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Trace metal concentrations in genetically dissimilar earthworms: (*Aporrectodes longa*: Ude, *Libyodrillus violaceus*: Beddard) from municipal dumpsites in Benin-City, Nigeria

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ABSTRACT: The study explored the use of earthworms as sentinels for biomonitoring heavy metal contaminations in the terrestrial environment. Steady state metal concentrations (Mn, Zn, Fe, Cu, Pb, Cd and cr) were measured in genetically dissimilar earthworms (*Aporrectodea longa:* Ude, *Libyodrillus violaceus:* Beddard) and soil samples from contaminated site also located in ancient city of Benin, Nigeria. Generally metal concentrations in the earthworms were higher than levels in the soil. Significant interspecific differences occurred in the metal concentrations in the biotaand soil and amongst the study sites ($P< 0.05$, df16). The pattern of metal concentrations in earthworms reflected the pattern in the soil. (Mn > Zn > Fe > Cu > Pb > Cd > Cr) Bioconcentration also depended on the concentration of metal in soil with bioconcentration factor being greater at lower soil concentrations. *L. violaceus* appears to be a better sentinel, accumulating more metals than *A. longa* in all sites. Voiding time played vital role as an experimental variable in levels of metals in earthworms, Zn, Cu, Pb, CD, Mn increased as voiding time increa/sed (tissue sequestration) while Fe and cr decreased with increase in voiding time (non-tissue sequestration). The bioavailability of metals in soil samples were also co-determined by soil texture. Reduced metal uptake and availability was observed, in soil with high clay content, since metals remained tightly bonded to clay site.

Keywords: Earthworm, metals, biokinetics, dumpsites, soil.

Introduction

Increasing incidence of indiscriminate dumping of municipalk solid waste (MSW) on land has raised concern over the increase in metal pollution in the affected soil. Possible infiltration of potentially toxic metals into the adjoining terrestrial and aquatic environments through runoffs has also been documented (Ezemonye and kadiri, 2000), which implies resultant bioaccumulation of metals in soil biota and metal enrichment along trophic levels (Ezemonye, 1992; Neushanser, *et al*., 1995). The above concern has necessitated the use of geochemical methods, such as sequential or selective chemical extraction, for assessing these ecological problems and provide indirect evidence on the availability of metals in organisms (Perez *et al*., 1998; Sandoval *et al*., 2001).

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Earthworms are known to be major component of soil biota and wildlife food chain. They have been described to be particularly suitable for the assessment of contaminants bioavailability. They are proven metal accumulators and are in full contact with the substrate they consume (Edwards and Bohlen, 1992; Aziz *et al*., 1999; Reinecke, *et al*., 1999; Vijver, 2001). Uptake of substantial levels of metals by earthworms from their surrounding environment have been well documented (Ireland, 1979; Wright and Stringer, 1980; Sandoval *et al*., 2001). Voiding of gut contents in earthworm can affect the proportion of total ash contributed to the earthworm tissue by soil present in gut. Voiding time have been observed to have effect on the final earthworm metal concentrations. This is dependent on the type of metal and degree of tissue sequestration (Neuhauser *et al*., 1995).

In the ancient city of Benin, Nigeria indiscriminate dumping of untreated municipal solid waste abound. At Uwelu and Evbareke suburbs in Benin metropolis, solid wastes dumpsites exist. These dumpsites contain various automobile scraps, metals and steel scraps, deposition of spent lubricants and solid wastes from electrical, automobile and iron smelters workshops. Thus heavily contaminating the surrounding soil with associated heavy metals. Since earthworms are known to consume a significant amount of dirt from dumpsite (Edwards and Lofty, 1997), where they are capable of inhabiting for a long period of time (Stafford and Mcgrath, 1986; Bambose *et al*., 1999), this study is therefore designed to further evaluate the suitability of earthworms as sentinels for biomonitoring heavy metals in municipal waste dumpsites.

The present survey, focused on the evaluation of the impact of municipal waste dumpsites on heavy metal concentrations in two genetically dissimilar species composing the lumbricid community close to the sites. Lubricids from less polluted "control" sites were sampled and voiding experiment complemented the assessment.

