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HEAVY METALS CONCENTRATION IN SOILS AND BIOACCUMULATION IN EARTHWORM (*Lumbricus terrestris*) AT LEMNA SOLID WASTES DUMPSITE, CALABAR, CROSS RIVER STATE

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ABSTRACT

This study investigated the concentrations of heavy metals and their bioaccumulation in earthworm in Lemna solid wastes dumpsite in Calabar, Cross River State, Nigeria. Three composite samples of the depth of 30 cm each were collected from Lemna dumpsites and control from the University of Calabar Botanical garden, making a total of four composite soil samples, while samples of earthworms were collected at each sampling point. The samples were analysed for Pb, Cd, Zn and Cu. The result showed that the levels of heavy metals were all higher in dumpsite and significantly different from the control. The respective means of metal concentrations from both Lemna and control were Pb(323.99mg/kg , 89.88mg/kg), Cd(1.12 mg/kg, 0.072 mg/kg), Zn(281 mg/kg, 7.60 mg/kg) and Cu(21.58 mg/kg, 4.89 mg/kg). Pb, Cd, Zn and Cu were also higher in earthworm of dumpsite than earthworm of the control soil. Correlation between Cadmium and Zinc concentration in soil and Cadmium and Zinc concentration in earthworm displayed strong and positive relationship while weak and negative correlation was observed between Cu and Pb concentrations in soil and Cu and Pb concentrations in earthworm. Bioaccumulation of the heavy metals by earthworm was in the order of Zn (0.382) > Cd (0.170) > Pb (0.076) > Cu (0.020). The dumpsite soil was found to be contaminated with Cd, Pb and Zn as their concentrations were high above maximum permissible limits except Cu. For safe consumption of crops from Lemna dumpsite soil, remediation of heavy metals in the soil in advocated.

KEYWORDS: Dumpsite, soil, heavy metals, earthworm, bioaccumulation, correlation

INTRODUCTION

Increase in world population with its consequent high industrial activities has resulted in the generation of large quantities of domestic municipal and industrial wastes (Lagerkvist and Dahlen, 2019; Twumasi *et al.,* 2016). The increased wastes production is however not commensurate with capacity in waste management especially in developing countries. This has led to wide instances of improper waste disposal and management, which pose serious threats to the environment and development of major cities around the globe especially Africa (Lebreton and Andrady, 2019). A major contemporary concern with indiscriminate disposal and burning of wastes is that they yield substances that contain toxic metals known as heavy metals (Sari *et al*, 2019). Wastes dumpsites are considered as the major sources of heavy metals pollution in the environment (Olayinwola *et al.* 2017).

Aboyade, (2004) reported that concentrations of heavy metals in soil around waste dumpsites are influenced by types of waste, topography, run-off and level of scavenging. Heavy metals pollution is a major problem which causes negative effect in soil properties and limitation of productive and ecosystem functions (Friedlora, 2010). Heavy metals can alter soil properties especially soil biological population and properties. They affect population, diversity, characteristics and activities of soil microorganisms in the environment (Pawlowska and Charvet, 2004). Heavy metals in the environment due to anthropogenic activities can be inimical even if their concentration is very low in soils (Bradl, 2004). Soil represents a major sink for metals released into the environment from a variety of anthropogenic activities.
Earthworms are able to accumulate various organic and inorganic contaminants (Morrison *et al*., 2000) present in the soil. They accumulate efficiently and tolerate high tissue metal concentrations using a variety of

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sequestration mechanism (Peijnenburg, 2002; Andre *et al*., 2009). Bioaccumulation is species-special (Heikens *et al*., 2001; Hendrickx *et al*., 2004; Nahmani *et al*., 2007) and is influenced by the physicochemical properties of the pollutants and of the environmental scenario (Vijver *et al*., 2005). Infact it depends on factors such as metal speciation and concentration (Heikens *et al.,* 2001; Hobbelen *et al.,* 2006; Spurgeon *et al.,* 2006; Nahmani *et al.*, 2007), soil type and characteristics (Hendrickx *et al.,* 2004; Hobbelen *et al.,* 2006; Spurgeon *et al*., 2006), temperature (Olchawa *et al*. 2006), and exposure duration (Nahmani *et al*. 2007).

