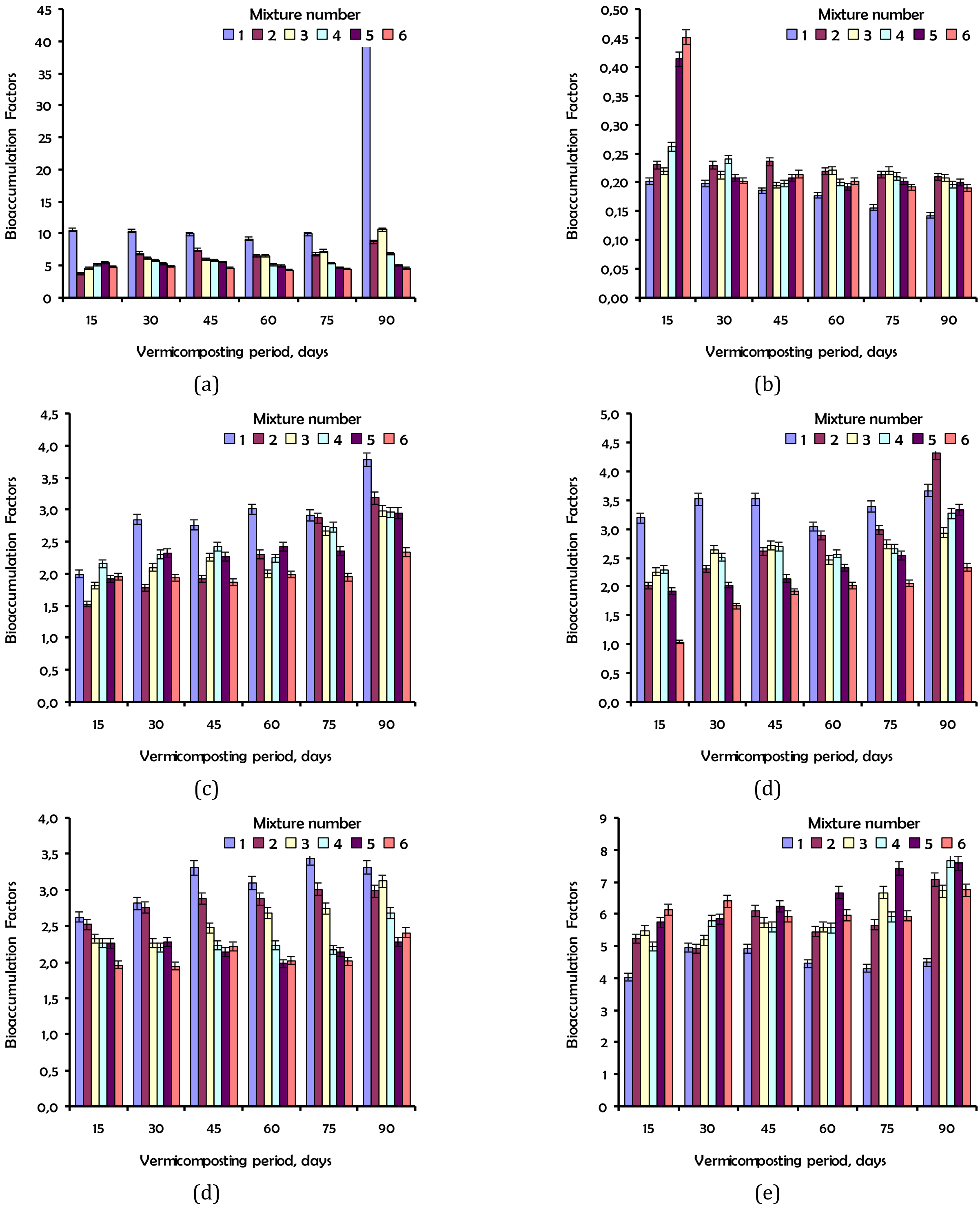

Figure 6. Bioaccumulation factors of heavy metals accumulated in earthworm *Eisenia feotida* body in relation with metal concentrations in different feed mixtures. Vertical bars indicate standard error of mean of three replicates at 95% confidence level. a) Zn b) Cu c) Cd d) Pb e) Ni f) Cr

