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Research Article

THE BIOREMEDIATION POTENTIAL OF *EISENIA FETIDA* AND *EUDRILUS EUGENIAE* IN CADMIUM AND COPPER POLLUTED COW DUNG

Md. Imran Khan[1*], Md. Abul Kashem[1] and Md. Abdul Aziz[1]

[1]*Department of Soil Science, Sylhet Agricultural University, Sylhet-3100, Bangladesh*

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***Corresponding author**

Md. Imran Khan

E-mail: imrankhan1802008@gmail.com

Abstract

An experiment was conducted from March to November 2024 at the vermicompost shed and laboratory of the Department of Soil Science, Sylhet Agricultural University, to see the bioremediation potential of earthworms through vermicomposting from cow dung. Two factors, where A- Two earthworm species viz., *Eisenia fetida* and *Eudrilus eugeniae*; and B- cadmium and copper concentrations viz., control (natural presence of Cd and Cu in cow dung), Cd (0.15 mg kg^{-1}), Cu (5 mg kg^{-1}), Cd+Cu (0.15+5, mg kg^{-1}), were employed completely randomized design (CRD) with three replications. Twenty-five earthworms were released into 500 g of cow dung in each bowl. The moisture level of cow dung was maintained between 60-80%. Data were recorded on the number of earthworms, cocoons, hatchlings, length, biomass, cadmium and copper contents in the earthworm's body. The vermicompost weight, its physical and chemical properties changes were also recorded. The result showed that the vermicompost was dark brown, ammonia off-odour, non-granular and not sticky. The highest number of earthworms, cocoons and hatchlings, was 26.83, 41.83 and 88.17 $bowl^{-1}$ found in control, respectively. Earthworm length (9.78 cm), biomass (12.32 g $bowl^{-1}$), and vermicompost weight (282.74 g $bowl^{-1}$) were the highest in control. The highest concentrations of Cd and Cu (0.068 and 4.694 mg kg^{-1}, respectively) were found in the earthworm's body in Cd+Cu (0.15+5, mg kg^{-1}) treatment, whereas the lowest Cd (0.133 mg kg^{-1}) and Cu (6.565 mg kg^{-1}) were recorded in the vermicompost of control. *E. fetida* and *E. eugeniae* showed statistically similar performance in case of the Cd and Cu accumulated in their bodies. The result indicated that earthworms would reduce Cd and Cu contents from cow dung and keep the environment clean from hazardous materials.

Introduction

The most hazardous contaminants heavy metals present in soils are cadmium, nickel, copper, lead, mercury and zinc (Ahemed and Malik, 2011). Because of their mobility, non-degradability in nature, heavy metals can affect a wide range of things and pose serious environmental concerns when deposited in soils in large amounts (Xu *et al*., 2021). These hazardous elements accumulate in living beings, including humans, plants, animals and microorganisms. Those are the cause of many metabolic and physiological problems for living beings. Bioremediation is considered one of the most effective management approaches among all other biological techniques. It is an advanced and attractive cleaning technique for reducing heavy metal concentration from cow dung, soil, water and also from the environment. This new approach helps to keep the surroundings free from pollution. It is a more cost-effective technique than other remediation

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technology which typically doesn't result in harmful byproducts. Because the environmental pollutants have completely mineralized, it also offers an environmentally friendly alternative (Abioye, 2011). According to Wu *et al*. (2015), earthworms are significant soil ecosystem engineers that affect soil functioning through their feeding, burrowing, and casting activities. Earthworms feed on heavy metal-contaminated feeding substances and soil, which causes bioaccumulation and produce quality vermicompost (Basha and Latha, 2016; Sarker and Kashem, 2020). They are highly sensitive and vulnerable to pollutants, making them significant species in soil eco-toxicity assessment studies (Chen *et al*., 2018). They are frequently exposed to soil pollutants through their permeable skin and gut, which causes them to build up significant amounts in their tissues. The present study was undertaken to determine the cadmium and copper content reduction potential through earthworms as well as the physicochemical changes in vermicompost produced from cow dung.

Materials and Methods

The experiment was conducted from March to November 2024 at the vermicompost production shed and the laboratory of the Department of Soil Science at Sylhet Agricultural University, Sylhet. The following treatments were adopted for the experiment.