The role of earthworms in the decomposition of organic matter and subsequent cycling of nutrients has raised the interest of their use as indicator organisms for the biological impact of heavy metal pollutants. This in turn has led to a large body of work on earthworm ecotoxicology (Spurgeon *et al.,* 2003). Earthworms are suitable organism for soil ecotoxicology research, and because of their interactions with soil, they are significantly affected by pollutants reaching the soil system. The earthworm skin is extremely permeable to water (Wallwork, 1983) and it represents a main route for contaminant uptake (Jajer *et al*., 2003; Vijver *et al.,* 2005). These organisms ingest large amounts of soil, therefore they are continuously exposed to contaminants adsorbed to soil particles through their alimentary tract (Morgen *et al*., 2004). Vijver *et al*. (2005) measured the contribution of each pathway for heavy metals uptake in the earthworm *Lubricous rubellus*. Earthworms can provide important information about environmental risks and could serve as useful biological indicators of contamination because of the fairly consistent correlation between the concentration of some contaminants in their tissues and soils (Nannoni *et al.,* 2004).

Ediene and Umoetok (2017) reported the concentration of heavy metals in soils at the municipal dumpsite. Oju *et al*. (2020) assessed the toxicity of Lemna solid waste dumpsite, Calabar, Nigeria using different extraction methods and toxicological response of PLHC-1 cells. Previous works in Lemna dumpsite only reported the concentrations of heavy metals and few toxicological indices. Moreover, due to the complexity of soil matrix, the damaging effects of soil contaminants like heavy metals that are continually released to the environment by the solid wastes are not well understood, hence, further assessment of the bioaccumulation of these heavy metals in the tissue of earthworm in the Lemna dumpsite would be an important toxicological base data in risk assessment of indiscriminate dumping of solid wastes with respect to soil conservation.

Table 1: Maximum permissible limits for heavy metals in soil

Heavy metals Mg/kg	EU STD Mg/kg	UK STD Mg/kg	US STD Mg/kg	WHO Mg/kg	Ranges for uncontaminated soil(Nangia,2001) Mg/kg
Fe	-	-	-	-	700-55000
Zn	300	200	200-300	12-60	10-300
Hg	-	-	-	0.001-0.004	0.001-0.3
Cu	140	63	80-200	1-12	2-100
Cd	3.0	1.4	400	0.002-0.5	0.01-0.7
Cr	180	6.4	400	0.002-0.2	5-300
Pb	300	70	300	0.3-10	2-200
Ni	-	-	-	0.1-5	10-1000

*EU= Europe, *UK= United Kingdom, *US= United States, *WHO=World Health Organisation, *STD= Standard
Source: [Hong *et al*, 2014; Asemave and Anhwange, 2012; Nangia, 2001]

MATERIALS AND METHODS

Geographical Location of the Study Area

The Lemna wastes dumpsite which is located in Calabar Municipality is very popular. Calabar is the capital city of Cross River State, with a population of about 372,000 (Oju *et al.,* 2020). The Geographic Positioning System (GPS) revealed that the dumpsite is located at latitude 4^0 57'N and longitude 8^0 20'E, and has been existing for more than three decades (Oju *et al.,* 2020). The dumpsite is the major waste deposition site in the region and repositories for several kinds of unsorted industrial and household wastes including plastics products, oils, electrical/electronic gadgets, paints, batteries, tyres, automobile parts, among others (Oju *et al.,* 2020).

Sampling Design

Random sampling was adopted in this ecological study where three stations; S_1, S_2 and S_3 (Fig 1) at the Lemna dumpsite were used for soil and earthworm collection. In each station, a composite soil sample was obtained from three different points and earthworms sourced from within one metre (1m) radius of the soil sampling point.

