To appear in: International Journal of Recycling of Organic Waste in Agriculture (IJROWA)
Online ISSN: 2251-7715 Print ISSN: 2195-3228

Received: 12 Apr 2023 Revised: 11 Aug 2023

Accepted: 13 Dec 2023

DOI: https://doi.org/10.57647/ijrowa-3kgw-nr28

ORIGINAL RESEARCH

Vermicomposting: A technology for vermiremediation of heavy metals from sewage sludge and animal dung

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Abstract

Purpose: The present study to investigate the earthworm *Eisenia fetida* was a chief organism for accumulation of heavy metals (Pb, Cr, and Cd) in their body tissue from sewage sludge with different combinations of animal dung and converted into rich organic vermicompost which plays a beneficial role for environment, animals and human health.

Method: Animal dung and sewage sludge were collected from Gorakhpur city and earthworm *E. fetida* an epigeic species collected from Vermibiotechnology Laboratory, Department of Zoology, D. D. U. Gorakhpur University, Gorakhpur. Analysis of heavy metals in different animal dung, sewage sludge, final vermicompost, and earthworm bodies were measured by Shimadzu AA-7000 atomic absorption spectrometer.

Results: There was a significant ($P<0.05$) reduction of Pb, Cr, and Cd concentration observed in different animal dung such as cow, buffalo and goat dung with sewage sludge in ratios of 1:1, 1:2, and 1:3 before and after vermicomposting. Cr was significantly decreased in cow dung (CD) and goat dung (GD) and Cd in cow dung (CD) and cow dung with sewage sludge (CD+SS, 1:1) the at BDL level whereas the concentration of Pb significantly decreased by 76.470% in the combination of GD+SS (1:1). The Cr and Cd were significantly increased in the body of *E. fetida* 3.384% and 11.801%, respectively, in the combination of CD+SS (1:3) whereas Pb significantly increase by 23.018% in the combination of BD+SS (1:3).

Conclusion: *E. fetida* is a suitable species for the accumulation of heavy metals such as Pb, Cr, and Cd in different vermibeds during vermicomposting and plays an important role in the remediation of heavy metals from animal dung with sewage sludge. Vermibiotechnology is a useful technique for the management of heavy metals.

Keywords: Vermiremediation, *Eisenia fetida*, Sewage sludge, Heavy metals, Vermicomposting

Introduction

The production of sewage sludge is parallel to the increasing rate of population in India. It is a serious problem for society and it causes environmental hazards (Kaviraj and Sharma 2003; Suthar 2006; Yadav and Garg 2011; Jatav et al. 2021). Cow, buffalo, sheep, goat, and horse excreta cause odor and pollution problems (Gupta 2005; Garg et al. 2006; Nath et al. 2009). Animal dung and sewage sludge contain heavy metals that cause ill effects on soil as well as human health (Prajapati and Singh 2013; Rusănescu et al. 2022). Heavy metals are generally referred to as those metals which possess a specific density of more than 5 g/cm^3 and adversely affect the environment and living organisms (Järup 2003). The safe value of Lead is 0.03 mg/kg, Chromium is 0.01 mg/kg and according to the FAO/WHO, the recommended tolerable daily Cd uptake is 0.01 to 0.035 mg/d (WHO 1985; 1993). The toxic metals, persistent organic compounds, pathogenic bacteria, and parasite eggs, could pollute the soil and groundwater (Dume et al. 2022). High exposure to lead causes severe damage to the brain and kidneys, anemia, increased blood pressure, miscarriage in pregnant women, and reduced fertility in males (Cleveland et al. 2008). Ahmed et al. (2016) reported that chromium eventually accumulates in crops from contaminated soils and imparts health risks to humans and animals, through the food chain. Cadmium is primarily toxic to the kidney and it causes damage to the proximal tubules and failure of the tubular reabsorption leading to renal dysfunction (Lee et al. 2006).