Factor A: Two earthworm species –

i) *Eisenia fetida*
ii) *Eudrilus eugeniae*

Factor B: Four concentrations –

i) Control (natural presence of Cd and Cu in cow dung)
ii) Cd (0.15 mg kg^{-1})
iii) Cu (5 mg kg^{-1})
iv) Cd+Cu (0.15+5, mg kg^{-1})

The study was followed by two factors completely randomized design (CRD) with three replications. Cow dung was used collected from ''Sylhet Govt. Dairy Farm''. Cow dung was pre-decomposed for 30 days before used.

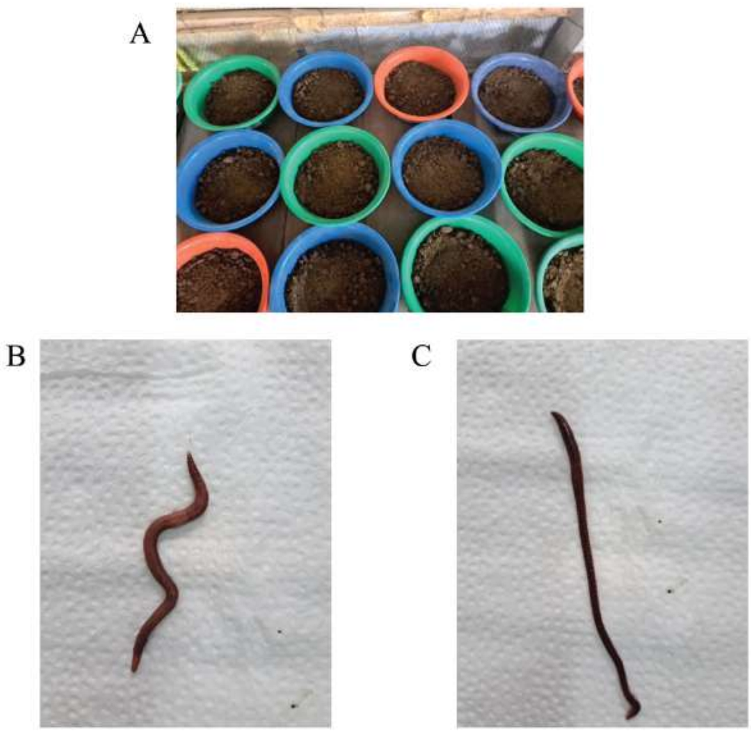

Figure 1. Experimental setup (A), *Eisenia fetida* (B) and *Eudrilus eugeniae* (C)

Experimental setup

The experiment used twenty-four plastic bowls, each measuring 28 cm in diameter, 9 cm in height, and 2770.89 cm^3 in volume. 500 g of decomposed cow dung was used in each plastic bowl as feeding material and covered with perforated plastic lids. Cadmium and copper were applied as $Cd(NO_3)_2$ and $CuSO_4$ as per treatment. Twenty-five earthworms per bowl were weighed and released after three days. The temperature and humidity were recorded at 4 days intervals. The temperature and moisture content of cow dung were also measured on the same day. The moisture level was maintained between 60-80%. Vermicompost was harvested when cow dung was fully utilized by the earthworms. The number of earthworms, cocoons, and hatchlings was counted after they were manually sorted out of the vermicompost. The earthworms were then cleaned and weighed. A centimetre scale was used to measure the earthworms' length. The separated vermicompost was spread out onto plastic sheets to reduce the moisture. Then kept in storage. The isolated earthworms were put in glass petri dishes and allowed for a whole day to reduce the excreta from earthworm's body. After that, they were cleaned with deionized water and allowed to dry for 72 hours at 45˚C in an oven. then a mortar and pestle were used to grind the tissues into a paste. The paste was then used to analyze cadmium and copper accumulated in the earthworm mass.

Chemical analysis of cow dung, earthworms and vermicompost was done to determine pH (Mclean, 1982), organic carbon (Nelson and Sommers, 1982), total N (Bremner and Mulvaney, 1982), available P (Yoshida *et al*., 1976), exchangeable K (Yoshida *et al*., 1976), available S (Chapman and Pratt, 1961), Cd and Cu contents (Yoshida *et al*., 1976).

