DISTRIBUTION OF HEAVY METALS CONTAMINANTS IN SURFACE SOIL, WATER, LIVER, HEART AND KIDNEY OF AMPHIBIANS *Ptychadenapumilio*AND *Amietophrynusmaculatus*IN SELECTED AREAS OF LAGOS STATE.

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ABSTRACT

The increasing rate of pollution necessitates proper and effective management of environmental health by monitoring the soil, water and animal components of the environment. The study investigated distribution of selected heavy metals contaminants in soil, water samples and bioaccumulation in heart, liver and kidney of two amphibian species, Amietophrynusmaculatus and Ptychadenapumilio in selected sites such as Ibafo (bordering Lagos and Ogun States), Iwaya, Makoko 1, Makoko 2 and University of Lagos (Unilag) located in Lagos state. Heavy metals were analyzed using the Analyst 200 Perkin Elmer series of Atomic Absorption Spectrophotometer. The overall mean concentrations of the heavy metals (mg/g) in the soil samples were as follows: Fe 3.12, Cu 0.41, Ni 0.30, Cr 0.05, Cd 0.07, Pb 0.04, Zn 0.19 and Mn 0.39 thus Fe > Cu >Mn> Ni > Zn > Cd > Cr >Pb which were significantly ($P < 0.05$) different across the sites except for Mn, which was not significantly ($P > 0.05$) different ($df = 4$, $x^2 = 2.739$). In water samples, the mean concentrations (mg/L) were as follows: Fe 1.80, Cu 0.74, Ni 0.78, Cr 0.06, Cd 0.05, Pb 0.03, Zn 0.54 and Mn 0.30. Zinc bioaccumulated highest in both species and in all the locations, followed by Mn and Pb. Mn, Zn and Pb concentrated more in the liver, while Ni was highest in the heart, not significantly ($P > 0.05$) different in both anuran species. Cr concentration in the liver of both anuran species was significantly ($P < 0.05$) different ($df = 8$, $x^2 = 2.717$). The study has revealed the prevailing environmental health with anurans as excellent bioindicators acting as an early warning signal of potential harm to the environment.

Key words: Heavy metals, bioaccumulation, anuran and bioindicator

INTRODUCTION

Global biodiversity loss is currently a major international concern (Stuart *et al*., 2004; Beebee and Griffiths, 2005; Mandal, 2011; Mfunda and Roskaft, 2011). Amphibian populations are no exception and they have been declining at an unprecedented rate with nearly one-third of the world's species threatened with extinction. Concern about amphibians is due in part to their value as indicators of environmental stress (Blaustein and Wake, 1995). Many are in close contact with water as larvae, and most have some contact with land as adults. Therefore they experience both aquatic and terrestrial stressors.
Amphibians have moist, permeable skin and unshelled eggs that are directly exposed to soil, water, sunlight and that can readily absorb toxic substances. Moreover, amphibians are important components of many ecosytems, acting as prey, predators or herbivores. Due to their contribution to trophic dynamics, loss of amphibians will probably affect other organisms (Blaustein*et al*., 1994; Blaustein and Kiesecker, 2002).

Pollutants that affect amphibian include pesticides, herbicides, fungicides, fertilizers, heavy metals and numerous others (Sparling *et al*., 2000; Boone and Bridges, 2003). Pollutants may be spread globally or act on a local scale. They are transported atmospherically and have the potential to affect amphibians in remote, relatively undisturbed environments. Even low levels from atmospheric deposition are potentially harmful. In many cases, heavy metals from industrial and agricultural activities have been implicated (Maheswaran*et al*., 2008). Intensive agricultural, industrial production and pollution from mines have increased the prevalence of heavy metals in surface waters that may ultimately affect amphibian populations. Metals such as aluminum, lead, zinc, cadmium, mercury, silver, copper, arsenic, manganese, molybdenum and antimony have a number of effects on amphibians. These heavy metals can be lethal or induce sub lethal effects such as slow growth and development, reproductive failure and behaviourial changes (Lefcort*et al*., 1998, 1999; Raimondo*et al*., 1998; Blaustein*et al*., 2003). In many cases, the effects of heavy metals on amphibian survival have been linked closely to acidification because they leach from soils in contact with acidic water (Blaustein*et al*., 2003).

Laboratory experiments have shown that aluminium levels as low as 10 to 20 ppb at pH 4.7 cause reduced hatching success of *Bufoamericanus and Ranasylvatica*eggs (Clark and LaZerte, 1987).

Heavy metals are natural components of the earth's crust occurring in varied concentrations in the ecosystem. Heavy metals are present in rocks, soils and air in small amounts from geological sources that influence the chemical composition and the nature of airborne particulates and dusts inhaled or ingested (Don-Pedro, 2009). Heavy metals are usually detected in measurable levels in industrial effluents because metallic compounds are common constituents of several raw materials. Such materials that serve as feed stocks, catalysts and lubricants are employed in industrial production processes (Ezemonye and Enuneku, 2005). Adult amphibians can acquire heavy metals through their skin or orally by comsumption and inhalation. Larvae may also absorb them through their skin (Ezemonye and Enuneku, 2006).
One of the major problems associated with the persistence of heavy metals is the potential for bioaccumulation and biomagnification causing increased exposure for some organisms than is present in the environment. Bioaccumulation means an increase in the concentration of a chemical in an organism over time, compared to the chemical's concentration in the environment. When exposed to higher concentrations, organs of aquatic animals may accumulate heavy metals (Ezemonye and Enuneku, 2005, 2011). Compounds accumulate in living things when taken up and stored at a faster rate than they are broken down (metabolized) or excreted. The involvement of bioaccumulating organisms such as amphibians in the food chain therefore, increases the potential for these accumulated metals to be transferred along the food chain, and subsequently become biomagnified in organisms further up the food chain.