Vermicomposting of sewage sludge with animal dung by *Eisenia fetida* is an ecological method of sewage sludge management (Spurgeon and Hopkin 1999; Iwai et al. 2013). The worms break down these organic wastes and modify them into nutrient-rich organic manure i.e. vermicompost. *E. fetida* is often

used in vermicomposting as it reduces the C/N ratio, the pathogenic load of various wastes but increases the total Kjeldahl nitrogen (TKN) and total phosphorus (TP), hence obtaining good quality fertilizer for soil (Li et al. 2011; Iwai et al. 2013; Panday et al. 2014). Earthworm *E. fetida* biochemically degrades sewage sludge and homogenizes the material through the muscular actions of the intestine and releases nutrient-rich vermicompost for plants (Yadav et al. 2012; Purdea et al. 2019) by accumulating heavy metals in their body during the vermicomposting (Suleiman et al. 2017; Singh and Bhartiya 2021). The aim of the present study was to investigate the accumulation of heavy metals in earthworm *E. fetida* in vermicomposting of animal dung with sewage sludge and converted rich organic vermicompost which is ecologically sound and culturally most acceptable for agriculture.

Materials and methods

Collection of animal dung, earthworm, and sewage sludge (SS)-

Dung of cow, buffalo, and goat dung and sewage sludge were collected from different parts of Gorakhpur city. These wastes were exposed to sunlight for 5 to 10 days for the removal of various harmful and noxious gases. *E. fetida* is an epigeic species used for this experiment collected from Vermibiotechnology Laboratory, Department of Zoology, D.D.U., Gorakhpur University, Gorakhpur. In the laboratory condition, the temperature was 20 ^{0}C to 30 ^{0}C and the moisture was maintained up to 40% to 60% for proper growth and survival of earthworms (Bhartiya and Singh, 2011; Nath and Singh 2016).

Experimental setup for vermicomposting

Vermicomposting of different combinations of animal (cow, buffalo, and goat) dung with sewage sludge in 1:1, 1:2, and 1:3 ratios was used for the preparation of vermibeds which was conducted on the cemented earth surface. The size of each vermibed is (3m length × 1m width × 12cm depth). 2kg of cultured *E. fetida* inoculated in each bed and covered with jute pockets for proper moisture maintenance up to 40 to 50 days. Each vermibed manually turned over up to 5 weeks after a one-week interval. After 60 days, tea granules like vermicompost appeared on the upper surface of each bed. The prepared vermicompost and earthworms were used for chemical analysis and heavy metals Pb, Cr, and Cd concentrations (Bhartiya and Singh 2012b; Nath and Singh 2016).

Analysis of heavy metals in initial feed mixture, final vermicompost, and earthworm body

Heavy metal content of the initial feed mixture (IFM) and final vermicompost (FVC) was measured by the method of Maboeta and Rensburg (2003). 1 g of IFM and FVC is required for the preparation of samples. These samples were digested by adding nitric acid (1:1) placing on a hot plate and heating it for 4 hours at 90 ^{0}C to 100 ^{0}C and keep watching that the sample did not dry up after digestion sample was poured into a100 ml flask filter through Whatman No 41 paper.

The heavy metals in the earthworm's body tissue are digested using the method of Katz and Jenneis (1983). Earthworms dried, ground, and burned to ash at high temperatures. The ash was placed in a test tube with about 10 ml to 15 ml of 55% nitric acid (HNO_3) added to it. The solution was left for 12 hrs at room temperature. The solution heated at 40 ^{0}C to 60 ^{0}C for 2 hours and followed by 120 ^{0}C to 130 ^{0}C for one hour and 1 ml of 70% perchloric acid ($HClO_4$) was added to it. The solution

was allowed to cool and 5 ml of distilled water was added thereafter. The solution was again heated up to 130 ^{0}C until white fumes emitted. The solution was allowed to cool and filtered through the Whatman No 41 filter paper. This solution allowed for the analysis of heavy metals of the initial feed mixture, final vermicompost, and earthworm body by Shimadzu AA-7000 atomic absorption spectrometer (Najah et al. 2015).

Calculation of heavy metals concentration in vermicompost and earthworm body

$$\% \text{ decrease HMCs in VC} = \frac{(\text{IFM} - \text{FVC})}{\text{IFM}} \times 100 \text{ mg/kg}$$

$$\% \text{ increase HMCs in earthworm body} = \frac{(\text{EfBFV} - \text{EfBC})}{\text{EfBC}} \times 100 \text{ mg/kg}$$

HMCs	=	heavy metals concentration
IFM	=	initial feed mixture
FVC	=	final vermicompost
EfBC	=	earthworm *Eisenia fetida* body (control)
EfBFV	=	earthworm *Eisenia fetida* body in final vermicompost

Statistical analysis

All the data are mean ± SD of 6 replicates. Students 't' test was applied to determine the significant ($P<0.05$) difference between combinations of cow, buffalo, and goat dung with sewage sludge (SS) of initial, final vermicompost, before inoculation and final earthworm *E. fetida* body (Sokal and Rohlf 1973).