Materials and Methods

Study area and sample material

Soil and earthworm samples were collected from two dumpsites at Uwelu and Evbareke and a control uncontaminated sites at the Ugbowo campus of the University of Benin, Nigeria with similar soil type. Polluted sites [1} (Uwelu) and [2] (Evbareke) are dumpsites contaminated with automobile scraps, non-metals, steel scraps, spent oil lubricants, grease, burnt and condemned tyres (see Fig. 1). Chemicals from automobile, electrical and iron smelters workshops are also deposited at these sites.

Soil and earthworm collections were carried out according to methods described by Terhivuo *et al* (1994) and Spiegel (2202), respectively. The earthworms were extracted by digging and hand sorting into plastic labeled bags from subsurface litters. They were taken to the laboratory for identification, counting and weighing. Before weighing they were washed free from adhering soil particles and then left to dry on absorbent filter paper for about 1min. The earthworms were allowed to void their gut content for 24hrs in a petric dish with moist filter paper at 27°C (room temperature) after which they were washed with distilled water and frozen pending analysis. Soil samples were also collected at each of the dumpsite and the control site with the aid of a plastic spoon and transferred into celophene bags.

Erathworm samples identification

Earthworm species common to all the three sites were *A. longa* (Ude) and *L. violaceus* (Beddard). They represent epigeic (or macrophagous, litter-dwelling) species which live among litter and or close to the surface. The nomenclature adopted is according to Sims and Gerrard (1985) and Nature Watch, 2003.

Sample Identification

Aporrectodea longa: Colour:- red-violet with dark anterior segments; length: 9cm-12.3cm clitellum:- saddle shaped and non-flared; prostomium:- prolobous; Number of segments: 153-164; Tubercular Pubertis: bar-shaped; genital Tumescence: alternating in paris (nature watch, 2003).

Libiodrilus violaceus: Colour: red-violet; length: 9.2cm – 12cm; clitellum: annular and non-flared, occupying XIV to XVI segments; body segment:-triannula and numbers 130-171; tubercular pubertalis: absent, anterior segments triannulate (Nature Watch, 2003).

Chemical and Statistical Analysis

Soil samples were air dried, thoroughly mixed and sieved through a 4mm mesh to remove rocks and plant debris. Sub-samples of the pre-treated soil were used to obtain data for soil textural composition and pH using standard methods. Soil pH was determined in 1:1 soil/water suspension with a combination of glass electrode pH meter as described by Folsom *et al* (1981) and Bambose *et al* (1999). 20g pre-treated soil sample was weighed into a 50ml beaker and 20ml of water added. The mixture was allowed to stand for 30mins stirring occasionally with a glass rod. The electrodes of the calibrated pH meter were inserted into the suspension and the pH of the soil measured:

Soil texture was determined according to the method of Bouyoucos (1928) earlier described by day (1965) and was classified by the soil survey manual USA method (USDA, 1951). Textural Classes were obtained through particle size distribution pattern and texture analysis expressed as % Clay, % Silt, % Sand. This involved the dispensing of soil with sodium hexametaphosphate (algon) and taking hydrometer and temperature readings at specific intervals.

The soil samples for heavy metal analysis were pre-treated by allowing it to dry at room temperature and passed through 2mm sieve. 5g of sieved soil sample weighed and 10ml of concentrated nitric acid was added. In a beaker covered with a watch glass, the mixture was refluxed for 45 mins. The watch glass was then removed and the content of the beaker evaporated to dryness, 5ml aqua regia was added and evaporated to dryness after which 10mml of 1M nitric acid was added and the suspension filtered. The filtrate was diluted to volume with distilled water in a 50ml flask. This was followed by the determination of heavy metal (Mn, Zn, Fe, Cu, Pb, Cd and Cr) using flame atomic absorption Spectrophotometry duly calibrated with authentic standards.

Heavy Analysis in Earthworms

Erathworms were allowed to void their gut content for 24hrs in a Petri dish with moist filter paper at 27°C. They were washed with distilled water, killed by freezing and finally rinsed with distilled water before analysis for heavy metals.

The methods for heavy metal analysis are as described by bambose *et al* (1998). Pre-treated earthworms were weighed and 3g of earthworm samples digested with 2ml concentrated nitric acid and heated to dryness on a hot plate. The digest was re-dissolved in 1ml concentrated nitric acid and made up to 50ml with distilled water. The heavy metal analysis for Mn, Zn, Fe, Pb, Cu, Cd and cr was carried out with flame atomic absorption spectrophotometry.