It is clear that amphibian is used as indicators to monitor the health of the environment because of its morphological and physiological uniqueness. A lot of industrial activities are taking place in the Lagos metropolis resulting into a lot of untreated industrial effluents being released into the environment. Part of this environment includes the habitat of amphibian species. Thus this study was aimed at ascertaining the concentration of selected heavy metals in surface soil and water samples in the habitats of two amphibian species (*Ptychadenapumilio*and *Amietophrynusmaculatus*) in selected sites in Lagos State. These two species are endemic to West Africa and are of ‘least concern’ pertaining to their conservation status (Rodel, 2000). The study also entails the determination of the concentration of selected heavy metals in the liver, heart and kidney of these amphibian species.

MATERIALS AND METHODS

Description of Study Area

Lagos State lies in the South - Western part of Nigeria and shares boundaries with Ogun State both in the northern and eastern region and is bounded on the West by the Republic of Benin. In the South it stretches for 180km along the coast of the Atlantic Ocean. There were five sites selected in the state for the ecological study. These locations were Ibafo (bordering Lagos and Ogun States), Iwaya, Makoko 1, Makoko 2 and University of Lagos (Unilag). The habitats of the various sites were briefly described and geo-referenced with a Magellan Sport Track Global Positioning System (GPS) with accuracy of a metre shown in Table 1.

Table 1: Global Position System (GPS) Coordinates of the sampling locations

Sites	Location	G.P.S.	Short Habitat Characterization
1	IBAFO	3°25'19.47''E 6°44'24.53''N	Area located at the border of Lagos and Ogun States. A small town located near the Ogun river. Agricultural activities and cattle rearing are common occurrences.
2	IWAYA	3°23'25.81''E 6°30'12.60''N	Area located in the Lagos metropolis. A few metres from the Lagos lagoon. It is overcrowded with poor waste disposal system.
3	MAKOKO 1	3°23'1.64''E 6°29'40.19''N	Area situated along the Lagos lagoon. A slum dwelling which is heavily polluted with domestic wastes. Wastes being poured both on the terrestrial and aquatic habitats.
4	MAKOKO 2	3°23'34.47''E 6°29'55.23''N	Area also situated along the Lagos lagoon which is being polluted by the discharge of effluents from industries. Also a slum dwelling place where houses are built above the water. Domestic wastes are being dumped directly into the lagoon.
5	UNILAG	3°23'39.14''E 6°30'50.23''N	Campus of the University of Lagos. It is situated along the Lagos lagoon. The lagoon receives effluents from industries situated along its border. There are tertiary and secondary vegetation on the campus bordering the lagoon.

Collection of Soil, Water and Animal Specimens

Three replicates of surface soil samples were randomly collected from the five (5) selected experimental locations, properly homogenized, stored in a plastic container and transferred to the laboratory prior to analysis.

Surface Water samples from the five (5) experimental locations were collected randomly in three replicates and transferred into labeled 120ml plastic container. To prevent adsorption of metals into the walls of the container, the sample was acidified with two drops of concentrated HNO_3 to pH ≤ 2 (APHA, AWWA and WPCF, 1995)and stored in a refrigerator (4^0 C) until required for analysis.

The animals (amphibian) specimens were caught mainly during night surveys with sweep nets (mesh size 0.1cm x 0.1cm). The animals were collected, transferred to sacs and taken to the laboratory for weighing and dissection. Characteristic parameters such as the snout-vent-length (SVL), lengths of thigh, tibia, hind limbs and hind limb/SVL ratio were measured.

Chemical Determination of Heavy Metals in the Soil and Water Sample

Digestion of Samples for Atomic Absorption Spectrophotometry (AAS)

Surface soil samples were sieved using a 200 - µm sieve to normalize for particle size and properly homogenized. One grams (1g) of dried surface soil sample collected from experimental locations digested , according to the method adopted by Ageiman and Chau, (1976); Bryan and Langston, (1992). Cadmium (Cd), chromium (Cr), manganese (Mn), lead (Pb), iron (Fe), copper (Cu) and nickel (Ni) using the Analyst 200 Perkin Elmer series of Atomic Absorption Spectrophotometer (AAS).

Water samples from experimental sites were digested according to the methods ofAPHA/AWWA/ WPCF, (1995).

Determination of Heavy Metals in Animal Samples

Dissection of animal samples was performed with a medical dissecting set and in with strict adherence to pathological techniques and Organization for Co-operation and Economic Development (OCED) guidelines. Harvested liver, heart and kidney of the animals were placed in labeled plastic bags and stored at -20 ^{0}C to avoid tissue degradation prior to analysis. The liver, heart and kidney of the animals were properly washed to avoid contamination and oven dried using the fanning system (70^0 C). Each of the dried samples was then ground to powder in a mortar. Five grams of each sample was made into paste by adding a 2 ml of distilled water. This was digested with 10ml of HNO_3 by heating until the brown fumes disappeared. It was allowed to cool and then distilled water was added to make up to 50ml in a standard volumetric flask. The filtrate was analyzed for the levels of Cd, Cr, Ni, Mn, Pb and Zn using the Analyst 200 Perkin Elmer series of AAS.