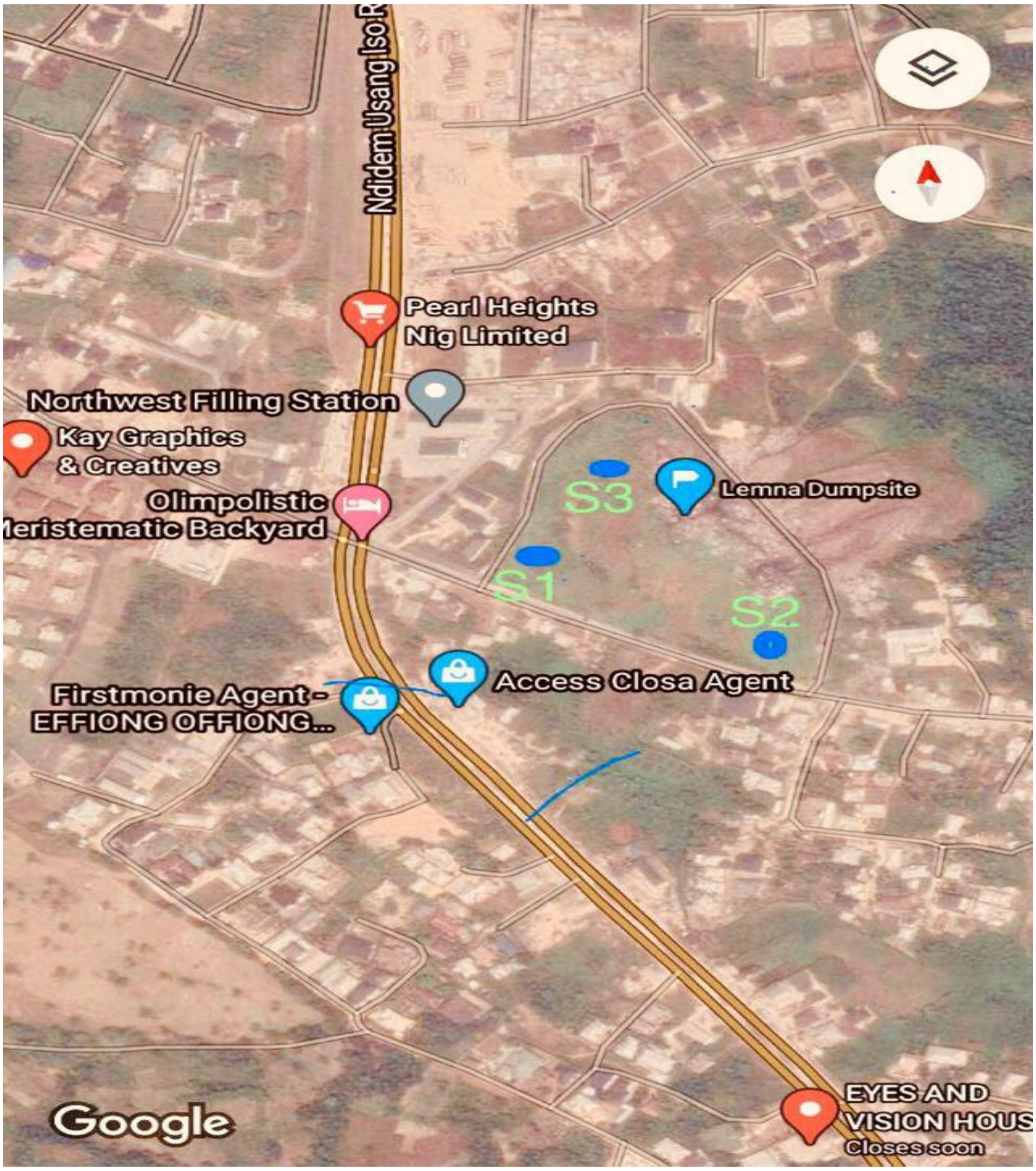

Figure 1: Map of Lemna Dumpsite Showing the Different Sampling Points

Legend
S1: Sampling point 1
S2: Sampling point 2
S3: Sampling point 3

COLLECTION OF SAMPLES

Earthworm Samples

Earthworms were collected in the month of May, during their period of abundance. Hand sorting as reported by Satchel (1969) was adopted in sampling the earthworms. The choice of hand method stemmed from the fact that chemical application like formalin, which is lethal to earthworms with consequent alteration of accumulated heavy metals is not involved. Shovel was used to excavate the soil volume of 30 cm x 30 cm x 30 cm and put in garbage bag and paced on a plastic sheet where the crumbs of soil were examined for earthworms. About ten earthworms were obtained from each station. They were quickly transported with a ball of soil to the laboratory for digestion and analysis. Prior to digestion and analysis, the samples were stored at -20 ^{0}C to avoid tissue deterioration.

Soil Samples

At each station, soil samples were collected at three different points between 0-30 cm, which represents the depth of arable cultivation, using soil auger at the Lemna Dumpsite. The soil samples collected from each station were made into composite samples. Earthworms and soil samples for control were however, collected accordingly from the University Calabar Botanical Garden without history of pollution.

DETERMINATION OF HEAVY METALS

Samples were first digested before analysis for heavy metals. Following digestion, samples were analysed for heavy metals (Pb, Cd, Zn, Cu) using Atomic Absorption Spectrophotometer (AAS) of UNICAM 919 model (Srikanth *et al.,* 2013).

The bioaccumulation factor (BAC)

The bioaccumulation factor (BAC) of heavy metals in earthworm, also known as the bioaccumulation coefficient, was calculated according to equation 1:

$$BAC = \frac{CE}{CS} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (1)$$

Where CE is the concentration of heavy metal per kg earthworm and CS is concentration of heavy metals per kg soil.

Statistical Analysis

The data obtained were statistically analyzed using one-way analysis of variance (ANOVA) by graph pad. Significantly different results were established. The accepted level of significance was 5%. Further analysis was done to evaluate the correlation between heavy metals in soil and in the tissue of earthworm.