Conclusion

Disposal of SS by environmentally acceptable means is a serious problem. Our trials demonstrated that vermicomposting could be an alternate technology for the management of primary SS mixed with HH and CM. Vermicomposting of SS, after mixing it with a HH and CM reduced in the concentration of heavy metals. However, in both feed mixture no. 3 (20% SS + 40% HH + 40% CM) and feed mixture no. 4 (30% SS + 35% HH + 35% CM) maximum increase in numbers and biomass production rates of earthworms as well as decrease in heavy metal concentrations was recorded during the vermicomposting period. The decrease in metal concentrations in the vermicompost indicated the capability of *E. feotida* in accumulating heavy metals in their body tissues. Although, earthworm *E.feotida* could efficiently reduce the contents of heavy metals in sludge, which could be further used for sustainable land restoration practices, but greater level of bioconcentrated metals in earthworm tissues could not be ignored due to high level of mortality was recorded in ≥ 60% SS feed mixtures. The results indicated that after the addition of primary SS in appropriate quantities (20–30%) to the HH and CM, it may be used as a raw material in the vermicomposting.

Heavy metals can be accumulated in earthworm tissues to reduce of heavy metal level in during the vermicomposting process. The metal contamination is a major problem during direct field application of such SS. Earthworm biomass production and reproduction performance was found

excellent in bedding those contained lower proportions of distillery sludge i.e. feed mixture no. 3 and 4. It is suggested that the numbers and biomass production rates of earthworms were significantly affected by the proportion of SS of their feed mixtures. Results indicated that SS mixed with HH and CM could be utilized as an efficient soil conditioner for sustainable land restoration practices, at low-input basis, after processed by epigeic earthworms *E.feotida*. The study also inferred that the application of SS-based vermicompost in the agricultural fields as a soil conditioner, would not have any adverse effect.

References

Abbasi, S.A., Ramasamy, E. V., 1999. Biotechnological Methods of Pollution Control. Orient Longman Ltd. UK. 168 pp.

Aranda, E., Barois, I., Arellano, P., Irisson, S., Salazar, T., Rodriguez, J., Patron, J.C., 1999. Vermicomposting in the tropics. In: Earthworm Management in Tropical Agroecosystems. Lavelle, P., Brussard, L., Hendrix, P., (Eds.), CABI, UK, pp. 253–287.

Bansal, S., Kapoor, K.K. 2000. Vermicomposting of crop residues and cattle dung with *Eisenia foetida*. *Bioresource Technology* 73(2): 95–98.

Barrera, I., Andrés, P., Alcañiz, J. M., 2001. Sewage sludge application on soil: Effects on two earthworms species. *Water, Air, and Soil Pollution* 129: 319–332.

Carter, G.S., Kenney, E.A., Guthrie, T.F., Timmenga, H., 1983. Heavy metals in earthworms in non-containated and contaminated agricultural soil from near Vancouver, Canada. In: Satchell, J.E. (Ed.). Earthworm Ecology. Chapman and Hall. London, UK. pp.267-274.

Dia, J., Becquer, T., Rouiller, J.H., Reversat, G., Bernhard-Reversat, F., Nahmani, J., Lavelle, P., 2004. Heavy metal accumulation by two earthworm species and its relationship to total and DTPA-extractable metals in soils. *Soil Biology and Biochemistry* 36(1): 91–98.

Edwards, C.A., 1996. Biology and Ecology of Earthworms. 3rd edition Chapman & Hall, New York. Usa. 426p.

Edwards, C.A., 1988. Breakdown of animal, vegetable and industrial organic wastes by earthworms. In: Earthworms in Waste and Environmental Management, Edwards, C.A., Neuhauser, E.F., (Eds), SPB Academic Publishing, The Hague, The Netherlands. pp. 21–31.

Edwards, C.A., Bater, J.E., 1992. The use of earthworms in environmental management. *Soil Biology and Biochemistry* 24(12): 1683-1689.

Elvira, C., Sampedro, L., Dominguez, J., Mato, S., 1997. Vermicomposting of wastewater sludge from paper-pulp industry with nitrogen rich materials. *Soil Biology and Biochemistry* 29(3-4): 759-762.

