Bioaccumulation of heavy metals in earthworms collected from abattoir soils in Abeokuta, south-western Nigeria

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Abstract

Activities in abattoirs and direct release of its waste into the environment are on the increase due to high protein demand in the country; and there is a need for proper assessment of abattoir soil for pollution. This study evaluated bioaccumulations of heavy metals in indigenous earthworm from abattoir soils as a measure of pollution. Five replicates each of earthworms, soil and cow dung samples were collected from three abattoir sites (Lafenwa, Gbonogun and Madojutimi) in Abeokuta, Ogun State and a control site (Federal University of Agriculture, Abeokuta, Arboretrum) beside an undisturbed stream with no cow dung. Samples were collected at 1m interval from sampling point at each site. Heavy Metal (Cu, Zn, Pb, Cd, Co, Cr, Ni and Mn) concentrations were measured in the earthworm species (*Libyodrilus violaceus, Eudrilus eugeniae* and *Alma millsoni*), soil and cow dung samples from the sites using Atomic Absorption Spectrophotometer. Some physico-chemical parameters (pH and percentage organic matter) of the soil and cow dung from the sample sites were also assessed using standard methods. The heavy metals concentrations, pH and the percentage organic matter recorded in the cow dung of all the abattoir locations were significantly higher ($p<0.05$) than the concentrations in their respective soil samples. Lafenwa abattoir had the highest concentrations of heavy metals and percentage organic matter (4.57 ± 0.06) in its soil. Concentrations of Cu, Zn, Pb, Cd and Mn were highest in the tissue of earthworm species obtained from Lafenwa abattoir. The bioaccumulation factors for the metals analysed were less than unity except for Cd. *Libyodrilus violaceus, Eudrilus eugeniae* and *Alma millsoni* bioaccumulate heavy metals from the abattoir soils and the accumulation is directly related to the concentration of such heavy metals in the soil. Indigenous earthworms are a good bioindicator of pollution for heavy metals.

Keywords: heavy metal, bioaccumulation, abattoir soils, earthworms.

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Introduction

As reported by Salehi and Tabari (2008), high protein demand in countries has significantly increased meat production. Meat processing facilities produce solid, liquid and gaseous wastes. Solid and liquid wastes tend to be worrisome due to the high content of putresible organic matter, which can lead to the depletion of oxygen and an impairment or disruption of water and soil eco-functionality and a preponderance of disease-causing organisms.

The meat processing wastes come from stockyards, abattoirs and packing plants. All these contain blood, fats, protein, gut contents, heavy metals, anti-bodies, hormones and other substances (Itodo and Awulu, 1991). In developing nations like Nigeria, many abattoirs dispose off their waste directly into streams or rivers and also use water from the same source to wash slaughtered meat (Adelegan, 2002). The situation is not any different in Ghana where most liquid wastes are released into the immediate environs of the abattoir (Weobong, 2011). In some instances, the solid wastes are deposited with other urban wastes some distance from the abattoir (Weobong, 2011).

Contamination by heavy metals, particularly lead and zinc, has been reported in abattoir areas in South-western region of Nigeria (Coker *et al*, 2001). The accumulation of heavy metals in soil poses many risks to human and the ecosystem (Odoh *et al*, 2011). Due to the non biodegradable property of the heavy metals in soils, their release to the environment should be restricted. Earthworm constitute a major component in soil functioning and they play an important role in chemical element transformation (Lee, 1985). Studies have shown that earthworms accumulate heavy metals in their body tissues from contaminated substrates (Gupta *et al*, 2005; Suthar, 2008). Metal concentration in earthworm tissues has been shown to be directly correlated to the level of their contamination in the substrates and soil (Hobbelen *et al*, 2006). Due to this limited mobility, earthworms are particularly suited to be bioindicators of heavy metals in the soil ecosystem (Suthar *et al*, 2008). Among the heavy metals, Cd and Zn are accumulated most by earthworms (Hobbelen *et al*, 2006). Earthworms are located at the beginning of the consumer food chain and can transfer the contaminated food to their predators and ultimately to humans. Also, high protein demand in the country has lead to increase in meat processing from abattoirs which releases waste containing heavy metals into the environment. This study is therefore aimed at assessing the bioaccumulation of heavy metals in indigenous earthworms from abattoir soil environment as a measure of heavy metal pollution.