Data analysis

Data were analyzed by Levene's test (t - test) using SPSS 16 package. Possibilities less than 0.05 were considered statistically significant ($P < 0.05$).

RESULTS

Mean Concentrations of Heavy Metals in the Soil and Water

Results of overall mean concentrations of the heavy metals (mg/kg) in soil samples showed that, Fe 3.12, Cu 0.41, Ni 0.30, Cr 0.05, Cd 0.07, Pb 0.04, Zn 0.19 and Mn 0.39. This lead to the following ranking: Fe > Cu >Mn> Ni > Zn > Cd > Cr >Pb. Figure 1 showed that the concentration of iron (Fe) in the soil was highest at Makoko 1, followed by Ibafo, Iwaya, Unilag and Makoko 2. Ni, Mn and Cu had almost the same concentrations at Unilag, Ibafo and Iwaya, while the least concentrations occurred at Makoko 2 and Ibafo. Cd, Cr Pb and Zn were at very low concentrations across the sampling sites. The concentrations of these heavy metals in the soil were significantly ($P < 0.05$) different across the sites except Mn that was not significantly ($P > 0.05$) different ($df = 4$, $x^2 = 2.739$).

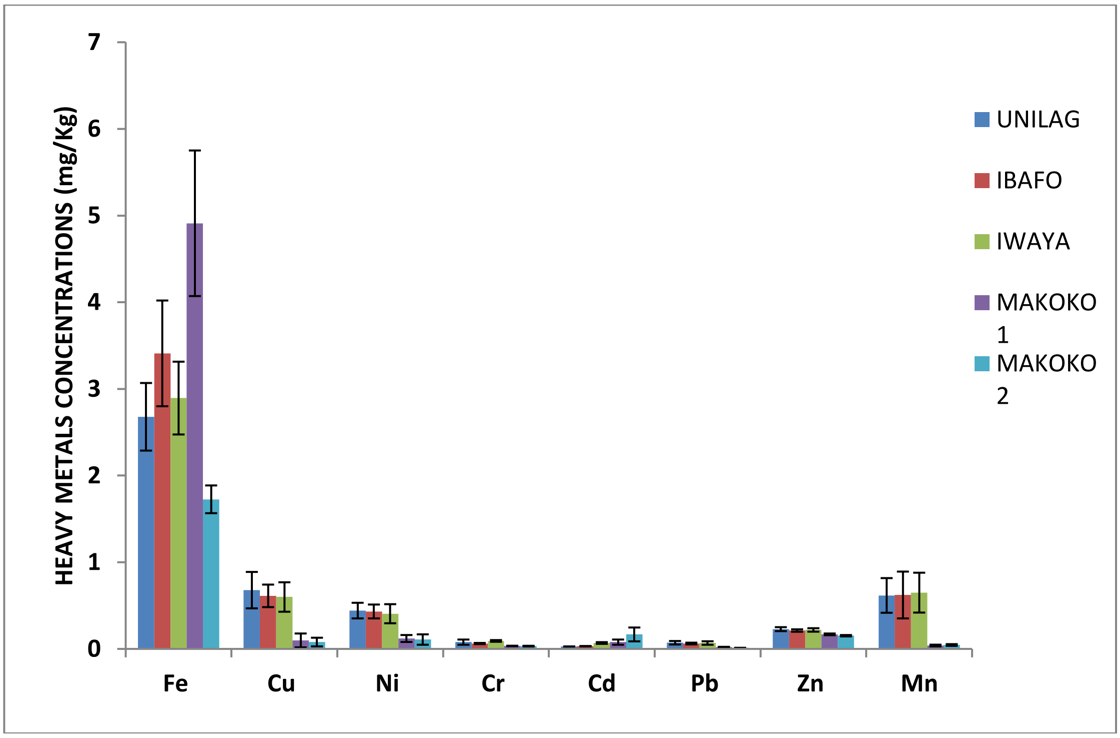

Fig. 1: Mean concentrations of heavy metals in the soil from the sampled locations in Lagos State

Calculations of the overall average concentrations of the heavy metals (mg/L) in the water samples showed the following results: Fe 1.80, Cu 0.74, Ni 0.78, Cr 0.06, Cd 0.05, Pb 0.03, Zn 0.54 and Mn 0.30. This lead to the following ranking: Fe > Ni > Cu > Zn >Mn> Cr > Cd >Pb. Similarly as in the soil samples, the concentration of iron (Fe) in the water was highest at Makoko 1, followed by Ibafo, Iwaya, Unilag and Makoko 2 (Figure 2). Ni, Mn and Cu had almost the same concentrations at Unilag, Ibafo and Iwaya, while the least concentrations occurred at Makoko 2 and Ibafo. Cd, Cr, Pband Zn were detected at very low concentrations across the sampling sites. The concentrations of these heavy metals in the water samples were all significantly ($P < 0.05$) different across the sites.