Garg, V.K., Kaushik, P., 2005. Vermistabilization of textile mill sludge spiked with poultry droppings by an epigeic earthworm *Eisenia foetida*. *Bioresource Technology* 96(9): 1189–1193.

Goats, G.C., Edwards, C.A., 1988. The Prediction of Field Toxicity of Chemicals to Earthworms by Laboratory Methods, In: Earthworms in Waste and Environmental Management, Edwards, C.A., Neuhauser, E.F., (Eds), SPB Academic Publishing, The Hague, The Netherlands. pp. 283-294.

Graff, O., 1982. Vergleich der Regenswurmaten Eisenia feotida und Eudrilus eugeniae hinsichlich ihrer Eignung zur Proteinwinnung aus Abfallstoffen. *Pedobiologia* 23 : 277-282.

Gupta, S.K., Tewari, A., Srivastava, R., Murthy, R.C., Chandra, S., 2005. Potential of Eisenia foetida for sustainable and efficient vermicomposting of fly ash. *Water, Air, and Soil Pollution* 163: 293–302.

Gupta, R., Garg, V.K., 2008. Stabilization of primary sewage sludge during vermicomposting. *Journal of Hazardous Materials* 153(3): 1023–1030.

Hartenstein, R., 1983. Assimilation by the earthworm *Eisenia fetida*. In: Earthworm Ecology: from Darwin to Vermiculture. Satchell, J.E. (Ed), Chapman and Hall, London, pp. 297-308.

Hartensein, R., Hartenstein, F., 1981. Physicochemical changes effected in activated sludge by the earthworm *Eisenia foetida*. *Journal of Environmental Quality* 10(3): 377–382.

Harstenstein, R., Mitchell, M.J., 1978. Utilization of earthworms and microorganisms in stabilization, decontamination and detoxification of residual sludges from treatment of wastewater. Final Report, US Department of Commence, National Technical Information Services, PB 286018, Springfield, Virginia. 34pp.

Hopkin, S.P., 1989. Ecophysiology of Metals in Terrestrial Invertebrates. Springer Netherlands, 366p.

Hsu, M.J., Selvaraj, K., Agoramoorthy, G., 2006. Taiwan's industrial heavy metal pollution threatens terrestrial biota. *Environmental Pollution* 143(2): 327–334.

Ireland, M.P., 1983. Heavy metal uptake and tissue distribution in earthworms. In : Earthworm Ecology: From Darwin to Vermiculture. Satchell, J.E. (Ed), Chapman and Hall, London, UK. pp. 247-265.

Ismail, S.A., 1997, Vermicology: The Biology of Earthworms, Orient Longmans, Hyderabad, 92 pp.

Jain, K., Singh, J., 2004. Modulation of fly ash induced genotoxicity in vicia faba by vermicomposting. *Ecotoxicology and Environmental Safety* 59(1): 89-94.

Jain, K., Singh, J., Gupta, S.K., 2003. Development of a modified vermireactor for efficient vermicomposting: a laboratory study. *Bioresource Technology* 90(3): 335-337.

Kagi, J.H.R., Kojima, Y., 1987. Chemistry and biochemistry of metallothionein. In: Metallothionein II. Kagi, J.H.R., Kojima, Y. (Eds). Birkhauser, Verlag. Basel, Switzerland. pp 25–61.

Kaplan, D., Hartenstein, R., Neuhauser, E.F., Malecki, M.R., 1980. Physicochemical requirements in the environment of the earthworm Eisenia foetida. *Soil Biology and Biochemistry* 12(4): 165-171.

Katayama, A., Ker, K.C., Hirai, M., Shoda, M., Kubota, H., 1987. Stabilization process of sewage sludge compost in soil. *Soil Science Plant Nutrition* 33(1): 123-135.

Kaushik, P., Garg, V.K., 2003. Vermicomposting of mixed solid textile mill sludge and cow dung with epigeic earthworm Eisenia foetida. *Bioresource Technology* 90(3): 311–316.

Kızılkaya, R., 2004. Cu and Zn accumulation in earthworm *L. terrestris* L. in sewage sludge amended soil and fractions of Cu and Zn in casts and surrounding soil. *Ecological Engineering* 22(2): 141–151.

