

Bioaccumulation Potential of Heavy Metals in *Lumbricus Terrestris* and Associated Soils in Municipal Open Dumpsites Uzoka Christopher Ndubuisi¹, Ibe Colman Chikwem², Egbuawa Irene Ogechi¹, Ezikeudu Chinonso Eric¹, Nwauhiara Michael Obinna¹

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ABSTRACT

Heavy metal concentrations in Earthworm (Lumbricus terrestris) and in soil samples from three domestic dumpsites located in Owerri, Nigeria were measured spectrophotometrically . Mean metal concentration in dumpsite soils were, 2.02-2.94mg/kg, 7.15-13.45mg/kg, 42.27-52.27mg/kg, 42.51-53.44mg/kg, 1.62-2.84mg/kg, 24.61-36.74mg/kg, 2.74-5.56mg/kg, and 3.12-4.52mg/kg for Cu, Pb, Fe, Zn, Cd, Mn, Cr, and Ni respectively. The mean concentration range recorded in Lumbricus terrestris were, 1.51-2.09mg/kg for Cu, 5.64-9.36mg/kg for Pb, 28.44-35.61mg/kg for Fe, 27.34-39.01mg/kg for Zn, 1.21-2.14mg/kg for Cd, 18.36-21.12mg/kg for Mn, 1.96-3.43mg/kg for Cr, and 2.02-3.26mg/kg for Ni. Control samples of soil and Lumbricus terrestris collected from an area devoid of municipal waste dump were also analysed for these heavy metals. Control soil sample values were 0.62mg/kg, 2,06mg/kg, 5.96mg/kg, 5.43mg/kg, 0.24mg/kg, 4.99mg/kg, 1.25mg/kg and 0.94mg/kg for Cu, Pb ,Fe, Zn, Cd, Mn, Cr, and Ni respectively while Lumbricus terrestris had the values of 0.41mg/kg, 1.37mg/kg, 3.97mg/kg, 5.25mg/kg, 0.15mg/kg, 2.99mg/kg, 0.83mg/kg, and 0.62mg/kg for Cu, Pb, Fe, Zn, Cd, Mn, Cr and Ni respectively. The biota-to-soil accumulation factors (F) calculated were all less than unity for all metals while the order of bioaccumulation of the metals both in soil and in Lumbricus terrestris followed the trend Zn>Fe>Mn>Pb>Cr>Ni>Cu>Cd. Since reptiles, birds and some other vertebrates feed on Lumbricus terrestris, transfer of these metals across the food chain is most likely. Therefore proper waste treatment before disposal in the city of Owerri should be enforced. Keywords : Bioaccumulation, Heavy Metal, Lumbricus Terrestris, Soil, Open Dumpsites

I. INTRODUCTION

Heavy metals are essential for optimum crop production, they can also act as toxicants to soil and crops at elevated concentrations. Heavy metals such as Mn, Ni, Cd, etc are required at micro level for optimum crop performance and in most cases they play key roles in the growth and development of crops (Mustapha and Loks 2005). At high concentration, the same metals can result to stunted growth of crops (Rosen, 2002) and consequently adverse effect on the final consumer of these crops. Beside the land discharge of chemical waste which is usually associated with heavy metals, the major pathways of heavy metal into the soil are mainly through application of compost and chemical fertilizer (Kaonga et al, 2010). There was also evidence that heavy metals could enrich the agricultural soil and pose environmental and health risks of the plant through plant uptake of the

discharged waste water and sludge (Onweremadu, 2008). Runoff containing heavy metals from solid waste dump sites are deposited onto sediments, variations in characteristics of these heavy metals are evident in their varying mobility rates and distributions. In a study of evaluation of heavy metals in core sediments of southeast of India, there were heavy presence of lead, cadmium, and zinc at the bottom layer of the sediments whereas chromium and copper dominated the upper layer. There was also a measure of variability in the heavy metal distributions within the soil matrix. Mustapha et al (2010) observed that values of extractable Zn, Cu, Fe, and Mn were predominant in the respective depth of 0.48-0.75, 0.18-0.26, 18.40-21.91, and 30.54-38.58 respectively of a natural sandy loam soil of northern Nigeria. The apparent distribution variation observed was as a result of different soil physical, chemical and biological properties, In their