The analysis of variance (ANOVA) was done following the principle stated by Gomez and Gomez (1984). The means were separated using the least significant difference (LSD) method.

Results and Discussion

Physical properties of vermicompost

Vermicompost had appeared as dark and brownish colour (Figure 2). It had a non-granular, powdery, and non-stick texture with strongly of ammonia gas smell. These findings correlate with Bagum *et al*., 2022; Ahmed, 2015. They found that vermicomposts are peat-like, dark, and homogeneous mixtures.

Figure 2. Vermicompost

Number of earthworms

The different concentrations of Cd and Cu significantly affected the earthworm number. The highest number of earthworms (26.83 $bowl^{-1}$) was recorded in control and the lowest number of earthworms (23.83 $bowl^{-1}$) was found due to the application of Cd+Cu (0.15+5, mg kg^{-1}) at harvest (Table 1). The possible reason for the reduction of earthworms for toxicity developed due to increase of cadmium and copper in the cow dung. The outcomes are consistent with the findings of Lapinski and Rosciszewska (2008), who reported that the adults number of *E. fetida* decreased in the cadmium-contaminated substrates.

Table 1. Effects of earthworms species, concentration of cadmium and copper their interactions on number of earthworm, cocoons and hatchlings

Treatment	Number of earthworms (bowl^{-1})	Number of cocoons (bowl^{-1})	Number of hatchlings (bowl^{-1})
Earthworm species			
E. fetida	25.58	35.58	81.00a
E. eugeniae	24.75	32.67	60.25b
Level of significance	NS	NS	***
Concentrations of Cd and Cu			
Control	26.83a	41.83a	88.17a
Cd (0.15 mg kg^{-1})	25.17b	37.50ab	75.33ab
Cu (5 mg kg^{-1})	24.83bc	31.67bc	65.67bc
Cd+Cu (0.15+5, mg kg^{-1})	23.83c	25.50c	53.33 c
Level of significance	**	**	*
Earthworm species × Concentrations of Cd and Cu			
E. fetida × Control	27.33	45.67	93.67
E. fetida × Cd (0.15 mg kg^{-1})	24.33	38.33	83.00
E. fetida × Cu (5 mg kg^{-1})	24.00	31.67	77.00
E. fetida × Cd+Cu (0.15+5, mg kg^{-1})	23.333	26.67	70.33
E. eugeniae × Control	26.33	38.00	82.67
E. eugeniae × Cd (0.15 mg kg^{-1})	26.00	36.67	67.67
E. eugeniae × Cu (5 mg kg^{-1})	25.67	31.67	54.33
E. eugeniae × Cd+Cu (0.15+5, mg kg^{-1})	24.33	24.33	36.33
Level of significance	NS	NS	
Co-efficient of variation (%)	3.97	20.07	15.69

This means the following dissimilar letters in a column are significantly different at 0.1%, 1% and 5%.

Number of cocoons

The cocoon number was negatively affected by the application of cadmium and copper in cow dung (Figure 03). The highest number of cocoons (41.83 bowl^{-1}) was found in control while the lowest number of cocoons (25.50 bowl^{-1}) was recorded in the case of Cd+Cu (0.15+5, mg kg^{-1}) treatment (Table 1). Duan *et al.* (2016) reported that the decreased cocoon production due to the exposure of earthworms to increased Cu concentration.

A B

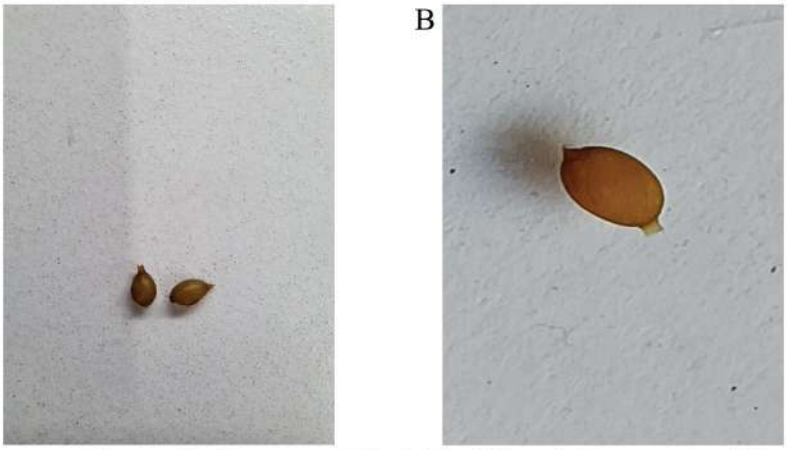

Figure 3. Cocoons of *E. fetida* (A) and *E. eugeniae* (B)