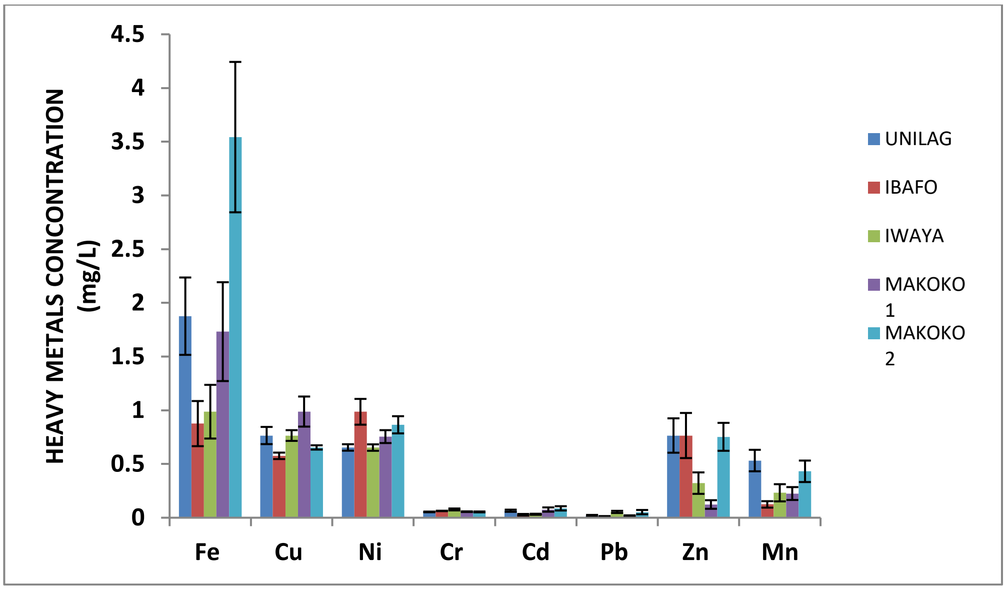

Fig. 2: Mean concentrations of heavy metals in the water from the sampled locations in Lagos State.

Parameters Measurement of Amphibian Species

Results of the parameters measurements show that *Amietophrynusmaculatus*has a much larger morphology than *Ptychadenapumilio*(Table 2)

Table.2: Mean parameters of amphibian species (±) = range of parameters

Parameters (cm)	***Ptychadenapumilio***	***Amietophrynusmaculatus***
SVL	3.69 ± 0.76 (2.68 – 4.69)	6.52±1.54 (4.34-8.77)
Thigh	1.79 ± 0.42 (1.40 – 2.44)	2.42±0.74 (1.56-3.30)
Tibia (Shank)	2.09 ± 0.55 (1.58 –3.00)	2.46±0.75 (1.60-3.40)
Foot	3.22 ± 0.64 (2.24 – 4.21)	3.63± 0.81 (2.32-4.51)
Hind limb	7.12 ± 1.59 (5.51 – 9.65)	8.51± 2.28 (5.48-11.21)
Hind limb/SVL	1.93 ± 0.07 (1.83 – 2.07)	1.31± 0.09 (1.19-1.46)
Weight (g)	5.31± 3.05 (2.31 – 11.03)	36.66± 22.58 (7.4-70.58)

Selected Heavy Metals Levels in the Heart, Liver and Kidney of *A. maculatus*and *P. pumilio*

Heavy metals accumulation in the heart, liver and kidney of *A. maculatus* and *P. pumilio* generally showed that the concentration of Zn was the highest compared to other heavy metals in both species and in all the locations, followed by Mn and Pb. In the liver, Mn, Zn and Pb were concentrated, while Ni occurred at highest concentration in the heart. Ni, Cr, and Cd occur at very low concentrations which were almost the same in *A.maculatus* and *P. pumilio* from all the sampled locations (Figures 3 – 12). Comparism of the concentrations of the selected heavy metals in the organs of both anuran species, showed that they were not significantly ($P > 0.05$) different. Table 3, showed that the concentration of Cr in the liver of both anuran species was significantly ($P < 0.05$) different ($df = 8$, $x^2 = 2.717$).

The variation of bioaccumulation of heavy metals with respect to the tissues in descending order is as follows: In *A. maculatus*;

Heart: Zn>Mn>Pb>Ni>Cd>Cr   Liver: Zn>Pb>Mn>Cd>Cr>Ni   Kidney: Zn>Mn>Pb>Cd>Cr>Ni
In *P. pumilio;*
Heart: Zn>Mn>Pb=Ni>Cr>Cd   Liver: Zn>Mn>Pb>Cd>Ni>Cr   Kidney: Zn>Mn>Pb>Cd>Cr>Ni