study they observed that high accumulation of heavy metals affected adversely the biological and physicochemical status of the soil, These manifested in low soil carbon mineralization rate, impairment of respiration of soil microbes and low soil microbial biomass carbon. In the physico-chemical sense, heavy metal concentration upsets the pH, salinity and electrical conductivity (EC) balance of the soil (Sanchez et al, 2007). Beside the effects on the physico-chemical properties of the soil, bio-accumulation of heavy metals on certain invertebrates has been recorded and could be a reliable indicator of heavy metal toxicity of the environment (Alloway and Ayres 1997) and owing to the ecological importance of Lumbricus terrestris in most temperate and tropical soils, it can be used as bio-indicators to assess the bioavailability of heavy metals in terrestrial environment (Onweremadu, 2008).

Earthworm (Lumbricus terrestris) constitute a major component in soil functioning and plays an important role in chemical element transformation. It utilizes a significant amount of soil organic matter for feeding, produce huge amount of biogenic structures and determine the activities of micro-organisms and other smaller invertebrates (Lavelle 1997). The influence of heavy metals in soils on earthworms and their bioaccumulation has been subject of many studies. According to Ireland (1983) and Bamgbose et al (2000) earthworms can accumulate in their tissues heavy metals from the environment. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted, (Gilbert et al 2006).

The use of earthworm as a bio-indicator for soil pollution was shown by Morgan and Morgan (1998). Stafford and Mc Grath (1986) also reported positive correlation between earthworm and total soil Cu, Pb and Zn concentration from various metal contaminated sites. Metal bioavailability to earthworm can be evaluated both in terms of relative toxicity and through bioaccumulation determination yielding a Biota-to-Soil Accumulation Factor (BSAF), (Cortert and Weiss 1999). Agbarie and Emoyan (2012) carried out a study on Heavy metal bioaccumulation by *Lumbricus terrestris*,

the study confirms that earthworms accumulated some amount of heavy metals from domestic dumpsite soils and so can be used as a bio-indicator for pollution. They also discovered that the availability of metals in soils was influenced by the soil pH and the soil organic matter accounting for the variation in metal concentrations from one site to the other. In a study by Atulegwu et al (2013), on modeling lateral distribution of Heavy metal and bio-accumulation in Earthworms in varying acidic surface horizon of waste polluted soil, showed that heavy metal distribution in the background unit was natural and typical of natural soil-formation while that of the polluted site showed apparent heavy metal pollution with the metals concentration distribution decreasing along the gradient of decreasing pollution of the site. It was also noted that the earthworms bio-accumulated more of the lead and cadmium.

Uba *et al* (2009), carrying out a study on content of Heavy Metals in *Lumbricus terrestris* and associated soils in dump sites, confirms that earthworms accumulated some amount of heavy metals from dumpsite soils and levels of these metals accumulated in the earthworms tissues were less than unity for Cd, Cu, Pb and Mn while the ratio was higher than unity for Zn metal. Bamgbose *et al* (2000), in a study on the physicochemical properties and heavy metal accumulation in contaminated and uncontaminated soil, shows that the concentration of heavy metals influences the pH and organic matter of the soil. Dumpsites usually contain various kinds and concentrations of heavy metals, which is dependent on the age, location and type of waste, (Ebong *et al* 2007).

To establish a bio-monitoring system using earthworms, the effects of various chemical pollutants on earthworms have been studied. Among metals, methyl mercury might be more easily absorbed by and accumulated in earthworms, suggesting that the earthworm is an ideal candidate for monitoring methyl mercury (Lee *et al* 2009). He also mentioned that metal bioaccumulation by earthworms could be used as an ecological indicator of metal availability. Natal-de-Luz *et al* (2011) investigated the effects of sludge contaminated with chromium, copper, nickel, and zinc, and soils freshly spiked with the same mixture of metals, on *Eisenia andrei*. They detected a decrease in the metal bioavailability for earthworms, promoted by the high organic matter content of the sludge. The binary mixture effects of cadmium and zinc on the mortality of Aporrectodea caliginosa were also investigated by (Qiu et al 2011). They reported that the effects of the cadmium and zinc mixtures on the mortality of Aporrectodea caliginosa were mainly antagonistic, and the magnitude of the antagonism was dependent upon both the relative concentrations of cadmium and zinc and their concentration magnitudes. The effects of combinations of metals and other chemical agents, such as pesticides, on earthworms were also investigated. Lister et al (2011) studied the effects of a binary mixture of nickel and chlorpyrifos, an organophosphate insecticide, on Lumbricid earthworms, and found that both chemicals were rapidly accumulated to equilibrium. Although the nickel uptake followed the same pattern as the single chemicals, the rates of chlorpyrifos uptake and elimination were faster, suggesting that a mixture of chemicals in soil might enhance the toxicity to organisms. This research therefore further examines the potentials of Lumbricus terrestris in the bioaccumulation of heavy metals found in open dumpsites.