Voiding Experiment

Voiding experiment was conducted on the *A. longa* species, which is the most abundant in the study areas. Worms collected from site [2] (Evbareke) were used to determine the effect of voiding time on earthworms metal content. They were analyzed for metal content on the first day (day 0) and also after 1,2, and 3 days of voiding. The worms were allowed to void their gut content ina petric dishes with moist filter paper at 27°C (room temperature). The filter papers were changed daily. Two sub-samples of 15 worms each, were analyzed for metal concentrations in each of the four voiding days.

The student t-test was applied to test the validity of the Null hypothesis on the concentration of earthworms and soil samples. The single factor analysis of variance (ANOVA) was used to test for significant difference between the metal concentrations in three sampling locations for both soil and earthworms. Where significant difference occurred, the post – Hoc test (Duncan Multiple Range Comparison) was used to locate sources of difference.

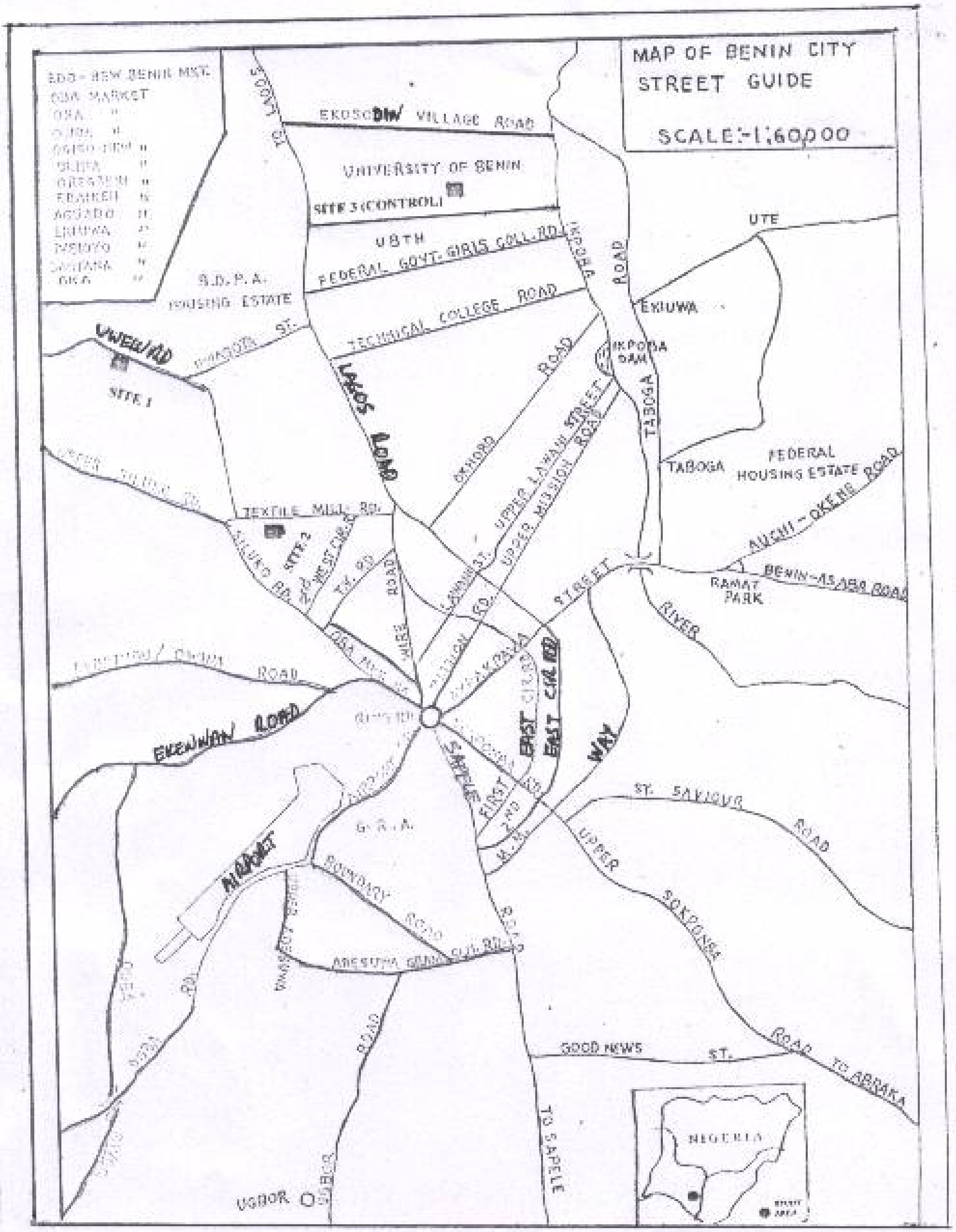


FIG. 1: MAP OF BENIN – CITY SHOWING THE SAMPLING STATIONS
(MAP OF NIGERIA IS INSERTED)