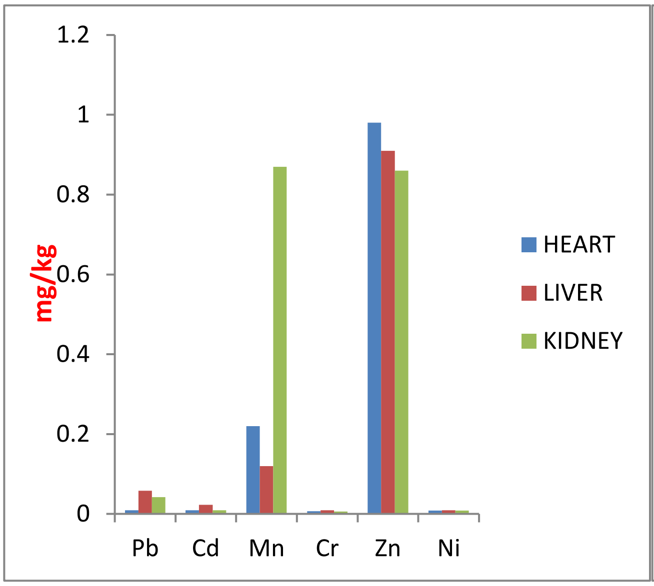

Fig. 3: Mean concentration of heavy metals in the heart, liver and kidney of *A. maculatus*from Unilag

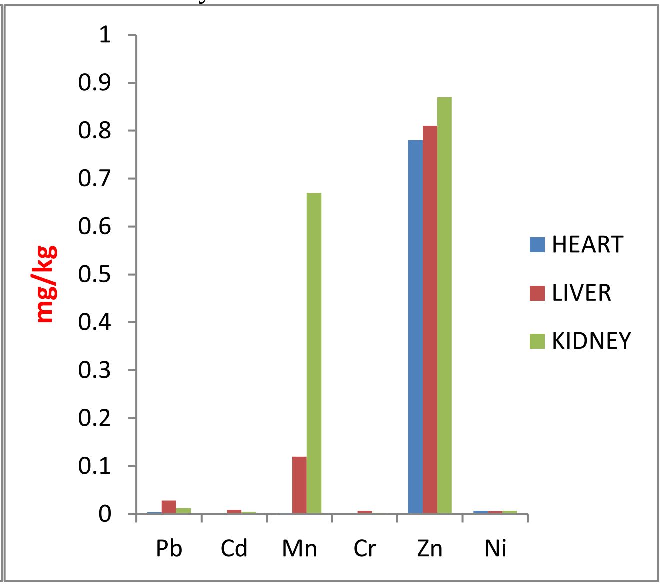

Fig. 4: Mean concentration of heavy metals in the heart, liver and kidney of *P. pumilio*from Unilag

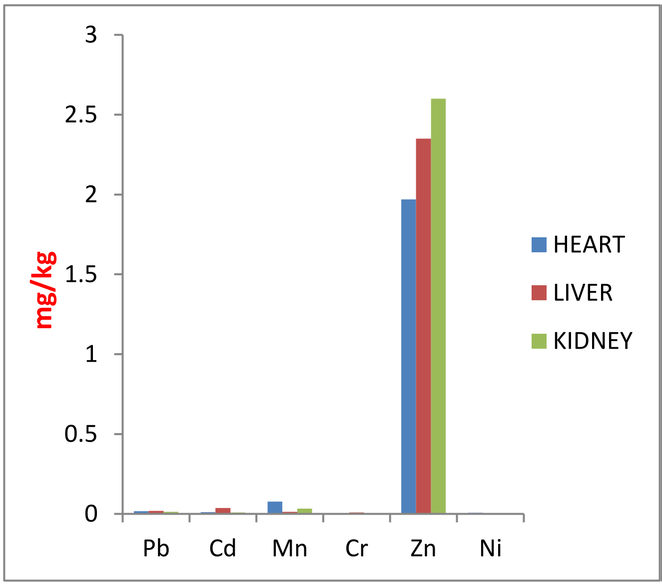

Fig. 5: Mean concentration of heavy metals in the heart, liver and kidney of *A. maculatus*from Ibafo

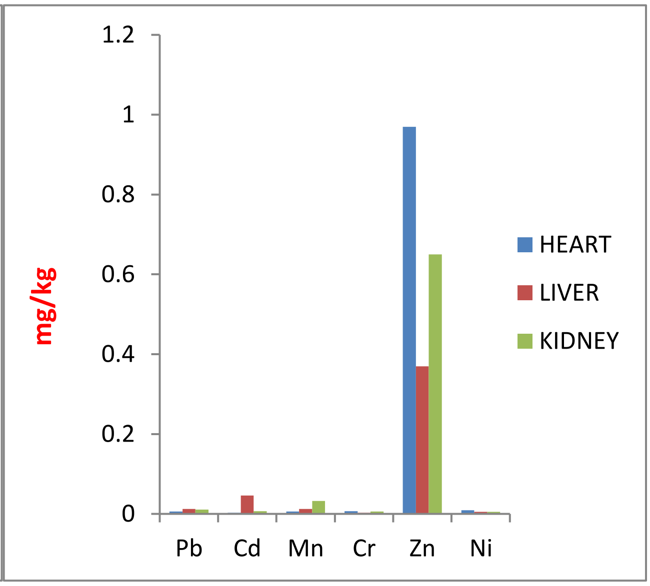

Fig. 6: Mean concentration of heavy metals in the heart, liver and kidney of *P. pumilio*from Ibafo