II. METHODS AND MATERIAL

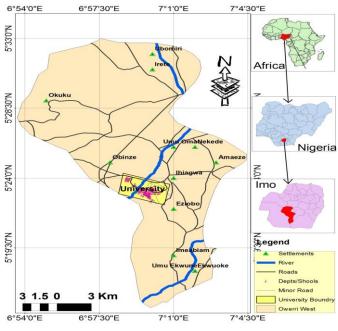


Figure 1. Map of the Study Area

SAMPLE COLLECTION: Soil samples from three domestic dumpsites namely: Old Nekede, Naze, and Obinze were used. A control sample was also collected from a point that is devoid of a dumpsite in the premises

of Federal University of Technology Owerri. The areas used for sampling in each site were divided into four quadrants, (Nuonamo *et al*, 2002). At each sampling site the surface debris was removed and the subsurface soil dug to a depth of between 0-20cm with a hand soil auger. About 50g of soil sample from each site was taken into a nylon bag and labeled. For earthworms (*Lumbricus terrestris*) they were collected by digging into the soil within the quadrant and then placed in sample bottles and labeled.

PREPARATION AND PRESERVATION OF EARTHWORM SAMPLES:

The earthworm samples were placed in Petri dishes, then refrigerated for 24hrs in order to purge the soil in the gut; thereafter they were removed, rinsed slightly with deionized water and then frozen pending analysis, Bamgbose *et al* (2000).

PREPARATION AND PRESERVATION OF SOIL SAMPLES:

The procedure employed in soil sample preparation and preservation include,

Oven Drying: Freshly collected soil sample are often moist, the first step in preparation of soil sample is to dry with air or oven-dry as soon as possible to halt all biological transformation activities. The soil samples were spread out on a flat surface sheet of paper or polyethylene bag and left in the oven at the temperature of 105°C for three days to fully dry all moisture present.

Sieving of soil samples: This was done to remove gravel, stones and plant debris. The materials that are able to pass through the 2mm mesh ordinarily called "fine earth materials" were selected and kept for analysis.

HEAVY METAL DETERMINATION IN SOIL AND EARTHWORM: 3g of the thawed earthworm sample was digested with 2ml concentrated nitric acid and heated to dryness on a hotplate. The digest was then re-dissolved in 1ml concentrated nitric acid, after which it was made up to 50ml with distilled water. For the soil samples, 5g of the sieved soil samples were weighed and 10ml concentrated nitric acid added. In beaker the mixture was covered with a watch glass and refluxed for 45 mins. The watch glass was then removed and the content evaporated to dryness. 5ml aqua regia was added and evaporated to dryness after which 10ml of 1M nitric acid was added and the suspension filtered. The filtrate was then diluted to volume with distilled water in a 50ml volumetric flask. Triplicate digestion of both soil and earthworm samples was carried out with samples labeled A, B and C. The digested extract was used to determine the heavy metal concentration using Atomic Absorption Spectrophotometer (AAS).

pH DETERMINATION:

20g of the air dried soil sample was weighed into a 50ml beaker and 20ml of distilled water added. The mixture was allowed to stand for 30min. with occasional stirring with a glass rod. Using a pH meter (ATC pH meter HI 8915) the pH reading of the partly settled suspension was noted. The result was recorded as the soil pH in distilled water.

ORGANIC MATTER DETERMINATION:

The preserved soil samples were ground and sieved using 0.5mm sieve, after which they were weighed in duplicate and transferred to a 250ml Erlenmeyer flask. Exactly 10ml of 1M potassium dichromate was pipette into each flask and swirled gently to disperse the soil. This is followed by addition of 20ml concentrated sulphuric acid. The flask was swirled gently until soil and reagents were thoroughly mixed. The mixture was then allowed to stand for 30minutes on a glass plate. 100ml distilled water was later added, followed by addition of 3-4 drops of ferroin indicator. This was titrated with 0.5N ferrous sulphate solution. The organic matter value is determined by multiplying the value of Soil Organic Carbon (SOC) by a factor 1.729. This is known as WALKLEY-BLACK WET OXIDATION METHOD of organic matter determination.