Results and discussion

Data emerging from results show there was a significant reduction of Pb, Cr, and Cd observed in prepared vermicompost of different animal dung and sewage sludge in the ratios 1:1, 1:2, and 1:3. Chromium (Cr) was significantly decreased in cow dung (CD) and goat dung (GD) and cadmium (Cd) in CD, CD+SS (1:1), BD and GD at BDL level. The Lead (Pb) was significantly decreased by 76.470 % (0.527±0.004 mg/kg to 0.124±0.003 mg/kg) in the goat dung with sewage sludge GD+SS 1:1 ratio (Table 1 and Fig. 1), whereas Pb accumulated and significantly increased (5.248±0.007 mg/kg to 6.456±0.009 mg/kg) 23.018% in earthworm *Eisenia fetida* body in the combination of buffalo dung with sewage sludge (BD+SS, 1:3). The Cr and Cd were significantly increased in the *E. fetida* body 3.384 % (11.435±0.012 mg/kg to 11.822±0.009 mg/kg) and 11.801 % (3.347±0.008 mg/kg to 3.742±0.010 mg/kg), respectively, in the combination of buffalo dung with sewage sludge (BD+SS) in ratio 1:3 (Table 2 and Fig. 2). Khwairakpam and Bhargava (2009) reported that the combination of metal decreased

during vermicomposting with all worm with *Eudrilus eugeinae*, *Perionyx excavates* and *Eisenia fetida* slightly more in case of lead.

Table 1. Concentration of heavy metals (mg/kg) in a combination of different animal dung with sewage sludge and vermicompost.

Particulars	Ratios	Heavy metal concentration in mg/kg								
		Pb			Cr			Cd		
		IFM	FVC	% Decrease	IFM	FVC	% Decrease	IFM	FVC	% Decrease
SS	-	2.196±0.010	-	-	0.794±0.006	-	-	0.538±0.009	-	-
CD	-	0.331±0.005	0.105±0.003*	68.278	0.199±0.004	BDL*	100	0.045±0.005	BDL*	100
CD+SS	1:1	0.530±0.003	0.297±0.005*	43.963	0.356±0.005	0.085±0.003*	76.123	0.209±0.005	BDL*	100
	1:2	0.905±0.005	0.340±0.004*	62.431	0.467±0.004	0.162±0.004*	65.310	0.381±0.004	0.112±0.006*	70.603
	1:3	1.560±0.008	0.890±0.004*	42.948	0.598±0.004	0.178±0.006*	70.234	0.390±0.005	0.129±0.005*	66.923
BD	-	0.452±0.004	0.119±0.005*	73.672	0.236±0.003	0.092±0.004*	61.016	0.062±0.004	BDL*	100
BD+SS	1:1	0.702±0.006	0.255±0.004*	63.675	0.367±0.005	0.156±0.005*	57.493	0.173±0.003	0.043±0.004*	75.144
	1:2	1.025±0.006	0.368±0.006*	64.097	0.402±0.003	0.189±0.003*	52.985	0.210±0.004	0.085±0.005*	59.523
	1:3	1.614±0.008	0.795±0.005*	50.743	0.678±0.003	0.308±0.004*	54.572	0.295±0.007	0.106±0.004*	64.067
GD	-	0.149±0.004	0.055±0.002*	63.087	0.127±0.004	BDL*	100	0.196±0.004	BDL*	100
GD+SS	1:1	0.527±0.004	0.124±0.003*	76.470	0.250±0.005	0.084±0.006*	66.400	0.309±0.003	0.068±0.003*	77.993
	1:2	0.851±0.004	0.209±0.005*	64.027	0.603±0.003	0.196±0.005*	67.495	0.371±0.006	0.096±0.004*	74.123
	1:3	1.272±0.009	0.423±0.006*	66.745	0.675±0.003	0.205±0.008*	69.629	0.467±0.006	0.145±0.005*	68.950

* Significant P<0.05 “t” test between animal dung, sewage sludge, and final vermicompost. Each value is the Mean ±SD of six replicates
BDL-Below detectible limit i.e. 0.0001 mg/kg
SS-sewage sludge, IFM- initial feed mixture, FVC- final vermicompost, CD-cow dung, BD-buffalo dung, GD-goat dung

Table 2. Concentration of heavy metals (mg/kg) of earthworm *Eisenia fetida* body in different combinations of animal dung with sewage sludge after vermicomposting.