Results

Soil pH has been reported as a significant parameter excerting controlling influence on the bioavailability of trace metals in the soil. The pH values of soil from contaminated sites had mean values of 6.34 (Uwelu) and 5.91 (Evbareke) while the control site at the Ugbowo campus of the University of benin had a mean of 6.25 (Table 1). The pH were generally acidic with that of Evbareke been slightly lower.

Soil texture, a reflection of the gravilometric composition of the soil, showed that the soils were generally composed of sandy, silt and clayey formations. The Uwelu (Site 1) presented a 70% sand, 8.50% silt, and 21.33%clay, Evbareke (Site 2) had 30% sand, 3.80% silt, and 66.30% clay, while the control (Site 3) presented a similar composition as Uwelu, 60.10% sand, 10.10% silt, and 29.80% clay (Table 1). Site 1 and 3 were predominantly sandy soil, while Site 2 was more of clayey soil.

Concentration of heavy metals in biota and soil

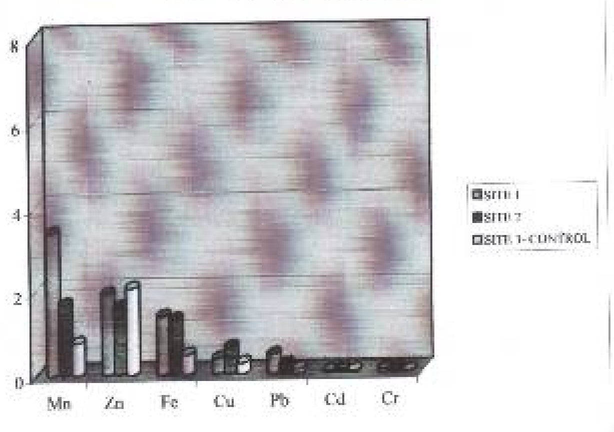
The results obtained for the levels of accumulated metals in biota and soil from contaminated and uncontaminated sites are shown in Table 1, 2 and 3, with further illustrations in Fig. 2 – 6.

Fig. 2 shows the levels of metals in soils in the three sites. The general trend shows that Mn and Zn were highest while Cd and Cr were the least. Within the exception of Zn the soil of the contaminated sites had higher levels of most metals than the control sites. Again amongst the contaminated sites with the exception of Cu, the soil Site 1 had more metal concentrations sites with the exception of Cu, the soil of Site I had more metal concentrations than Site 2. The general trend in the soil was Mn > Zn > Fe > Cu > Pb > Cd > Cr.

The levels of metals in the earthworm showed some variations in the metal concentrated. In nearly all metals the concentrations in both earthworms (*A. longa* and *L. violaceus*) were higher than those of the soil samples as shown in Figure 3. The result of the student t-test comparison of mean metal concentrations in the soil and earthworm showed that the metal concentrations were significantly different at $P < 0.05$, df 16.

The levels of metals in the earthworms from the contaminated sites were higher than that from the control (Figure 4). The Post hoc (Duncan Multiple Range test) showed that significant difference between and within earthworms and soil metal concentrations was observed at $P < 0.05$, df 16. mean metal concentration of earthworms and soil in the polluted sites were different from the control site while the concentrations of the polluted Sites were similar. This was so far for most metals except Zn.

FIGURE 2
HEAVY METAL CONCENTRATIONS (µg/g) IN THE SOIL OF THE THREE (03) STUDY SITES



The trend in the metal levels in the earthworm was a reflection of the soil metal concentration (Mn > Zn > Fe > Cu > Pb > Cd> Cr). The comparison of levels of metals in both earthworms are reflected in Figure 4. *L. violaceus* concentrated more metals than *A. longa* in all sites with the exception of Cr.in Site 3.