Materials and methods

Study site

This study was carried out in Ogun State situated at 7°00′ N, 3°35′ E, a state in south-western Nigeria. It borders Lagos State to the south, Oyo and Osun States to the north, Ondo State to the east and the Benin Republic to the west. The total area of the state is 16,762 km². The earthworm and soil samples were collected from three different abbatoir sites and a control site within Abeokuta metropoly in Ogun State. The sampling sites were; Lafenwa Abattoir (07.163^{0}N 003.328^{0}E), Gbonogun Abattoir (07.179^{0}N 003.406^{0}E), Madojutimi Abattoir (07.163^{0}N 003.376^{0}E) and the control site, Federal University of Agriculture Abeokuta Arboretum (07.224^{0}N 003.450^{0}E).

Sample collection

Earthworm, cow dung and soil samples (0-20 cm depth) were collected from the three abattoir sites. While earthworm and soil samples were only collected from the control site by digging and hand sorting method according to standard method of Owa (1992). Five replicates of earthworms, cow dung and soil samples were collected at 1 m interval at each sampling site. The samples were transported to the laboratory in plastic containers. The earthworms were washed in running tap water and placed on wet filter paper for 24 hours to void out gut contents (Owa, 1992).

Laboratory analysis: Physico-chemical analysis of the soil and cow dung sample

Analysis of the soil pH

Soil and cow dung samples were collected and pH was measured according to the method of Davey and Conyers (1988). Air-dried samples (20 g) was weighed into a 50 mL beaker and 20 mL of distilled water added. The mixture was allowed to stand for 30 minutes with occasional stirring with a glassrod. The electrodes of a calibrated pH meter were then inserted into the partly settled suspension and pH of the samples was measured.

Determination of the percentage organic matter

The percentage organic matter of the soil and cow dung was determined by the method described by Nelson and Bomers (1982). The samples were sieved through a 0.5 mm mesh after which they were weighed in duplicate and transferred to a 250 mL erlenmeyer flask. Exactly 10 mL of 1 M potassium dichromate was pipetted into each flask and swirled gently to disperse the sample followed by addition of 20 mL concentrated sulphuric acid. The flask was swirled gently until samples and reagents were thoroughly mixed. The mixture was then allowed to stand for 30 min. on a glass plate. 100 mL distilled water was added followed by addition of 3-4 drops of ferroin indicator, after which it was titrated with 0.5 N ferrous sulphate solutions. A blank titration was similarly carried out.

The percentage organic carbon is calculated by the equation:

$$\frac{(\text{Mek}_2\text{Cr}_2\text{0}_7\text{-MeFeSO}_4) \times 0.0031 \times 100 \times \text{F}}{\text{Mass (g) of air dried samples}}$$

F = Correction factor (1.33).
Me = Normality of solution x ml of solution used.
% organic matter in samples = % organic carbon $\times$ 1.729.

Heavy metal analysis

This involves sample digestion and determination of the heavy metal concentration in the digested samples according to the method of Association of Analytical Chemists, AOAC (2000). Sample digestion is by wet oxidation. To 0.2 g of earthworm and rumen waste sample, and 5 g of soil sample, 2 mL sulphuric acid, 4 mL perchloric acid and 20 mL nitric acid were added and the mixture was heated to boil on heating mantle until the mixture turns colourless. The mixture was allowed to cool and then diluted with distilled water to 100 mL.

Following sample digestion, an atomic absorption spectrophotometer (Perkin Elmer Model 403, North Chicago USA.) was used to determine the concentration of the following heavy metals in the digest of earthworm tissue, rumen waste and soil sample: Lead (Pb), Zinc (Zn), Cupper (Cu), Chromium (Cr), Cadmium (Cd), Cobalt (Co) and Nickel (Ni).

Bioaccumulation factor (BAF)

The indices of metal accumulation by organisms in their body tissue is known as BAF. It is the ratio of the level of metals in organisms to the soil 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 = Cbiota/Csubstrate,

Where Cbiota and Csubstrate were the total concentrations of heavy metals in earthworms and soil substrate respectively.

Data analysis

Data were presented as the mean ± standard deviation (SD). Statistical analyses for all measurements were performed by Statistical Package for Social Sciences (SPSS) version 16.0. Post Hoc test was also carried out using Duncan Multiple Range Test (DMRT). $p<0.05$ was considered to be statistically significant.