Particulars	Ratios	Heavy metal concentration in earthworm *Eisenia fetida* body (mg/kg)								
		Pb			Cr			Cd		
		EfBC	EfBFV	% Increase	EfBC	EfBFV	% Increase	EfBC	EfBFV	% Increase
CD	-	5.248±0.007	5.445±0.006*	3.753	11.435±0.012	11.552±0.008*	1.023	3.347±0.008	3.552±0.005*	6.124
CD+SS	1:1	5.248±0.007	5.406±0.004*	3.010	11.435±0.012	11.659±0.006*	1.958	3.347±0.008	3.565±0.005*	6.513
	1:2	5.248±0.007	5.619±0.003*	7.069	11.435±0.012	11.806±0.006*	3.244	3.347±0.008	3.704±0.004*	10.666
	1:3	5.248±0.007	6.040±0.008*	15.091	11.435±0.012	11.822±0.009*	3.384	3.347±0.008	3.742±0.010*	11.801
BD	-	5.248±0.007	5.662±0.004*	7.888	11.435±0.012	11.588±0.004*	1.337	3.347±0.008	3.411±0.007*	1.912
BD+SS	1:1	5.248±0.007	5.769±0.005*	9.927	11.435±0.012	11.592±0.005*	1.372	3.347±0.008	3.463±0.006*	3.465
	1:2	5.248±0.007	6.045±0.003*	15.186	11.435±0.012	11.685±0.006*	2.186	3.347±0.008	3.475±0.005*	3.824
	1:3	5.248±0.007	6.456±0.009*	23.018	11.435±0.012	11.762±0.006*	2.859	3.347±0.008	3.650±0.008*	9.052
GD	-	5.248±0.007	5.450±0.003*	3.849	11.435±0.012	11.505±0.007*	0.612	3.347±0.008	3.501±0.004*	4.601
GD+SS	1:1	5.248±0.007	5.601±0.004*	6.726	11.435±0.012	11.560±0.005*	1.093	3.347±0.008	3.545±0.006*	5.915
	1:2	5.248±0.007	5.670±0.005*	8.041	11.435±0.012	11.723±0.008*	2.518	3.347±0.008	3.673±0.005*	9.740
	1:3	5.248±0.007	6.186±0.005*	17.873	11.435±0.012	11.775±0.009*	2.973	3.347±0.008	3.705±0.007*	10.696

* Significant P<0.05 “t” test between earthworm body of each vermibeds before inoculation and after vermicomposting
Each value is the Mean ±SD of six replicates
SS-sewage sludge, EfBC- earthworm *Eisenia fetida* body (control)
EfBFV- earthworm *Eisenia fetida* body in final vermicomposting, CD-cow dung, BD-buffalo dung, GD-goat dung

Eisenia fetida had a significant degree of accumulation of Cr, Pb, and Zn compared to *Eudrilus eugeniae*, and the bioaccumulation factor was higher in the case of *E. fetida* (Parseh et al. 2021). The bioaccumulation of metals in the body of *E. fetida* and *E. andrei* was thus Cd > Zn > Cu > Ni > Pb (Rorat et al. 2016). The metallothioneins (MTs)—small, cysteine-rich proteins of *Lumbricus rubellus* and common red earthworm (*Eisenia fetida*) that bind d10 metal ions (Zn(II), Cd(II), or Cu(I)) in clusters. Three MT homologues are known for one of which, wMT-2, is strongly induced by exposure of worms to cadmium (Kowald et al. 2016). Earthworms have the ability to accumulate heavy metals, including Cu, Cd, Pb, and Zn by earthworm occurs through two pathways, which include absorption following dermal contact and adsorption through the intestinal tissues (Nirola et al. 2016; Wang et al. 2018; Lanno et al. 2019; Srut et al. 2019).