RESULTS AND DISCUSSION

Results obtained from the determination of heavy metal contents of soil and earthworm (*Lumbricus terrestris*) obtained from Lemna dumpsite in Calabar, Cross River State are presented in Table 2 and Table 3 respectively. Correlation coefficient used to determine the association between metal levels in soil and earthworm at Lemna dumpsite is presented in Table 4. Table 2 indicates that the concentrations of lead in soil obtained from Lemna dumpsite ranged from 291.22 mg/kg - 352.36 mg/kg. The highest value was recorded at sampling point 2 and the lowest at sampling point 3. The concentration of Lead (Pb) in soil from the control site ranged between 70.05 mg/kg and 102.20 mg/kg. The difference in Pb concentration between Lemna dumpsite and control was statistically significant ($p < 0.05$). Cadmium (Cd) concentration in soil from Lemna dumpsite ranged from 1.09 mg/kg to 1.14 mg/kg, with sampling point 3 having the lowest value and that of sampling point 1 recording the highest value. The levels of Cd in the soil collected from the control site ranged from 0.06mg/kg to 0.09mg/kg. There was significant difference ($p<0.05$) between the levels of Cd from dumpsite and control. Table 2 indicates that the concentrations of Zinc (Zn) obtained from Lemna dumpsite ranged from 240.50 mg/kg to 305.30mg/kg. The result reveals that sampling point 3 recorded the highest value against sampling point 1 which had the lowest value. The concentrations of Zn from the control site ranged from 6.51mg/kg to 8.70mg/kg. Statistically, there was difference between the levels of Zn from Lemna dumpsite and control site at 5% level of significance. The concentrations of Cd, Pb, and Zn were above the maximum permissible limit of heavy metals in soils recommended by recognized authorities (Table 1)

Copper (Cu) concentration in soil from Lemna dumpsite ranged from 19.36 mg//kg to 25.00 mg//kg. Sampling point 3 had the highest value while sampling point 1 recorded the lowest value. The concentration of Cu from the control site ranged from 4.35 mg/kg to 5.60 mg/kg. The difference in Cu concentration between Lemna dumpsite and control was statistically significant ($p<0.05$).