Results

The age of sample sites and the earthworm species identified from each site are shown in Table 1. At Lafenwa and Gbonogun abattoirs, we collected *Eudrilus eugeniae* and *Libyodrilus violaceus*; *Alma millsoni* and *Libyodrilus violaceus* were collected from Madojutimi Abattoir while only *Alma millsoni* was collected from the control site.

Table 1: Age of the sample sites and the earthworm species identified from each of them.

Sample Site	Age of the sample sites (years)	Species found
Lafenwa Abattoir	60	*Eudrilus eugeniae* *Libyodrilus violaceus*
Gbonogun Abattoir	25	*Eudrilus eugeniae* *Libyodrilus violaceus*
Madojutimi Abattoir	8	*Alma milsoni* *Libyodrilus violaceus*
Control	–	*Alma milsoni*

The value of pH was highest in the cow dung of all the abattoir locations than their respective soils (Figure 1). The highest percentage organic matter was recorded in the soil of Lafenwa Abattoir (4.57±0.06) while the soil of the control site had the lowest organic matter (2.11±0.05). The level of organic matter in the cow dung followed the order Gbonogun > Lafenwa Abattoir > Madojutimi Abattoir. There was no significant difference ($p>0.05$) in the value of organic matter in the soils of Gbonogun and Lafenwa Abattoirs.

The heavy metals concentrations recorded in the cow dungs of all the abattoir locations were significantly higher ($p<0.05$) than the metal concentrations in their respective soil samples. The soil samples obtained from the control site had significantly lower metal concentrations than the soils and cow dung samples from the abattoirs (Table 2). Values recorded for Cu, Pb, Cd and Co in the soil and cow dung samples, from all the abattoir locations, were significantly different ($p<0.05$). Lafenwa abattoir had the highest concentrations of Zn (28.06 ± 0.30), Pb (30.71 ± 0.35), Cd (10.78 ± 0.19) and Ni (25.81 ± 0.18) in its soil and Zn (40.23±0.01), Pb (40.92±0.01), Cd (25.12±0.01) and Ni (31.22±0.01) in its cow dung, while Gbonogun abattoir had the highest concentrations of Cu (40.60 ± 0.62), Co (8.56 ± 0.50) and Mn (4.12 ± 0.39) in its soil. The concentration of Cu, Zn, Co, Ni, Cr and Mn in the abattoir soil, cow dung and from the tissues of earthworm species collected from the abattoir locations follow the order: Cow dung>abattoir soil>earthworm tissues.

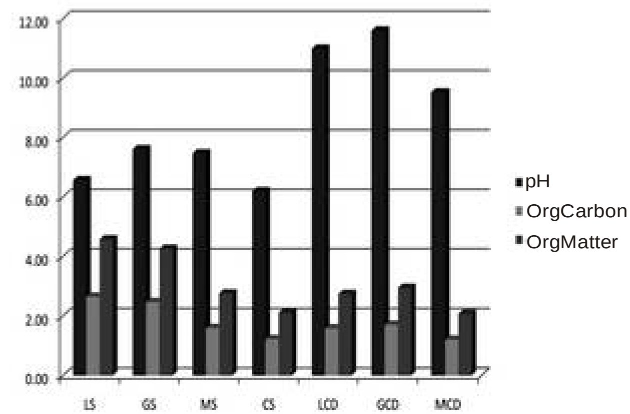

Figure 1. Physico-chemical parameters of the soils and cow dung obtained from the sampling locations. LS – Lafenwa Abattoir soil, GS – Gbonogun Abattoir soil, MS – Madojutimi Abattoir soil, CS – Control site soil, LCD- Lafenwa Abattoir cow dung, GCD – Gbonogun Abattoir cow dung, MCD – Madojutimi Abattoir cow dung.