Number of hatchlings

The two earthworm species significantly affected the number of hatchlings (Table 1). The higher number of hatchlings (81.00 bowl^{-1}) was found in *E. fetida* over *E. eugeniae* (60.25 bowl^{-1}). The hatchling's number was negatively affected due to the addition of different concentrations of Cd and Cu in cow dung (Figure 4). The highest number of hatchlings (88.17 bowl^{-1}) was recorded in the control treatment, and the lowest number of hatchlings (53.33 bowl^{-1}) was found due to application of Cd+Cu (0.15+5, mg kg^{-1}). The possible causes due to the toxicity levels were increased with the elevated concentration of cadmium and copper in the cow dung. Lapinski and Rosciszewska (2008) found similar results, namely that the increase of cadmium concentration decreases in young individuals.

A B

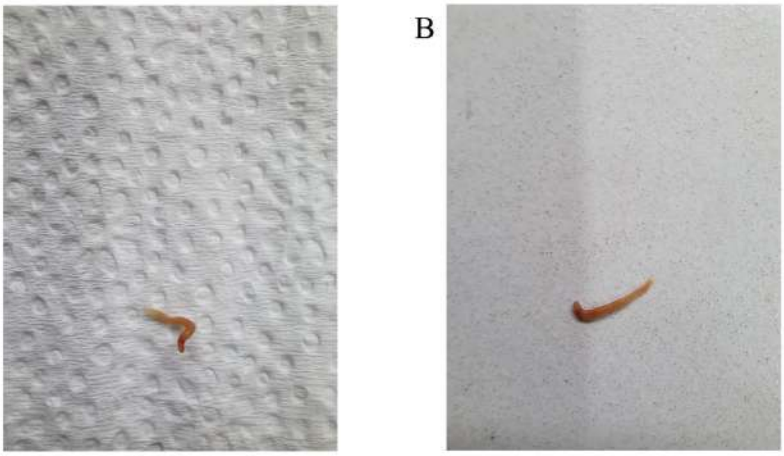

Figure 4. Hatchling of *E. fetida* (A) and *E. eugeniae* (B)

Earthworm biomass

The earthworm biomass differed due to the two earthworm species. The higher biomass of earthworms (11.18 g bowl^{-1}) was gained in *E. eugeniae* and the lower biomass of earthworms (9.84 g bowl^{-1}) in *E. fetida*. The different concentrations of Cd and Cu significantly affected the earthworm biomass where the highest earthworm biomass (12.32 g bowl^{-1}) was found in control and the lowest earthworm biomass (9.04 g bowl^{-1}) in Cd+Cu (0.15+5, mg kg^{-1}) treatment (Table 2). The growth of earthworms was retarded due to the presence of cadmium and copper in cow dung. The findings correlate to the outcomes of Malecki *et al*. (2007). They found that cadmium had the strongest negative effect on the biomass of earthworms among the other metals. Duan *et al*. (2016) also found that earthworm body weight negatively affected due to Cu toxicity.

Earthworm length

Significantly, the longer length of the earthworm (10.49 cm) in *E. eugeniae* and the lower earthworm (7.13 cm) in *E. fetida* were recorded (Table 2). *E. eugeniae* showed larger length than *E. fetida.* The different concentrations of Cd and Cu also negatively affected the earthworm length. The longest length of earthworm (9.78 cm) was found in control as well as the shortest length of earthworm (8.17 cm) was recorded in Cd+Cu (0.15+5, mg kg^{-1}). The cadmium and copper negatively affected the earthworm's body. As a result, the earthworm length was reduced. Spurgeon and Hopkin (1995) studied that the addition of cadmium and copper caused a decreased in the growth of the earthworms and increased the mortality of the *E. fetida* earthworms.