The results of this study reveals that heavy metals were highly concentrated in the dumpsite soils than control soils. Amongst the four heavy metals studied, Pb and Zn had the highest concentrations in both dumpsite and control. Higher concentration of heavy metals in dumpsite than control maybe due to higher content of heavy metal bearing wastes in the dumpsite. Higher Pb level in the dumpsite soil than other heavy metals and also than in control is in accordance with the observation of Ediene and Umoetok (2017) who in their study rated Pb as the third most abundant heavy metal in a related study in Calabar metropolis. Similarly, in related studies, Agbashie and Banunle (2020), Amos-Tautua *et al*., (2014) and Obasi *et al*. (2012) had similar views with these findings as they recorded higher values of heavy metals in dumpsite soil than control.

Cadmium, Zn and Cu were also far higher in the dumpsite soils than the control. Ediene and Umoetok (2017) attributed higher levels of heavy metals in dumpsite than control to higher content of heavy metals in wastes. Cadmium mean value obtained in dumpsite in this study was higher compared to the dumpsite in Ghana assessed by Agbashie and Banunle (2020) but lower than the concentration recorded in dumpsite in Bayelsa by Amos-Tautua *et al.,* (2014). These differences may be attributed to the differences in composition of wastes in the dumpsites and also differential ages of dumpsites and consumption capacity of regions. The concentration of Pb was above the maximum permissible limit of 30-300 mg/kg recommended by USEPA (1986), 300 mg/kg recommended by Hong *et al.* (2014), 2-200 mg/kg by Nangia (2001), for agricultural soils. The control soil had lead level within or below the permissible limit recommended by the above mentioned authorities. Cadmium concentration in dumpsite soil was above the maximum permissible limit of 3mg/kg and 0.1 o 0.7 mg/kg recommended by Hong *et al.,* (2004) and Nangia, (2001) accordingly while in control soil it was within safe limit. Zinc was above the maximum permissible limit of 300 mg/kg and 10-300 mg/kg recommended by Hong *et al*., (2014) and Nangia (2001) respectively in dumpsite soil and within the permissible limit in the control. Copper concentration in both dumpsite and control soil was within the maximum permissible limits of 140 mg/kg and 2-100 mg/kg recommended for soils by Hong *et al*. (2014) and Nangia (2001) respectively.

The concentrations of Pb, Cd and Zn being above maximum permissible limits recommended by several authorities means that humans and animals stand the risk of suffering from heavy metal toxicities, poisoning or ailments caused by heavy metals toxicities if crops grown in such dumpsites are consumed by man or used as forages.

Table 2: Metal Concentration in Soil Obtained from Lemna Dumpsite, Calabar, Cross River State, Nigeria

Sampling Station	sampling point	Pb mg/kg	Cd mg/kg	Zn mg/kg	Cu mg/kg
	1	328.40	1.14	240.50	19.36
	2	352.36	1.13	297.20	20.38
Lemna	3	291.22	1.09	305.30	25.00
	Mean	323.99[a]	1.12[a]	281[a]	21.58[a]
	Range	291.22-352.36	1.09-1.14	240.5-305.3	19.36-21.58
	1	101.20	0.07	8.70	5.6
Control	2	98.40	0.09	7.60	4.72
	3	70.05	0.06	6.51	4.35
	Mean	89.88[b]	0.073[b]	7.603[b]	4.89[b]
	Range	70.05-101.2	0.16-0.09	6.51-8.7	4.35-5.6