The concentrations of heavy metals in the tissues of all the earthworm species obtained from the abattoir locations, was significantly lower ($p<0.05$) than their respective abattoir soils (Table 3). However, *Libyodrilous violaceus* recorded higher concentrations of all the metals in its tissues than other earthworm species from all the abattoir locations (Table 2). The metal concentrations of Cu, Zn, Pb, Cd and Ni was highest in the tissues of earthworms from Lafenwa and Gbonogun abattoirs while the earthworm species obtained from the control site had the lowest metal concentrations in their tissues (Table 3). There was no significant difference ($p>0.05$) between the values recorded for Mn, Cr and Co in the earthworm tissues obtained from all the abattoir locations. The relationship between the heavy metal concentrations in the soil and earthworms collected from the study locations is shown in Table 4. Pearson correlation revealed that the concentrations of Cu, Zn, Pb, Cd and Ni in abattoir soils were positively correlated with concentrations in the tissues of earthworms ($p<0.05$) collected from them. However, there was no correlation ($p>0.05$) between the concentrations of Co, Cr and Mn in the abattoir soil and the tissues of earthworms collected from them. The BAFs for all the metals tested for were less than unity except for Cd which was more than unity (Table 5).

Table 2: Concentrations of heavy metals (mg/kg) in the soil and cow dung obtained from abattoirs of Abeokuta, south-western Nigeria.

Heavy Metals (mg/kg)	Soil sample				Cow dung			
	CS	LS	GS	MS	LCD	GCD	MCD	MPL in soil (USDA, 2000)
Cu	16.36±0.21^{g}	38.98±0.47^{e}	40.60±0.62^{d}	36.46±0.56^{f}	66.36±0.01^{b}	68.61±0.01^{a}	63.57±0.01^{c}	4,300
Zn	5.30±0.13^{e}	28.08±0.30^{c}	27.03±0.22^{d}	26.66±0.87^{d}	40.23±0.01^{a}	37.41±0.01^{b}	37.88±0.01^{b}	7,500
Pb	10.72±0.28^{g}	30.71±0.35^{d}	27.64±0.68^{e}	24.60±0.57^{f}	40.92±0.01^{a}	36.78±0.01^{c}	38.11±0.01^{b}	420
Cd	2.54±0.27^{f}	10.78±0.19^{c}	9.79±0.27^{d}	7.10±0.79^{e}	25.12±0.01^{a}	22.38±0.01^{b}	24.62±0.01^{a}	85
Co	1.58±0.23^{f}	6.46±0.17^{d}	8.56±0.50^{c}	4.78±0.88^{e}	16.81±0.01^{a}	16.16±0.01^{ab}	15.92±0.01^{b}	–
Ni	10.47±0.13^{e}	25.81±0.18^{c}	23.89±0.30^{d}	23.71±0.44^{d}	31.22±0.01^{a}	30.52±0.01^{b}	30.18±0.01^{b}	75
Cr	0.17±0.05^{c}	2.87±0.30^{b}	2.48±0.46^{b}	2.69±0.51^{b}	12.63±0.01^{a}	12.33±0.01^{a}	12.84±0.01^{a}	3,000
Mn	1.69±0.23^{d}	3.67±0.07^{c}	4.12±0.39^{c}	3.73±0.69^{c}	15.96±0.01^{b}	16.69±0.01^{a}	16.78±0.01^{a}	–

** Means with the same superscripts in a row are not significantly different (p<0.05).
CS – Control site soil, LS – Lafenwa Abattoir soil, GS – Gbonogun Abattoir soil, MS – Madojutimi Abattoir soil, LCD – Lafenwa Abattoir cow dung, GCD – Gbonogun Abattoir cow dung, MCD – Madojutimi Abattoir cow dung, MPL – Maximum Permissible Limit.

Table 3. Concentrations of heavy metals (µg/g) in the tissues of the earthworm species obtained from the control and abattoir sites.

Heavy Metals (µg/g)	Earthworm samples						
	AmC	EeL	LvL	EeG	LvG	AmM	LvM
Cu	7.82±0.56^{d}	21.52± 0.28^{b}	22.56±0.75^{ab}	21.48±0.77^{b}	21.79±0.15^{b}	20.03±0.73^{c}	23.07±0.76^{a}
Zn	3.47±0.30^{e}	12.23±0.63^{ab}	12.46±0.54^{a}	11.05±0.17^{cd}	11.83±0.90^{abc}	10.20±0.11^{d}	11.49±0.24^{bc}
Pb	2.31±0.20^{e}	9.46±1.16^{a}	10.11±1.38^{a}	6.35±0.34^{cd}	7.49±0.54^{bc}	5.32±0.43^{d}	8.08±0.56^{b}
Cd	2.37±0.35^{d}	14.39±0.37^{a}	14.94±0.77^{a}	11.62±0.43^{b}	12.31±0.29^{b}	10.38±0.24^{c}	11.76±0.57^{b}
Co	0.78±0.15^{c}	4.59±0.38^{b}	5.02±0.10^{b}	4.98±0.77^{b}	5.89±0.71^{a}	5.28±0.41^{ab}	6.01±0.35^{a}
Ni	7.61±0.28^{d}	23.13±0.28^{b}	24.00±0.30^{a}	20.39±0.44^{c}	20.68±0.51^{c}	20.08±0.19^{c}	22.45±0.82^{b}
Cr	0.12±0.08^{c}	1.37±0.18^{b}	2.24±0.42^{a}	1.55±0.22^{b}	1.67±0.21^{b}	1.51±0.26^{b}	2.22±0.34^{a}
Mn	0.16±0.05^{d}	3.32±0.37^{ab}	3.67±0.47^{a}	2.40±0.40^{c}	2.75±0.58^{bc}	2.35±0.30^{c}	2.76±0.09^{bc}