Vermicompost weight

Statistically similar weight was observed in vermicompost due to the various concentrations of cadmium and copper (Table 2). The highest vermicompost (282.74 g bowl^{-1}) was found in control and the lowest vermicompost (277.86 g bowl^{-1}) was found in Cd+Cu (0.15+5, mg kg^{-1}). The outcome correlates with the findings of Gupta *et al*. (2005). The effectiveness of the two earthworm species was different from each other species. *E. fetida* exhibited a greater effectiveness than *E. eugeniae*. The concentration of heavy metals reduced the efficiency of producing vermicompost by earthworms and therefore, the vermicompost productivity would be decreased with increasing of heavy metals.

Table 2. Effects of earthworm species, the concentration of cadmium and copper their interactions on earthworm biomass, length and vermicompost weight

Treatments	**Earthworm biomass (g bowl^{-1})**	**Length of earthworms (cm)**	**Vermicompost weight (g bowl^{-1})**
Earthworm species			
E. fetida	9.84b	7.13b	282.96
E. eugeniae	11.18a	10.49a	277.89
Level of significance	***	***	NS
Concentrations of Cd and Cu			
Control	12.32a	9.78a	282.74
Cd (0.15 mg kg^{-1})	10.90b	8.92ab	280.91
Cu (5 mg kg^{-1})	9.80c	8.38b	280.21
Cd+Cu (0.15+5, mg kg^{-1})	9.04c	8.17b	277.86
Level of significance	***	*	NS
Earthworm species × Concentrations of Cd and Cu			
E. fetida × Control	11.44	7.77	284.82
E. fetida × Cd (0.15 mg kg^{-1})	9.66	7.23	285.24
E. fetida × Cu (5 mg kg^{-1})	9.27	6.90	282.54
E. fetida × Cd+Cu (0.15+5, mg kg^{-1})	8.40	6.63	279.26
E. eugeniae × Control	13.19	11.80	280.66
E. eugeniae × Cd (0.15 mg kg^{-1})	11.54	10.60	276.58
E. eugeniae × Cu (5 mg kg^{-1})	10.33	9.87	277.88
E. eugeniae × Cd+Cu (0.15+5, mg kg^{-1})	9.68	9.70	276.46
Level of significance	NS	NS	NS
Co-efficient of variation (%)	3.97	8.76	3.85

This means the following dissimilar letters in a column are significantly different at 0.1% and 5%.

Nutrient composition of vermicompost

Initially, the pH value of cow dung was 6.8. Non-significant results were found in concentration effect of Cd and Cu as well as the interactions of earthworms with Cd and Cu but the pH value was slightly increased in vermicompost over cow dung (Table 3). This conclusion is consistent with the outcomes of Guoxue *et al.* (2001). Additionally, Tognetti *et al.* (2005) noted that the observed increase in pH during the process was likely caused by the breakdown of short-chain fatty acids and the conversion of organic matter into ammonia.

The organic carbon content in vermicompost was decreased over cow dung. Organic carbon (35.41%) in vermicompost produced by *E. fetida* was higher compared to organic carbon (34.46%) in vermicompost produced by *E. euginae* (Table 3). While vermicomposting, earthworms found energy from carbon sources. This result correlates with the findings of Loh (2005).