Mean with different superscripts indicates significant difference

Table 3 indicates that the concentrations of Pb in earthworm obtained from Lemna dumpsite ranged from 23.86 mg/kg – 25.04 mg/kg. The highest value was recorded at sampling point 2 and the lowest at sampling point 3. The concentration of Pb in earthworm from the control site ranged between 20.02 mg/kg and 50.50 mg/kg. The difference in Pb content between earthworm obtained from Lemna dumpsite and control was not statistically significant ($p > 0.05$). Cadmium concentration in earthworm obtained from Lemna dumpsite ranged from 0.17 mg/kg-0.21 mg/kg. The lowest value was recorded at sampling point 2 while the highest value at sampling point 3. The concentration of Cd in earthworm from the control site ranged from 0.01 mg/kg – 0.02mg/kg. There was significant difference ($p<0.05$) between the Cd content in earthworm obtained from Lemna dumpsite and control site. Table 3 indicates that the concentrations of Zn in earthworm obtained from Lemna dumpsite ranged from 102.61 mg/kg - 112.32 mg/kg. The lowest value was recorded at sampling point 1 and the highest value at sampling point 3. The levels of Zn in earthworm obtained from the control site ranged from 0.98 mg/kg –2.03 mg/kg. The difference in the levels of Zn in earthworm from Lemna dumpsite and the control site were statistically significant at 5%. Copper concentration in earthworm from Lemna dumpsite ranged from 0.36 mg/kg to 0.55 mg/kg. The lowest value was recorded at sampling point 1 and the highest at sampling point 3. The concentration of Cu in earthworm from the control site ranged between 0.10mg/kg and 0.90 mg/kg. The difference in lead content between earthworm obtained from Lemna dumpsite and control was not statistically significant ($p > 0.05$).

The concentration of Pb, Cd and Zn was higher in earthworm of dumpsite soil than earthworm in the control soils while Cu was more concentrated in earthworm obtained from the control soil than dumpsite soil earthworm. The concentration of heavy metals in earthworm obtained from the dumpsite shows that dumpsite soil has higher level of heavy metals compared to the control and that earthworms are capable of bio accumulating heavy metals. This is in accordance with Morgan and Margon (1991), who stated that earthworms are known to accumulate heavy metals. Similarly, Lemtiri (2015) opined that some earthworms can survive in heavy metal polluted soils and can accumulate heavy metals in several sequestrations. However, Edwards and Bohlen (1996) are of the view that heavy metals have adverse effect on earthworm populations which in turn affect the global functioning of ecosystem.

Table 3: Metal concentration in Earthworm (*L. terrestris*) obtained from Lemna Dumpsite, Calabar, Cross River State, Nigeria

Sampling Station	Sampling point	Pb mg/kg	Cd mg/kg	Zn mg/kg	Cu mg/kg
	1	24.74	0.19	102.61	0.36
	2	25.04	0.17	107.33	0.38
Lemna	3	23.86	0.21	112.32	0.55
	Mean	24.54[a]	0.19[a]	107.42[a]	0.43[a]
	Range	23.86-25.04	0.17-0.21	102.61-112.32	0.36-0.55
	1	50.50	0.02	2.03	0.90
Control	2	34.24	0.01	1.55	0.82
	3	20.02	0.01	0.98	0.10
	Mean	34.92[a]	0.013[b]	1.52[b]	0.60[a]
	Range	20.02-50.5	0.01-0.02	0.98-2.03	0.1-0.9

Mean with different superscripts indicates significant difference

Relationship between heavy metal concentrations in soil and earthworm (*Lumbricus. terrestris*) Obtained from Lemna Dumpsite

The summary of the relationship between the concentration of heavy metals in soil and earthworm (*L. terrestris*) obtained from Lemna dumpsite in Calabar is shown in Table 4. A weak negative correlation was observed between Pb in soil and Pb in earthworm (r = -0.431). The correlation was not significant both at 99 % and 95 % confidence levels. Cd in soil and Cd in earthworm displayed a strong positive correlation (r = 0.987). The correlation was significant at 99 % confidence level. Zn in soil and Zn in earthworm also displayed a strong positive correlation that was significant at 99 % confidence level (r = 0.995). Weak negative correlation was observed between Cu in soil and Cu in earthworm (r = -0.237). The correlation was not significant both at 99% and 95 % confidence level.