There was a significant increase of heavy metals in the body of inoculated earthworm *Eisenia fetida* after vermicomposting in a combination of animal dung with biological wastes during vermic-activity (Shahmansouri et al. 2005; Jordao et al. 2006; Bhartiya and Singh 2012a; 2012b; Singh and Bhartiya 2020a). Significant changes of fraction distribution and bioavailability of these metals (Zn, Pb, Fe, Mn, Cr, Co, and Cu) occurred during earthworm activity (Devliegher and Verstraete 1996). A significant concentration of Cd accumulation was reported in the earthworm body of *Eisenia fetida* (Conder et al. 2002; Shahmansouri et al. 2005; Singh et al. 2014; Singh and Bhartiya 2020b).

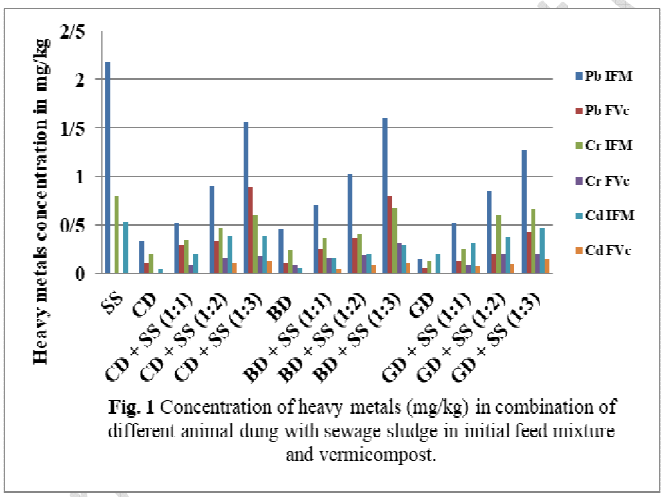

Fig. 1 Concentration of heavy metals (mg/kg) in combination of different animal dung with sewage sludge in initial feed mixture and vermicompost.

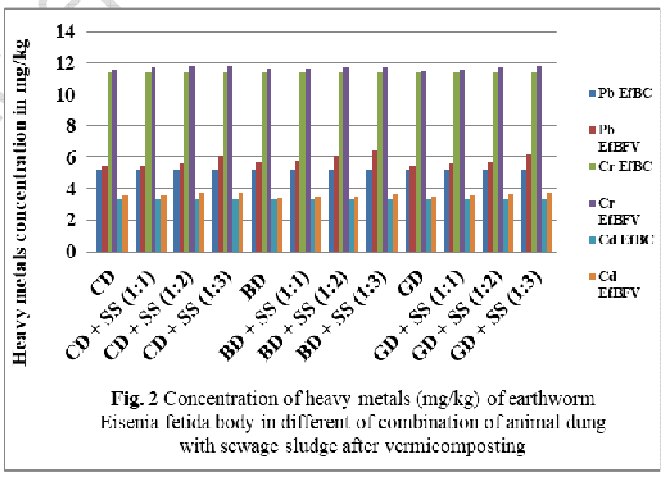

Fig. 2 Concentration of heavy metals (mg/kg) of earthworm Eisenia fetida body in different of combination of animal dung with sewage sludge after vermicomposting

Conclusion

Eisenia fetida accumulated heavy metals in their body tissues from different combinations of cow, buffalo, and goat dung with sewage sludge in the ratios 1:1, 1:2, and 1:3. There was a significant decrease in the concentration of Pb, Cr, and Cd in different animal dung with sewage sludge during vermicomposting. Cr and Cd were significantly decreased in cow dung (CD) and goat dung (GD) at the BDL level whereas the Pb concentration significantly decreased (89.261%) in the combination of GD+SS (1:1). *E. fetida* was a suitable species for the accumulation of Pb, Cr, and Cd from different waste during vermicomposting. The result of the study showed that earthworms play an important role in the remediation of heavy metals from sewage sludge with different combinations of animal dung.

Acknowledgments: The authors are thankful to Dr Jaswinder Singh, Associate Professor, Post Graduate Department of Zoology, Khalsa College, Amritsar, Punjab, India for technical support.

Author Contribution: All authors are contributed in experiment as well as paper drafting and writing.

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