Percentage (%) Organic carbon in soil is calculated as follows.

Percentage organic carbon = $(Meq K_2Cr_2O_7 - Meq feSO_4) \times (0.003 \times 100 \times F)$

Mass (g) of soil used

Where: F = correction factor = 1.33

Meq = normality of solution \times ml of solution used.

To obtain % organic matter in the soil = organic carbon (C) x 1.729.

ESTIMATION OF BIOTA TO SOIL ACCUMULATION FACTOR (f):

Biota to Soil Accumulation factor = Mean of Heavy metal concentration in Earthworm Mean of Heavy metal concentration in the soil.

III. RESULTS AND DISCUSSION

RESULTS

S/N	SAMPLING SITE	Cu	Pb	Fe	Zn	Cd	Mn	Cr	Ni
	~	2.0.1.0.00	715.000	40.07.0.01	10 51 0 00	0.04.0.02	24 61 0 05	2 22 0 02	2.12.0.05
1	NEKEDE	2.94 ± 0.09	7.15 ± 0.06	42.27±0.01	42.51±0.09	2.84 ± 0.03	24.61±0.05	3.22 ± 0.03	3.12 ± 0.05
2	OBINZE	2.10 ± 0.03	12.63±0.06	43.19±0.01	53.44±0.01	1.62 ± 0.03	32.84±0.09	5.56 ± 0.03	4.52 ± 0.07
3	NAZE	2.02 ± 0.03	13.45 ± 0.05	52.27±0.01	51.74 ± 0.11	2.73 ± 0.09	36.76±0.09	2.74 ± 0.04	3.98 ± 0.07
4	CONTROL	0.62 ± 0.01	2.06 ± 0.03	5.96±0.03	5.43±0.01	$0.24{\pm}0.05$	4.99 ± 0.02	1.25 ± 0.04	$0.94{\pm}0.01$

 Table 1. Heavy metal concentration of soil samples (mg/kg)

 Table 2. Heavy metal concentration in Lumbricus terrestris (mg/kg)

S/N	SAMPLING SITE	Cu	Pb	Fe	Zn	Cd	Mn	Cr	Ni
1	NEKEDE	2.09±0.03	5.64 ± 0.02	28.44±0.01	27.34±0.02	2.14±0.01	18.36±0.01	1.96±0.02	2.02±0.01
2	OBINZE	1.87 ± 0.03	9.36±0.02	31.07 ± 0.03	34.06 ± 0.02	1.21 ± 0.01	21.04 ± 0.01	3.45 ± 0.01	3.26±0.03
3	NAZE	1.15 ± 0.01	9.15±0.03	35.61 ± 0.03	39.09 ± 0.05	1.95 ± 0.02	21.12 ± 0.01	1.72 ± 0.02	2.86 ± 0.03
4	CONTROL	0.41 ± 0.01	1.37 ± 0.01	3.97 ± 0.02	3.25 ± 0.01	0.15 ± 0.01	2.99 ± 0.02	0.83 ± 0.02	0.62 ± 0.03

Table 3. Values of pH and organic matter of the soil.

S/N	SAMPLING	pН	ORGANIC
	SITE		MATTER %
1	NEKEDE	5.1±0.04	3.64±0.02
2	OBINZE	4.3±0.02	3.82±0.01
3	NAZE	5.2 ± 0.05	4.47 ± 0.01
4	CONTROL	6.6 ± 0.05	1.28 ± 0.03

TABLE 4. Biota to Soil Accumulation factor (BSAF)

S/N	SAMPLING SITE	Cu	Pb	Fe	Zn	Cd	Mn	Cr	Ni
1	NEKEDE	0.71	0.78	0.67	0.64	0.75	0.74	0.60	0.64
2	OBINZE	0.89	0.74	0.71	0.63	0.74	0.64	0.62	0.72
3	NAZE	0.74	0.68	0.68	0.75	0.71	0.57	0.62	0.72
4	CONTROL	0.66	0.66	0.66	0.59	0.62	0.59	0.66	0.65