**Means with the same superscripts in a row are not significantly different (p<0.05).
Key: AmC – *Alma milsoni* from the control site (stream adjacent FUNAAB arboretum), **EeL** – *Eudrilus eugeniae* from Lafenwa Abattoir, **LvL** – *Libyodrilous violaceus* from Lafenwa Abattoir, **EeG** – *Eudrilus eugeniae* from Gbonogun Abattoir, **LvG** – *Libyodrilous violaceus* from Gbonogun Abattoir, **AmM** – *Alma milsoni* from Madojutimi Abattoir, **LvM** – *Libyodrilous violaceus* from Madojutimi Abattoir.

Table 4: Pearson correlation coefficient between metal concentrations in all the abattoir soil samples and their respective earthworm tissues.

	Earthworms (r-values)							
Soil	**Cu**	**Zn**	**Pb**	**Cd**	**Co**	**Ni**	**Cr**	**Mn**
Cu	0.462*	0.555*	0.391	0.612**	0.331	0.504*	0.209	0.521*
Zn	0.459*	0.563**	0.383	0.613**	0.344	0.512*	0.206	0.538*
Pb	0.471*	0.590**	0.477*	0.671**	0.293	0.546*	0.228	0.599**
Cd	0.459*	0.568**	0.500*	0.672**	0.238	0.540*	0.223	0.581**
Co	0.425	0.471*	0.362	0.554*	0.249	0.451*	0.191	0.413
Ni	0.462*	0.578**	0.419	0.637**	0.333	0.526*	0.215	0.567**
Cr	0.431	0.536*	0.311	0.573**	0.358	0.483*	0.236	0.491*
Mn	0.437	0.504*	0.283	0.547*	0.361	0.478*	0.220	0.424

**Correlation (Pearson) significant at p<0.01; *Correlation (Pearson) significant at p<0.05.

Table 5: Values of BAFs for earthworm species obtained from the study sites.

	Control	Lafenwa		Gbonagun		Madojutimi	
Metals	***A. milsoni***	***E. eugeniae***	***L. violaceous***	***E. eugeniae***	***L. violaceous***	***A. milsoni***	***L. violaceous***
Cu	0.48	0.55	0.58	0.53	0.54	0.55	0.63
Zn	0.65	0.44	0.44	0.41	0.44	0.38	0.43
Pb	0.22	0.31	0.33	0.23	0.27	0.22	0.33
Cd	0.93	1.33*	1.39*	1.19*	1.26*	1.46*	1.66*
Co	0.49	0.71	0.78	0.58	0.69	0.1	0.26
Ni	0.73	0.04	0.93	0.85	0.87	0.85	0.95
Cr	0.71	0.48	0.78	0.63	0.67	0.56	0.83
Mn	0.09	0.90	1.00	0.58	0.67	0.63	0.74

* Values more than unity.

Discussion

The variation in the species of earthworm identified from the sample site could be attributed to the varying structure and composition of soil inhabited by the earthworms (Svendeson *et al*, 2004). This finding is comparable to the work of Hobbelen *et al* (2006) who also identified varying specieis of earthworms (*Lumbricus rubellus* and *Apporectodea caliginosa)* from an indusrial field soil.