In both earthworm and soil samples comparatively Zn was highest in Sites 1 and 3 while Mn was highest in Site 2 (Fig. 3). In all sites, Cd and Cr were the least. The essential metals (Mn, Zn, Fe, Cu) were higher than the toxic metals (Pb, Cd, Cr). Understandably, *L. violaceus* bio-concentrated more metals than *A. longa* with the exception of Cr at Site 3 (See Figure 5). With the exception of Zn, *A. longa* bio-concentration factor (BCF) was higher in the control site than the contaminated sites. *L. violaceus* showed a different pattern in the bio-concentration factor (BCF), where BCF of essential metals (Fe, Mn and Cu) were higher in the control sites. The contaminated sites had higher BCF for toxic metals (Cd, Pb and Cr) than the control site.

The results of the voiding studies are summarized in Table 4, using the mean values for the replicates on each day. Levels of Zn, Cu, Pb, Cd and Mn that are bio-concentrated in the earthworm (*A. longa*) increased as voiding time increased while levels of Fe and Cr decreased.

Table 1: Soil pH, texture and mean concentrations in the three sampling locations (µg/g)

Sample Site	pH	Soil texture%	Mn	Zn	Fe	Cu	Pb	Cd	Cr
Polluted Sites									
Site A (Uwelu	6.34	Sand, 70.15 Silt, 8.50 Clay, 21.35	3.39	1.93	1.38	0.37	0.45	0.04	0.08
Site B (Evbereke)	5.91	Sand, 30.00 Silt, 3.80 Clay, 66.22	1.69	1.62	1.31	0.64	0.21	0.07	0.04
Control Site									
Site C (Ugbowo Campus)	6.25	Sand, 60.10 Silt, 10.10 Clay, 29.80	0.76	2.06	0.47	0.26	0.01	0.04	0.02

Table 2: Mean total concentration in earthworm (*Aporrectodea longa*) from contaminated and control sites (µg/g)

	Mn	Zn	Fe	Cu	Pb	Cd	Cr
Polluted Sites							
Site A	6.98	4.10	2.63	1.13	0.45	0.72	0.19
Site B	3.54	4.13	1.95	1.32	0.41	0.20	0.07
Control Site							
Site C	1.40	3.77	1.40	0.58	0.38	0.12	0.06

Table 3: Mean metal concentrations in earthworm (*Libyodrilus violaceus*) from contaminated and control sites (µg/g)

	Mn	**Zn**	**Fe**	**Cu**	**Pb**	**Cd**	**Cr**
Polluted Sites							
Site A	10.77	11.21	5.21	2.89	1.26	1.10	0.23
Site B	10.58	7.17	5.13	1.93	0.87	0.79	0.16
Control Site							
Site C	5.53	7.10	2.50	1.57	0.43	0.33	0.03

Table 4: Voiding experiment (*Aporrectodea longa*)

	Mn	**Zn**	**Fe**	**Cu**	**Pb**	**Cd**	**Cr**
V_o (day 0)	1,23	2.71	1.50	0.80	0.52	0.16	0.18
V_o (day 1)	1.06	2.97	1.38	0.69	0.43	0.13	0.11
V_o (day 2)	1.59	3.43	1.56	0.96	0.74	0.28	0.21
V_o (day 3)	1.41	3.16	1.44	0.87	0.64	0.28	0.13

Discussion

The pH of the soil was generally acidic for both contaminated and control sites. This is typical of pH values reported for the soil in Benin City (Oguzie, 1998). The acidic nature of the soil may have influenced the adsorption of complexed metals into ionic forms thereby leading to enhanced bio-availability. (Graney *et al*., 1983; Bambose *et al*., al, 1999 and Sposito, 1992) reported pH values as high as 10.10 (Ita – Oshin and Iberekodo dump sites) and 7.8 (Dimino composite site) respectively suggesting that waste contaminated soil have relatively high pH values. However, the low pH values reported in this study further suggest that in addition to nature of waste material, soil composition or type may also influence the pH values.

Metal bio-availability is very much related to local situation with respect to soil texture (Marinussen, 1997). The contaminated soil, with predominant sandy texture had more bio-available metals except for Cu (Site 1) while in Site 2 where clay was predominate only Cu was higher. Cu is known to have a very strong affinity with clay (Spiegel, 2002). This may have contributed to the enhanced level of Cu in site 2. In terms of actual exposure concentration experienced by the biota, Site 1 had more while Site 2 (clayey) had lower values. Clay has been associated with very strong binding character which makes metals less bio-available, (Ezemonye, 1992). This further supports the influence of gravilometric properties on bio-availability of heavy metals in the soil.