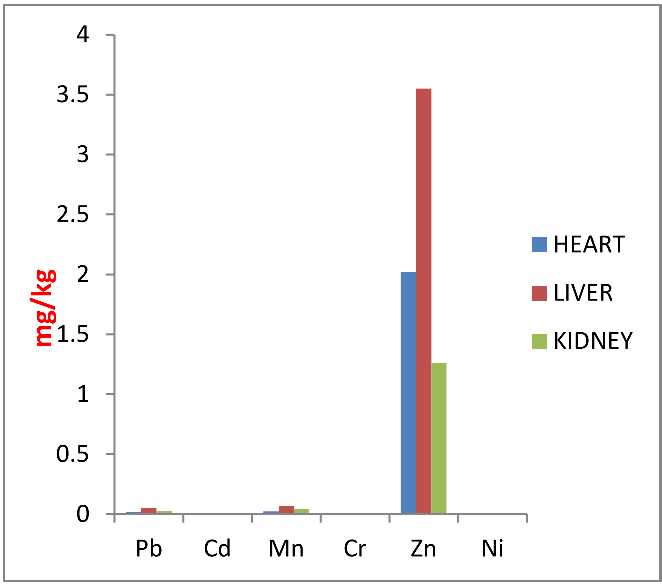

Fig. 7: Mean concentration of heavy metals in the heart, liver and kidney of *A. maculatus*from Iwaya

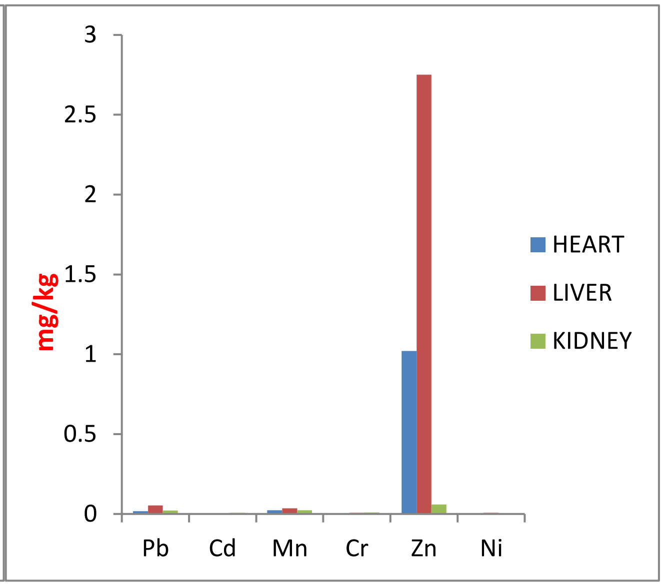

Fig. 8: Mean concentration of heavy metals in the heart, liver and kidney of *P. pumilio*from Iwaya

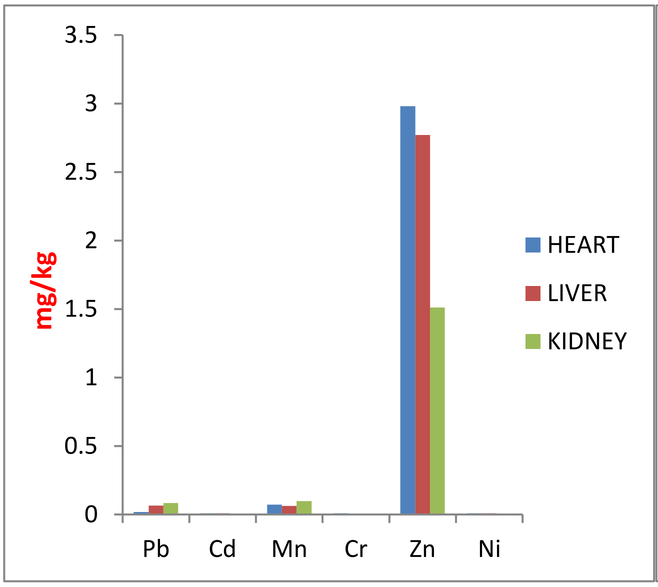

Fig. 9: Mean concentration of heavy metals in the heart, liver and kidney of *A. maculatus*from Makoko 1

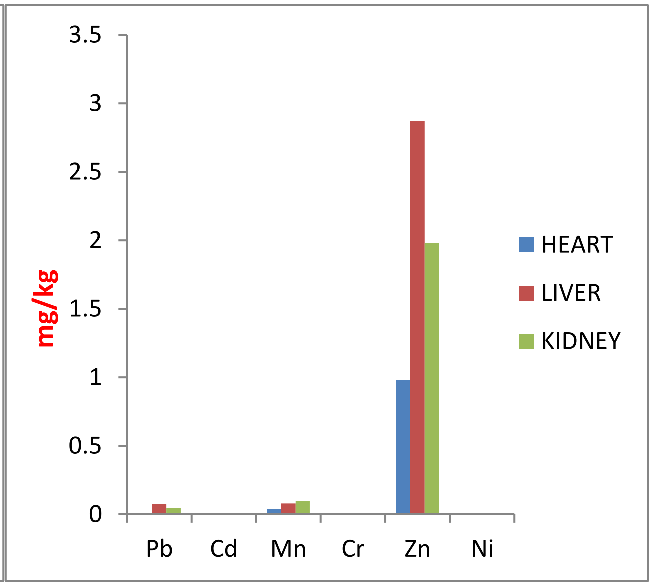

Fig. 10: Mean concentration of heavy metals in the heart, liver and kidney of *P. pumilio*from Makoko 1