The weak and negative correlation observed between Pb in dumpsite soil and Pb in earthworm dumpsite suggests that increase in Pb concentrations in dumpsite soil will result to no increase in Pb level of earthworm. This may be due to the toxic nature of Pb. Lead is a very toxic heavy metal that has deleterious effect on biological system (Pottee and Pain, 2003) which is as a result of the capacity of Pb^{2+} to interfere with several enzymes (Ewers and Schilipcoter, 1991). Similar observation of Pb was made for Cu. This negative relationship may be due to inability of earthworm to bioaccumulate Cu and Pb beyond certain levels. The relationship between Cd and Zn in soil and in earthworm showed that earthworm has the ability to continuously bioaccumulate Cd and Zn as they keep measuring in soil. The observation made for Cd and Zn agrees with the findings of Gruber *et al*. (2000) who stated that the normalization of glutathione concentration and antioxidant enzymes of earthworm observed after 28 and 21days of laboratory exposure to Pb and Zn respectively may be due to their capacity to sequester the metals in biologically inactive form by the induction of metal binding protein.

Table 4: Relationship between heavy metal concentrations in soil and earthworm (*L. terrestris*) from Lemna Dumpsite, Calabar, Nigeria

Metals	Soil against earthworm (r)	Inference
Pb	-0.431	Weak negative relationship
Cd	0.987	strong positive relationship
Zn	0.995	strong positive relationship
Cu	-0.237	Weak negative relationship

Co-relation ratings
0.80 – 0.99 Strong relationship
0.50 – 0.79 Slightly strong relationship
0.10 – 0.49 Weak relationship
0.00 – 0.09 No relationship

The average bioaccumulation factor for the metals under study were 0.076 for Pb, 0.170 for Cd, 0.382 for Zn and 0.020 for Cu (Table 5). The bioaccumulation factors obtained for the heavy metals showed that Zn and Cd were more bioaccumulated by the earthworm than Pb and Cu. Higher accumulation of Zn and Cd by earthworm than Pb and Cu is without doubt shown in the positive and strong correlation/relationship between Cd and Zn in earthworm and Cd and Zn in dumpsite soils. The bioaccumulation of heavy metals by earthworm was in the order of Zn > Cd > Pb > Cu

Table 5: Bioaccumulation Factor (BAC) of Heavy Metal in Earthworm (*L. terrestris*) from Lemna Dumpsite, Calabar, Nigeria.

Metals	Bioaccumulation Factor (BAC)
Pb	0.076
Cd	0.170
Zn	0.382
Cu	0.020

SUMMARY AND CONCLUSION

The result of heavy metal concentrations in dumpsite soil in Lemna and control revealed that all the metals were higher in dumpsite than control. Lead, Cd, Zn and Cu had mean values of 323.99 mg/mg and 89.88 mg/kg, 1.12 mg/kg and 0.073 mg/kg, 281 mg/kg and 7.60 mg/kg and 21.58 mg/kg and 4.89 mg/kg in the dumpsite soil and control respectively. All the heavy metals were significantly ($P<0.05$) different and higher in the dumpsite soils than the control. Lead, Cd, Zn and Cu had mean values of 24.546 mg/kg and 34.92 mg/kg, 0.19 mg/kg and 0.013 mg/kg, 107.42 mg/kg and 1.52mg/kg and 0.43mg/kg and 0.606mg/kg in earthworm sampled from dumpsite and earthworm sampled from the control correspondingly. Lead level in earthworms of the dumpsite and control was not significantly ($P>0.05$) different, Cd and Zn were significantly ($P<0.05$) different while Cu was not significantly different. Strong and positive relationship was observed between Cd and Zn level in dumpsite soils and Cd and Zn concentration in earthworm while reverse observation was obtained for Pb and Cu. Positive correlation between Cd and Zn concentration in earthworm of dumpsite and Cd and Zn concentration in soil of dumpsite was attributed to the ability of earthworm to sequester heavy metals.

Cadmium, Pb and Zn concentration in dumpsite soil was far above the maximum permissible limits of heavy metals in soil recommended by recognized authorities while Cu was within the maximum permissible limit in both dumpsite and control. It was also observed that Cd and Zn were far bioaccumulated than Pb and Cu by earthworm. For cultivation and safer consumption of crops from Lemna dumpsite in Calabar, remediation of heavy metal content of the soil is recommended.

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