Generally, concentration of metals in the earthworm varied slightly with metals exhibiting evidence of metal specificity earlier reported. The same species of biota have been reported to behave differently to a particular metal in the same location (Murphy *et al*., 1978). Landner (1986) observed that different species react differently to the same metal. The difference in concentrations observed in the earthworms may be due to varying capacity of metabolic regulation for both toxic and essential metals.

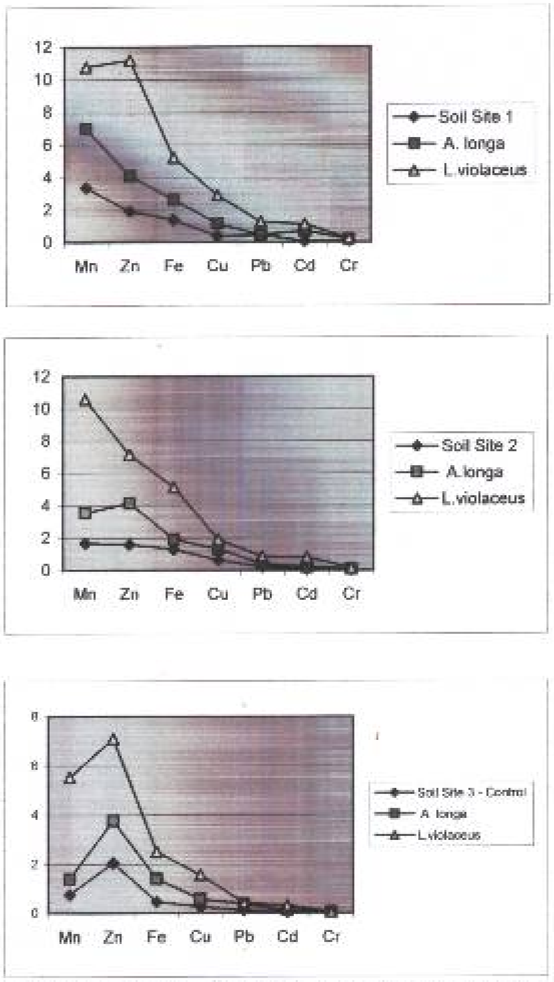


FIGURE 3 HEAVY METALS CONCENTRATIONS (μg/g) IN SOIL AND EARTHWORMS IN THE THREE STUDY SITES

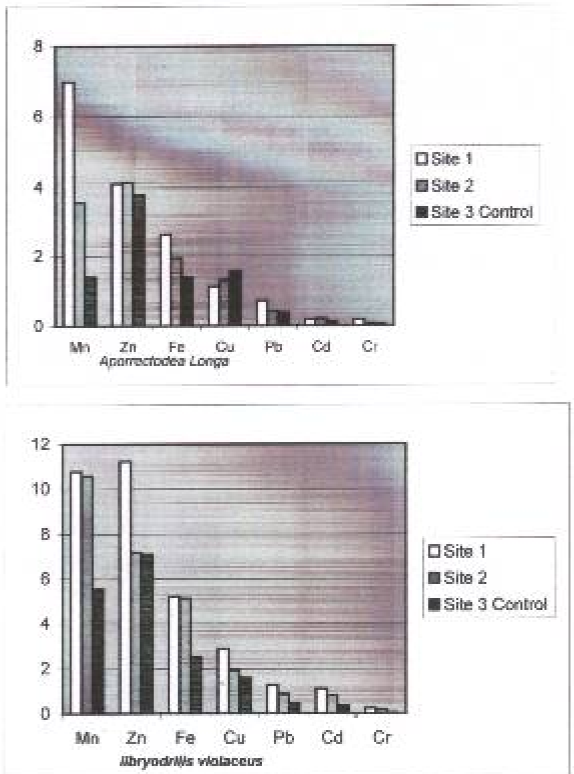


Fig (4) - Heavy Metals Uptake by Earthworms in the Three Sites

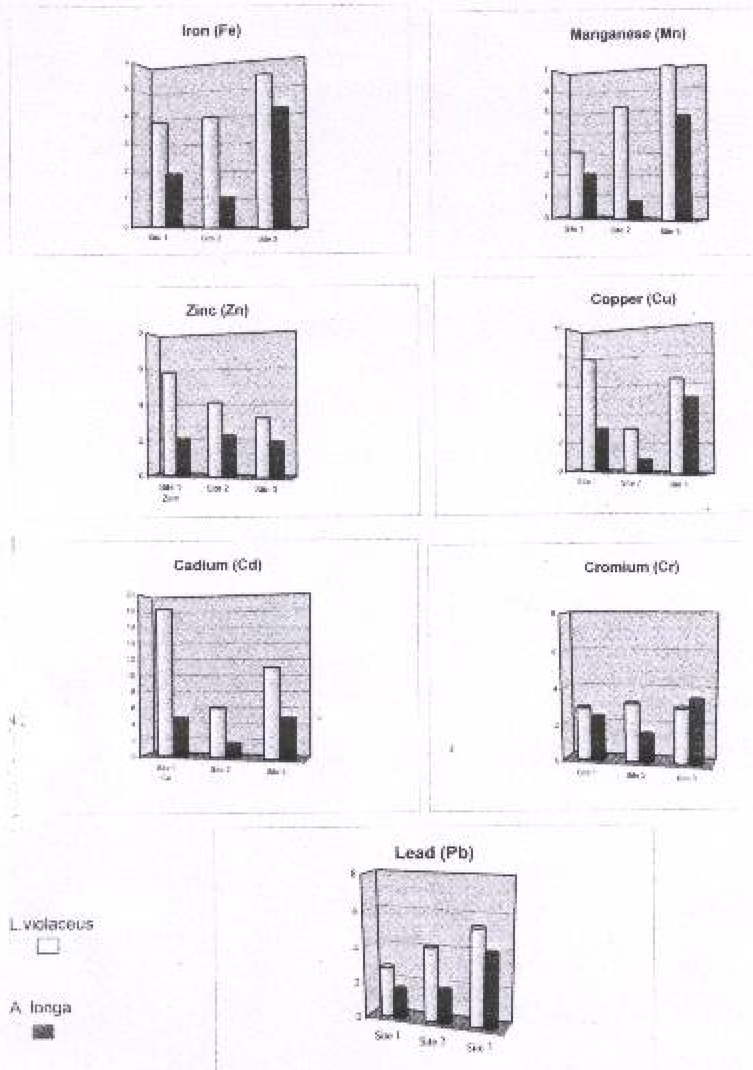


FIGURE 5: BIOCONCENTRATIONS FACTOR (BCF) OF L.violaceus *AND* A.longa IN THE THREE (03) STUDY SITES

The trend in metal concentration in the earthworms in both control and contaminated sites showed that the essential metals Mn, Zn, Fe and Cu were accumulated more than the toxic types (Pb, Cd, Cr). This is similar to the observation of Anderson and Laursen (1982) who worked on *Lumbricus terrestris* in contaminated soil and Bambose *et al* (1999) on *L. violaceus*. Cu and Zn, both essential metals, are used for the enzyme synthesis, DNA etc. (Vijver, 2001), which suggest possible reasons for their elevated levels. Due to these physiological attributes, the biota are able to regulate these essential metals. It usually takes large concentrations to exceed "the window of essentiality". Exceeding or deficiency of essential metals respectively above or below the optimum leads to adverse effects (Vijver, 2001).

Conversely non-essential metals like, Pb, Cd, Cr differ from essential ones by the absence of regulated use; here the "window of essentiality" is approximately zero, depending on the metal species. This accounts for their low level. Furthermore in the present study, the mean concentrations of metals in the earthworms were lower than the range obtained by Bambose *et al* (1999) (Mn 11.71-12.81µg/g; Pb, 5.21-5.44µg/g); Cd, 0.77-0.82µg/g)for the same earthworm *L. violaceus* from different dump sites. This again is a function of the amount of the metal in the soils of study. The higher levels were observed in the dump site, studied by Bambose *et al*., 1999. This may have influenced the high levels observed in the earthworm.

The level of metals observed in the earthworms were higher than soil metal concentrations in both the contaminated and control sites. Significant difference was observed at $P > 0.05$ for both earthworms in all soil locations. Metal concentrations in the earthworm were more in the polluted sites than in the control, for most metals and this conforms with the observations of Morgan and Morgan (1993); Weigmann (1991) who reported higher levels of metals in worms from polluted sites irrespective of degree of pollution. This observation fall within the generalization of the "donor – control principle" which state that external concentrations determine the uptake by an organism (Vijver, 2001).