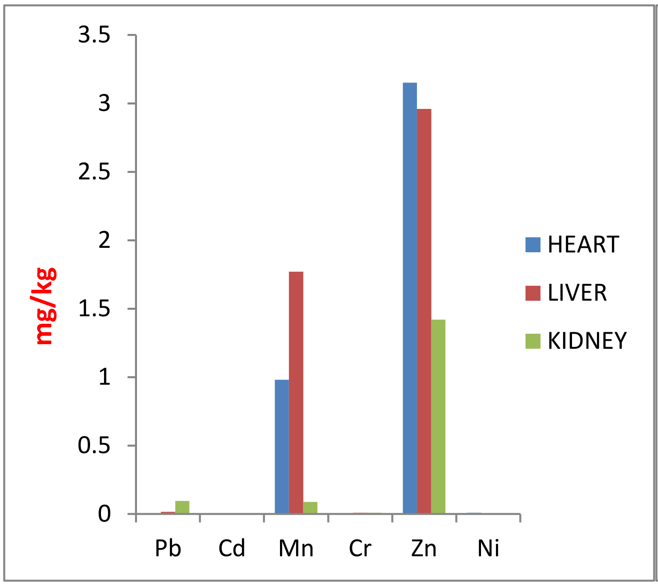

Fig. 11: Mean concentration of heavy metals in the heart, liver and kidney of *A. maculatus*from Makoko 2

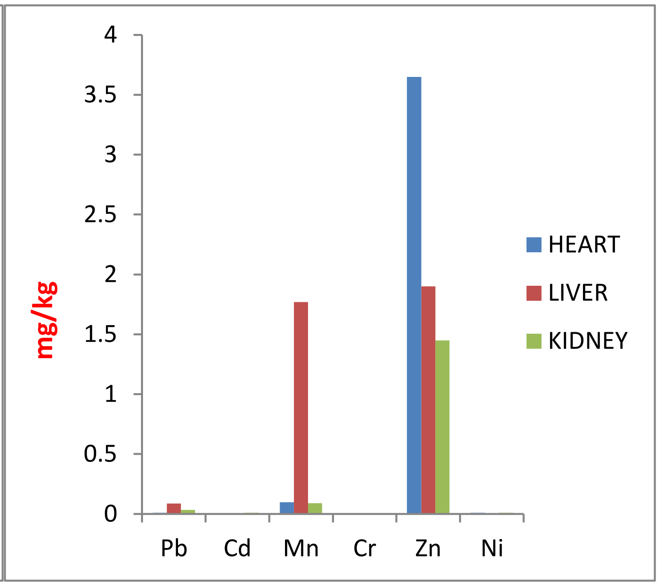

Fig. 12: Mean concentration of heavy metals in the heart, liver and kidney of *P. pumilio*from Makoko 2

Table 3. Overall mean concentration of heavy metals in the organs of *A. maculatus*and *P. pumilio.*

Heavy Metals **(mg/kg)**	Organs of Amphibian Species (kg)					
	Heart		Liver		Kidney	
	P. pumilio	*A. maculatus*	*P. pumilio*	*A. maculatus*	*P. pumilio*	*A. maculatus*
Pb	0.007±0.002	0.013±0.002	0.051±0.01	0.042±0.01	0.024±0.006	0.052±0.016
Cd	0.003±0.001	0.006±0.001	0.013±0.008	0.015±0.006	0.007±0.0006	0.007±0.0008
Mn	0.033±0.017	0.274±0.17	0.403±0.32	0.406±0.34	0.182±0.12	0.226±0.16
Cr	0.005±0.001	0.005±0.001	0.005±0.0008	0.008±0.0003	0.006±0.001	0.006±0.001
Zn	1.480±0.54	2.220±0.39	1.740±0.50	2.508±0.44	1.002±0.33	1.530±0.29
Ni	0.007±0.001	0.008±0.0003	0.006±0.0004	0.006±0.001	0.005±0.001	0.005±0.001

DISCUSSION

Part of this study demonstrated the concentration of heavy metals in soil and water samples from different study sites in Lagos state. The comparison between the sampling sites showed that the concentration of heavy metals varied from site to site and the variation could be related to variability in sources of metals input. The concentration of Iron (Fe) occurred highest in both water and soil samples from all the sites sampled. This finding agrees with Oyewo (1998) who estimated levels of heavy metals discharged into the Lagos lagoon. He observed that Fe concentration was one of the highest heavy metals detected as follows: Fe- 161,718kg, Mn- 205,989kg, Cu- 6,124kg, Zn- 7,026kg, Cr- 5,285kg, Pb- 2,259kg, Ni- 6,124kg, Cd- 538kg and Hg- 278kg per annum. Fe is commonly used both in industries and households and their fillings are easily transported to the soil and water bodies in surrounding environments.

At Makoko 1, the concentration of Fe was the highest in the soil of all the sampled sites. This is probably based on the location of a huge dumpsite within the sampled site. These dump sites consist of a mixture of both domestic and industrialized solid wastes. These refuse contain chemical substances, machines, gadgets and equipment that contain one or more heavy metals as their parts or components.
At Makoko 2, the concentration of Fe was the highest in the water sample. In Makoko 2, most homes are built above the water of the Lagos lagoon. The heavy metals from the refuse dump sites are usually leached into the nearby water body or ground water. The Lagos lagoon is surrounded by the densely populated and industrialized Lagos metropolis, thus a convenient dumping site for numerous industrial and domestic wastes (Don-Pedro *et al*., 2004).