Table 3. Nutrient compostion of vermicompost

Treatments	**pH**	**OC (%)**	**Total N (%)**	**Available P (%)**	**Exchangeable K (meq 100^{-1} g)**	**Available S (%)**
			Cow dung			
	6.8	47.23	1.16	0.43	0.84	0.47
			Vermicompost			
E. fetida	7.80	35.41	1.60a	0.68	1.06a	0.63
E. eugeniae	7.77	34.46	1.36b	0.66	0.98b	0.93
Level of significance	NS	NS	**	NS	*	NS
			Concentrations of Cd and Cu			
Control	7.71	35.12	1.67a	0.741a	1.120a	0.70a
Cd (0.15 mg kg^{-1})	7.83	35.52	1.56ab	0.718a	1.068a	0.66a
Cu (5 mg kg^{-1})	7.84	34.90	1.26b	0.640b	0.956b	0.62ab
Cd+Cu (0.15+5, mg kg^{-1})	7.78	34.23	1.43ab	0.573b	0.925b	0.54b
Level of significance	NS	NS	*	***	***	*
			Earthworm species × Concentrations of Cd and Cu			
E. fetida × Control	7.73	34.15	1.72	0.72	1.14	0.71
E. fetida × Cd (0.15 mg kg^{-1})	7.75	35.97	1.58	0.69	1.11	0.68
E. fetida × Cu (5 mg kg^{-1})	7.97	34.15	1.60	0.64	1.02	0.62
E. fetida × Cd+Cu (0.15+5, mg kg^{-1})	7.75	33.61	1.49	0.59	0.97	0.53
E. eugeniae × Control	7.69	36.08	1.63	0.76	1.10	0.71
E. eugeniae × Cd (0.15 mg kg^{-1})	7.90	35.08	1.53	0.75	1.03	0.64
E. eugeniae × Cu (5 mg kg^{-1})	7.70	35.66	0.92	0.64	0.90	0.61
E. eugeniae × Cd+Cu (0.15+5, mg kg^{-1})	7.79	34.85	1.37	0.56	0.88	0.55
Level of significance	NS	NS	NS	NS	NS	NS
Co-efficient of variation (%)	2.43	5.83	17.41	8.32	6.42	13.53

This means the following dissimilar letters in a column are significantly different at 0.1%, 1% and 5%.

The nitrogen content (1.60%) in vermicompost produced by *E. fetida* was significantly higher compared to the nitrogen content (1.36%) in vermicompost produced by *E. eigeniae.* Different concentrations of Cd and Cu showed significant effects on total nitrogen in vermicompost where the highest value (1.67%) in control and the lowest value (1.43%) in Cd+Cu (0.15+5, mg kg^{-1}) were recorded (Table 3). These results showed similar to the findings of Kiyasudeen *et al*., 2015. It is possible that earthworm activities contributed to the increased release of metabolic nitrogenous products through the excretory secretions of earthworms, such as mucus (Suthar and Singh, 2008).

The different concentrations of Cd and Cu affected the available phosphorus content in vermicompost, whereas control showed the value (0.741%), followed by the available phosphorus due to Cd (0.15 mg kg^{-1}) in vermicompost (Table 3). The findings correlate with the results of Azgin (2021).

The exchangeable potassium was significantly different in vermicompost produced by two earthworm species. In case of different concentrations of Cd and Cu, exchangeable potassium was also significantly increased in vermicompost. The highest value was 1.120 meq 100^{-1} g and the lowest value was 0.925 meq 100^{-1} g found in vermicompost (Table 3). These findings correlate with the output of Suthar and Singh (2007).

The different sulphur contents were recorded in vermicompost due to different concentrations of Cd and Cu. The highest value (0.70 %) was recorded in control, whereas the lowest value (0.54%) was found in Cd+Cu (0.15+5, mg kg^{-1}) in vermicompost (Table 3). A similar result was obtained by Rahman *et al*. (2014).

Cadmium contents in earthworm body

The cadmium contents in earthworm bodies varied with the different concentrations of cadmium. The highest Cd content (0.071 mg kg^{-1}) was found in earthworm body in Cd (0.15 ppm) treatment whereas the lowest Cd content was recorded (0.032 mg kg^{-1}) in Cu (5 mg kg^{-1}) application. The cadmium concentration was increased in earthworm bodies due to presence of cadmium in cow dung (Table 4). This finding correlates the results of Sarker and Kashem (2020). They stated that the earthworm significantly affected cadmium contents in earthworm's body during vermicomposting. Earthworm ingests cow dung and are exposed to heavy metals through their body and intestines. The concentrations of the studied metals in the tissues of earthworms exceeded the concentrations in the substrate many times (Lapinski *et al*., 2002; Li *et al*., 2010).