Kızılkaya, R., 2005. The role of different organic wastes on zinc bioaccumulation by earthworm *Lumbricus terrestris* L. (Oligochaeta) in successive Zn added soil. *Ecological Engineering* 25(4): 322-331.

Kızılkaya, R., 2008. Dehydrogenase activity in *Lumbricus terrestris* casts and surrounding soil affected by addition of different organic wastes and Zn. *Bioresource Technology* 99(5): 946–953.

Kızılkaya, R., Bayraklı, B., 2005. Effects of N-enriched sewage sludge on soil enzyme activities. *Applied Soil Ecology* 30(3): 192-202.

Kızılkaya, R., Hepşen, Ş., 2004. Effect of biosolid amendment on enzyme activities in earthworm (*Lumbricus terrestris*) casts. *Journal of Plant Nutrition and Soil Science* 167(2): 202-208.

Kızılkaya, R., Hepşen, Ş., 2007. Microbiological properties in earthworm cast and surrounding soil amended with various organic wastes. *Communications in Soil Science and Plant Analysis*, 38(19-20): 2861–2876.

Lukkari, T., Teno, S., Vaisanen, A., Haimi, J., 2006. Effect of earthworms on decomposition and metal availability in contaminated soil: Microcosm studies of populations with different exposure histories. *Soil Biology and Biochemistry* 38(2): 359–370.

Masciandaro, G., Ceccanti, D., Garcia, C., 2002. "In situ" vermicomposting of biological sludges and impacts on soil quality. *Soil Biology and Biochemistry* 32(7): 1015–1024.

Morgan, J.E., Morgan, A.J., 1992. Heavy metal concentrations in the tissues, ingesta and faeces of ecophysiologically different earthworm species. *Soil Biology and Biochemistry* 24(12): 1691–1697.

McBride, M.B., 1995. Toxic metal accumulation from agricultural use of sewage sludge: are usepa regulations protective?. *Journal of Environmental Quality* 24(1): 5-18.

Mench, M., Martin, E., Solda, P., 1994. After effects of metals derived from a highly metal-polluted sludge on maize (Zea mays L.). *Water, Air, and Soil Pollution* 7: 277-291.

Ndegwa, P.M., Thompson, S.A., 2001. Integrating composting and vermicomposting the treatment and bioconversion of biosolids. *Bioresource Technology* 76(2): 107–112.

Neuhauser, E.F., Loehr, R.C., Milligan, D.L., Malecki, M.R., 1985. Toxicity of metals to the earthworm *Eisenia fetida*, *Biology and Fertility of Soils* 1: 149-152.

Neuhauser, E.F., Loehr, R.C., Malecki, M.R., 1988. The potential of earthworms for managing sewage sludge. In: Earthworms in waste and environmental management, Edwards, C.A., Neuhauser, E.F., (Eds), SPB Academic Publishing, The Hague, The Netherlands. pp. 9–20.

Pearson, M.S., Maenpaa, K., Pierzynski, G.M., Lydy, M.J., 2000. Effects of soil amendments on the bioavailability of lead, zinc and cadmium to earthworms. *Journal of Environmental Quality* 29(5): 1611–1617.

Scaps, P., Grelle, C., Decamps, M., 1997. Cadmium and lead accumulation in the earthworm *Eisenia fetida* (Savigny) and its impact on cholinesterase and metabolic pathway enzyme activity. *Comparative Biochemistry and Physiology - Part C: Pharmacology, Toxicology and Endocrinology* 116(3): 233-238.

Suthar, S., 2008. Development of a novel epigeic-anecic-based polyculture vermireactor for efficient treatment of municipal sewage water sludge. *International Journal of Environment and Waste Management* 2 : 84–101.

Suthar, S., Singh, S., 2008. Bioconcentrations of metals (Fe, Cu, Zn, Pb) in earthworms (*Eisenia fetida*), inoculated in municipal sewage sludge: do earthworms pose a possible risk of terrestrial food chain contamination? *Environmental Toxicology* 24(1) : 25-32.

Szczeck, M.M., 1999. Suppressiveness of vermicompost against fusarium wilt of tomato. *Journal of Phytopathology* 147(3): 155–161.

Whittle, A.J., Dyson, A.J., 2002. The fate of heavy metals in green waste composting, *Environmentalist* 22: 13–21.