Copper was the second and third most concentrated heavy metal occurring in the soil and water samples respectively at all the sample sites. According to Tirkey*et al.* (2012), Cu is essential for human life, but in high doses it may cause anemia, liver and kidney damage, stomach and intestinal irritation. Ni was the second most concentrated heavy metal observed in the water samples in the study while Mn was the third in the soil samples. In the investigation of heavy metal pollution in Nigeria, these metals have been observed to occur in different concentrations in industrial effluents (Akinola*et al.,* 1981; Bhaleraoand Adeeko, 1981; Oyewo, 1998) and in segments of the ecosystem, particularly sediment and water column (Fodeke, 1979; Okoye, 1989; Chuckwu, 1991; Ogunsanya*et al.,* 1991). As stated earlier and observed in this study, variations in concentration of heavy metals is related to the variability in sources of the metal input. The Federal Environmental Protection Agency (FEPA) of Nigeria guidelines of the concentration of heavy metals showed that these sites surveyed were not heavily polluted (FEPA, 1991). However there is a tendency for the concentrations of these heavy metals to increase over the preceding years especially at the various sites close to the Lagos lagoon, a thing of concern. According to Singh *et al.*, (1995) an estimated 10,000 m^3 of industrial effluents are discharged into the Lagos lagoon per day. Don Pedro *et al.,* (2004) observed that the concentration of heavy metals detected in all the three principal media (water, sediment and animal) of the Lagos lagoon had a distinct upward trend from equivalent samples collected five years apart. This is due to the discharge of metal-laden industrial and domestic effluents entering the lagoon via drainage channels and streams (Oyewo, 1998).

Amphibian species inhabiting Makoko 1 and Makoko 2 were observed to have bioaccumulated the highest concentration of Zn in their organs. This shows that these sites were more polluted than the others sampled. Shaapera*et al.* (2013) observed that Zn had the highest concentration (1.81± 0.20 mg/kg) in the intestine while the concentration was the same in the liver and skin (1.50mg/kg) of *Ranasp* in Benue state. Zn is essential in nutrition and has low toxicity level; however high level of Zn is harmful to human health (ATSDR, 2004). High accumulation of Zn in the tissue of frog and toad could be based on specific adaptive mechanism to absorb Zn from the environment for onward transfer to the kidney where it is needed for metabolic process and co-enzyme catalyzed reactions as reported by Jaffar and Pevaiz (1989). Zinc also acts as a catalyst in metal biomolecules and sulphur legends to form tetrahedral zinc metalloproteins and metalloenzymes in kidney tissues (Ashraf, 2005).

In this study, it was observed that Mn and Pb occurred as the overall second and third highest concentration with the highest concentration occurring in the liver. Similar observations were made by Shaapera*et al.* (2013) revealing that Pb showed the highest unit of 1.89 ± 1.45mg/kg in the liver of *Rana*sp. The accumulation of heavy metals in the liver is likely linked to its role in metabolism (Zhao *et al.*, 2012). The liver is the organ responsible for the detoxification or biotransformation of toxicant in the system of organisms.

Generally, results showed that heavy metals were more concentrated in the liver, heart and kidney of the frog and toad samples from Makoko 2, Makoko 1 and Iwaya than those from Ibafo and Unilag. This could be due to the environment of the latter two sites were less populated, hence less indiscriminate waste disposal leading to a less polluted environment. These amphibian species were able to accumulate heavy metals proportional to the concentration or amount of pollutants in their environment giving them the position of being good bioindicators. Amphibians have a biphasic life exposing them to toxins in both the aquatic and terrestrial environments. They possess smooth moist skin; a partial breathing organ, with permeable eggs casing which can be penetrated by harmful chemicals. The amphibian larvae are often omnivorous, ingesting everything from sediments and phytoplanktons to conspecifics, hence accumulating high concentration of contaminants (Hoff *et al.,* 1999; Snodgrass *et al.,* 2004). Some amphibians are known to accumulate high concentrations of metalloids and organic contaminants in their tissues (Sparling, 2000; Hopkins *et al.,* 2006; Roe *et al.,* 2006).

It was observed from this study that the toad (*A. maculatus*) tend to accumulate more heavy metals than the frog (*P. pumilio*), though not significantly different. This may be due to their morphology as toads are stouter and have shorter legs than frogs, hence they usually hop or crawl during movements (Rodel, 2000). Thus they stay or spend more time on the substrates increasing the chance of bioaccumulation these heavy metals. Frogs morphologically have a more slender body and longer legs (Table 3, hind limb/SVL ratio) giving them the ability to jump more frequently with longer distance, hence reducing their contact with the substrate.

CONCLUSION

The results obtained from this study established the fact that amphibians are excellent bioindicator organisms and an insight on the prevailing health of the environment. The biological significance of this attribute is that these organisms give an early warning signal of potential harm to the environment. If these signals are checked immediately, this could prevent the risk of potential disruption of the delicate ecological balance of the ecosystem.

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