Copper contents in earthworm body

Copper contents were statistically different in earthworm bodies due to the copper concentrations added to cow dung. The highest content (4.694 mg kg^{-1}) was found in Cd+Cu (0.15+5, mg kg^{-1}), whereas the lowest content (2.972 mg kg^{-1}) was observed in the control (Table 4). Earthworms can store significant amounts of metal in their tissues. This makes them valuable biological indicators of concentration, as there is a consistent association between the concentrations of specific contaminants in earthworms and the surrounding environment (Suthar, 2008). Duan *et al*. (2016) state that copper concentrations in earthworms increased as linear functions increased Cu concentrations in the substrate.

Table 4. Cadmium and copper accumulation in earthworm's body and their interactions on vermicompost

Treatments	Cd Concentration ($mg\ kg^{-1}$)		Cu Concentration ($mg\ kg^{-1}$)	
	Earthworm	Vermicompost	Earthworm	Vermicompost
Earthworm species				
E. fetida	0.050	0.151	3.911	9.636
E. eugeniae	0.052	0.152	3.848	9.368
Level of significance	NS	NS	NS	NS
Concentrations of Cd and Cu				
Control	0.034b	0.133b	2.972c	6.565c
Cd (0.15 $mg\ kg^{-1}$)	0.071a	0.171a	3.075c	7.110c
Cu (5 $mg\ kg^{-1}$)	0.032b	0.133b	4.778a	11.810b
Cd+Cu (0.15+5, $mg\ kg^{-1}$)	0.068a	0.168a	4.694a	12.525a
Level of significance	***	***	***	***
Earthworm species × Concentrations of Cd and Cu				
E. fetida × Control	0.030	0.131	3.143	6.92
E. fetida × Cd (0.15 $mg\ kg^{-1}$)	0.071	0.171	3.112	7.02
E. fetida × Cu (5 $mg\ kg^{-1}$)	0.034	0.134	4.716	12.22
E. fetida × Cd+Cu (0.15+5, $mg\ kg^{-1}$)	0.067	0.167	4.673	12.39
E. eugeniae × Control	0.037	0.135	2.802	6.21
E. eugeniae × Cd (0.15 $mg\ kg^{-1}$)	0.072	0.172	3.040	7.20
E. eugeniae × Cu (5 $mg\ kg^{-1}$)	0.030	0.131	4.839	11.40
E. eugeniae × Cd+Cu (0.15+5, $mg\ kg^{-1}$)	0.070	0.170	4.714	12.66
Level of significance	NS	NS	NS	NS
Co-efficient of variation (%)	8.312	3.120	11.78	5.056

This means the following dissimilar letters in a column are significantly different at 0.1%.

Cadmium contents in vermicompost

Cadmium contents in vermicompost were elevated with the increase in cadmium concentration. A significant difference was recorded in due to Cd contents in vermicompost. The highest (0.171 $mg\ kg^{-1}$) and the lowest (0.133 $mg\ kg^{-1}$) contents of Cd in vermicompost were recorded (Table 4). The earthworms consume cow dung that would reduce heavy metals content through their gut and intestines. As a result, the rest amount of cadmium would be released with excreta. Therefore, the addition of cadmium caused the higher concentration of cadmium in vermicompost. The decomposition of organic matter during vermicomposting could concentrate and increase the heavy metal contents (Hsua and Lob, 2001; Zhu *et al.*, 2014).

Copper contents in vermicompost

Copper contents in vermicompost were varied with the application of different concentrations of copper in cow dung. The higher content was recorded as 12.525 $mg\ kg^{-1}$ significantly in vermicompost due to Cd+Cu (0.15+5, $mg\ kg^{-1}$) than the lowest content of 6.565 $mg\ kg^{-1}$ in vermicompost by the control (Table 4).

The earthworms ingest organic materials with heavy metals and thus the metal compounds are disintegrated in the gut of earthworms and released in vermicompost at harvest. The total content of heavy metals in final vermicompost and the effect of earthworms on that were reported by several authors (Goswami *et al*., 2014; Suthar *et al*., 2014).

Conclusion

The present study revealed that cadmium and copper accumulated in earthworm bodies with elevated levels of heavy metals. The earthworm fecundity was also negatively affected. Thus, the heavy metal concentration reduced by earthworms would be an effective biological approach. The concentration would remain under the threshold level and keep the cow dung, soil as well as the environment free from hazardous materials.

Conflict of interest

The authors declare that there are no conflicts of interest related to this research.

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