This study revealed a higher concentration of heavy metals in the abattoir soils than the control site. The information available at Ogun State Ministry of Agriculture indicated that Gbonogun and Lafenwa Abattoirs are the largest and oldest abattoirs with higher rate of slaughtering activities thereby leading to higher deposition of cow dung. Agbaire and Emoyan (2012) has shown that the soil organic matter acts as a "storehouse or reservoir" for many of these metals. It therefore influences micronutrient availability through chelation. Sharma *et al* (2003) had earlier reported positive correlation between some micronutrient availability and organic matters in the soil.

It was also observed that the cow dung deposited at the abattoirs contained higher concentrations of heavy metals than in their respective soils. Information gathered through group discussion with the workers at these abattoirs showed that most cattle slaughtered at the abattoirs were purchased from nomadic Fulanis who free-range their herds from the northern regions of Nigeria. In the course of such migration or movement down south, these cattle graze on roadside plant materials which have probably been contaminated by or accumulated heavy metals from cars and heavy duty trucks exhaust fumes. These loads of bio-accumulated heavy metals in the plants could therefore be transferred to the cattle some of which are then bioaccumulated in their tissues while the remaining loads bind with the organic content of the rumen which are then deposited unto abattoir soil as dung and rumen wastes during slaughtering activities.

The concentrations all the metals tested were higher in the tissue of *L. violaceous* than those of *A. milsoni* and *E. eugeniae*. Hopkin (1989) stated that earthworms showed a specific capacity to regulate metals, particularly trace metals such as Cu, and Zn in their bodies, and that the bioaccumulation and regulation could be species-specific. Hobbelen *et al* (2006) also submitted that accumulation behaviour of heavy metals in earthworms and their sensitivity to these pollutants can vary from species to species. The three earthworm species in this study showed considerable differences in metal accumulation in their tissues which could be due to variation in their metabolism and duration of exposure to the metal-contaminated soils (Morgan and Morgan, 1992).

The concentrations of Cu, Zn, Pb, Cd and Ni in earthworm tissues were found to be directly dependent on the metal concentrations of their respective abattoir soil as shown by the positive correlation between the metal accumulated and metal concentrations in the abattoir soil. This finding is in support of the work of Gupta *et al* (2005) who reported that earthworm tissue metal level was directly related to their proportion in a given soil. Similar pattern of metal bioaccumulation was observed by Suthar *et al* (2008), who further supported this finding that tissue-metal level reflects the metal availability in the soil. This may explain the reason why the average metal concentrations of Cu, Zn, Pb, Cd and Ni were higher in the tissues of earthworm species obtained from Lafenwa Abattoir as the concentrations of these metals were higher in its soil samples.

This study clearly shows that earthworms also bioaccumulate heavy metals in their body tissues from their soil substrates. According to Edwards and Bohlen (1996), ingested substrates by earthworms undergo changes through chemical and microbial activities during their passage through the gut; a great proportion of the organic fraction is converted into soluble forms that are more available to organisms. The increase in gut pH and microbial activities increased the possibility for metals to bound to ions and carbonates (i.e. more soluble fraction) in ingested materials (Morgan and Morgan, 1999). These soluble fractions can be accumulated in earthworm tissues during transit of waste through worm's gut (Gupta *et al*, 2005).

This study also shows that the BAFs were less than unity for all the metals except Cd which was more than unity. This implies that the Cd concentration in the tissues of earthworms exceeded the concentration in the soil. Earthworms easily accumulate Cd and retain it in their body tissues. These confirmed the findings of Liu *et al* (2010) who reported higher levels of metals in worms from more polluted site and that irrespective of the degree of pollution, the ratio of metal concentrations in earthworms to soil sample is less than unity with the exception of a few metals such as Cd. The observed difference for BAFs could be related to the differences in specific metabolism and regulating mechanism of organic matter fractions of organo-metal compounds in the earthworm body as reported by Lukkari *et al* (2009).

It was also observed that the abattoir soil samples contained high levels of heavy metals which was probably due to the direct deposition of cow dung that also contain high loads of heavy metals. All earthworm species accumulated heavy metals in their tissues but the accumulation was noted to be species-specific (being highest in *L. violaceous*), dependent on the load of heavy metal in the abattoir soil substrates, the soil pH and organic content. BAFs for the metals were less than unity except for Cd which was more than unity. This indicates Cd as the heavy metal with the highest accumulation potential in earthworms. This study reveals that earthworms accumulated some amount of heavy metals from abattoir soils, thus, can be used as a bio-indicator for pollution as regards heavy metals.

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