DISCUSSION

From Table 3, the mean pH values of soil from the dumpsites falls within 4.3-5.2 while that of the control is 6.6. The result shows that the pH of the soil from the dumpsites is more acidic indicating the effect of solid waste on the soil. Heavy metals are said to be mobile under acidic conditions, (Alloway 1996). Banjoko and Sobulo 1990 have reported similar figures for some Nigeria soils especially from the forest region. Correlation between soil pH and micronutrients availability has also been reported as evidenced by the works of Joshi et al (1983), Sharma et al (2003) and Akinrinde et al (2005). While Uba et al (2009) observed a moderately lower acidic pH range of 4.2-5.1. Atulegwu et al (2013) reported a more acidic pH range of 3.5-4.1 lower than that obtained from the study, the variation could be as a result of the nature of waste disposed in the dumpsite. Results from Atulegwu et al (2013) showed high concentration of Ni, Cd and Pb, these are major compositions of motor battery waste.

Soil organic matter acts as a "storehouse" for many of the Heavy metals; it therefore influences micronutrient availability through chelation. The organic matter content of the soil from the dumpsites falls between the values of 3.64 to 4.47% while that of the control site is 1.28%, the study therefore shows an increased organic matter with variations in each of the dumpsite. This variation could be as a result of the age of the dumpsite. Ahm 1970 have reported that a good forest top soil

contain between 5-7% organic should matter. Meanwhile a positive correlation between some micronutrients availability and organic matter has been shown by other researchers (Sharma et al 2003, Akinrinde et al 2005, Li et al 2007). Bamgbose et al (2000), Agbaire and Emoyan (2012) and Uba et al (2009) have reported values of organic matter content with the range of 3.66-4.43%, 1.97-4.24% and 3.82-5.63% respectively of different solid waste dumpsites. The variation is indicative of differences in decomposition and degradation processes of the waste materials in the dumpsites. These processes have a strong positive correlation with the age of the dumpsites studied.

The profile of the Mean heavy metal concentrations in dumpsites are Zn>Fe>Mn>Pb>Cr>Ni>Cu>Cd the (Table 1). Comparing the results obtained with that of the control site it could be observed that there is an appreciable degree of accumulation of heavy metals in the soil from municipal solid waste, the concentration of each metal being dependent on the composition of the wastes. The profile of mean heavy metal concentration as obtained by Agbarie and Emoyan (2012), Atulegwu al (2013) and Uba et al (2009) are et Mn>Zn>Pb>Cr>Fe>Ni, Ni>Cd>Pb>Zn>Mn>Cu>Cr, and Zn>Pb>Cu>Cd >Mn respectively. Significant differences do exist from the result of the present study and what is obtained by these researchers. The variation is indicative of the difference in the composition of the waste.

This study has indicated that Earthworms bioaccumulate heavy metals in their tissue having the soil as its habitat, they feed on the debris of dead plants and animals which are components of the soil organic matter, the degree of bio-accumulation of these heavy metals by Earthworm is dependent on the degree of accumulation in the soil. The accumulation in Earthworm samples maintains the same profile as mean heavy metal concentration of the soil from the dumpsites i.e. Zn>Fe>Mn>Pb>Cr>Ni>Cu>Cd. This trend is similar to the result obtained by Agbarie and Emoyan (2012), Atulegwu *et al* (2013) and Uba *et al* (2009).

From table four, the transfer factors were all less than unity in all the dumpsites studied. Generally levels of all the metals analyzed from dumpsite samples were higher than those in *Lumbricus terrestris*; this was not unexpected since soil has been described as reservoir of pollutants (Onyari *et al* 2003). Mineralization of dead earthworms releases accumulated heavy metals back to the soil (Morgan and Morgan 1988a). According to Ma *et al* (1983), the amount of metals accumulated within earthworm tissues is partly dependent on the absolute concentration of metal within a given soil and the physico-chemical interactions.

IV. CONCLUSION

Heavy metal pollution of the soil and Ecosystem has become something of a global concern, this is as a result of its adverse effect on living organisms (plants and animals) when they come in contact with these metals. They tend to be toxic and of a detrimental effect, this can be seen as a result of its ability to bio-accumulate in the body of living organisms thus interfering with the food chain. Macroorganisms such as *Lumbricus terrestris* has been used as an entity to justify this. This study therefore confirms the potentials of *Lumbricrus terrestris* to accumulate heavy metals from soils of domestic open dumpsites and so can be used as a bioindicator for pollution studies.

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