L. violaceus appears to have a concentrated more metals than *A. longa* in this study. Uptake and elimination kinetics are known to determine the accumulation rate of trace metals in biota. The resultant residual concentration inside the exposed organisms, depends on the uptake rate and the rate of excretion, the rate of toxicant conversion (degradation) or the rate of dilution by growth. Aziz *et al*., (1999) reported that long term earthworms colonizers of heavily polluted metaltiferous soils have responded genetically to selection pressure imposed upon them by the edaphic contaminants such that they have evolved into locally differentiated, metal – adapted ectotypic populations. Reinecks *et al* (1999) concluded that worms with long-term history of exposure to a metal develop resistance. One of these factors must have favoured *L. violaceus* high metal accumulation capacity and a preferred seintive.

Bio-concentration oof heavy metals may have been controlled by the ability of the exposed organism to reduce inherent metal toxicity by forming comparatively innoecus complexes (e.g. with methallothioeine, cytochrome P – 450). The resulting internal equilibrium concentration may be below or above the threshold concentration for effects (Aziz *et al*., 1999). Generally in this study, bio-concentration in both organisms irrespective of pathway and genetical dissimilarity followed the pattern of concentration of metals in soil. This is similar to the observations of Bambose *et al* (1999), Terhivuo *et al* (1994) and Neuhauser *et al* (1995) who reported that for each metal, evidence suggest that bio-concentration in organism depends on the metal concentration in the soil. The implication of this observation is summarized in the donor – control principle which states that the external concentrations determine uptake by an organism, whereas the internal concentration conduct the elimination of a metal (Vijver, 2001).

The bio-concentration factors (BCF) for *A. longa* with the exception of Zn were lower in the polluted sites than the 'control' soils indicating that the organism was able to regulate the other metals (Fe, Mn, Cu, Cr, Cd, Pb). This observation is similar to that reported by Tertivuo *et al* (1994), for *A. caliginosa, Lumbricus rubellus* and *L. castaneus*. In *L. violaceus*, the observation differed; only the essential metals of Fe, Mn, Cu had bio-concentration factors lower in the polluted sites than the "Control" soil. Which suggests that Zn, Cd, Cr and Pb were either poorly regulated or transformed into innocuous forms enhancing their levels in the biota in the polluted sites. Generally BCF is greater in lower soil concentration (Neuhauser *et al*., 1995).

The voiding time has been shown to be an important experimental variable in determining the measured levels of heavy metals in earthworms, because experimental measurements are usually made on aworm-soil complex (Neuhauser *et al*., 1995). The metal concentrations of Zn, Cu, Pb, Cd, and Mn which increased with voiding time in the earthworm (*A. longa*) must have done so because they were sequested in the worm tissue Fe and Cr levels decreased with voiding time in the earthworm an indication that these metals failed to bio-concentrate in the worm tissue. Neuhauser *et al*., (1995) have earlier reported that metals that are bio-concentrated in worm tissue, increasing the voiding period increases the concentration of the metals in the worm-soil complex (i.e. the soft tissues of the worm and the soil in the gut of the worm). Conversely, for metals that are not bio-concentrated increasing the voiding time leads to a decrease in concentration in the worm-soil complex. In their study on two earthworms (*Allolobophora tuberculata* and *Lumbricus rubellus*) Neuhauser *et al*., (1995) reported that only Cd and Zn levels increased with increase in voiding time while Pb, Cu and NI, did not. It is possible that sequestration of metals into tissue may be influenced by genetic configuration of the earthworm. Studies by Woodbury (1992) have shown that earthworms can adopt differently to toxic heavy metal polluted environments. They exhibit various molecular responses to acute heavy metal exposure.

In conclusion, metal concentrations varied among the earthworm species and within the locations. Variations in the soil metal concentrations may have accounted for substantial amount of variability observed among metal concentrations in earthworms, not excluding the role of adaptive mechanisms of the earthworms and the "window of essentiality" of the metals. Soil character may have influenced bio-availability of metals. *L. violaceus* shows evidence of better adaptation to metal pollution and is therefore as preferred bio-indicator in dump site environments.

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