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Heavy metal bioaccumulation and biomarkers of oxidative stress in the wild African tiger frog, *Hoplobatrachus occipitalis*

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Human activities can have dramatic effects on animal populations around urban areas with heavy metal contamination being a primary cause of harm. Amphibians, as residents of aquatic systems and with their semi-permeable skin are especially susceptible to heavy metal contamination. To better understand the effect of heavy metals on Wild African Tiger frogs (*Hoplobatrachus occipitalis)* and the resulting production of oxidative stress enzymes, the concentrations of the heavy metals, cadmium (Cd), copper (Cu), iron (Fe), zinc (Zn), lead (Pb) and nickel (Ni) were investigated in the tissues of *H. occipitalis* as well as in water and sediment samples collected from five different locations in Lagos State, Nigeria. The activities of superoxide dismutase (SOD), reduced glutathione (GSH) and level of lipid peroxidation product, malondialdehyde (MDA) were analyzed in the liver of the sampled frogs. Most measured physicochemical characteristics of the water varied significantly across the sampling locations (P<0.05). The levels of metals (mg/kg dry weight) in muscle tissues also varied significantly across the locations (P<0.05) and ranged as follows: Cd: 0.21-5.03, Cu: 0.74-13.40, Fe: 3.19-109.10, Zn: 3.70-120.20, Pb: 0.12-18.24 and Ni: 3.20-7.28. Zn was the most accumulated metal, followed by Fe, Cu and Ni, while Pb was the least. The mean of SOD and reduced GSH in the frogs indicate some responses to oxidative stress which varied significantly among sampling areas (P<0.05). MDA values however did not consistently correlate with either oxidative stress or heavy metal concentrations in the frogs. The water-sediment-tissue analysis for heavy metals demonstrated that the sediment concentrated more heavy metals than water, while the frog tissues accumulated these metals particularly in more polluted areas.

Key words: Heavy metal pollution, bioaccumulation, *Hoplobatrachus occipitalis,* biomarkers, oxidative stress.

INTRODUCTION

Heavy metal pollution is ubiquitous in our environment (Don-Pedro et al., 2004) and results from diverse activities such as industrial effluents, foundry wastes, wearing of metal parts and equipments, paints, automobiles, mining and rock weathering. These are subsequently deposited on soil surfaces and may be leached through municipal drainages to nearby ponds, streams and rivers which are common amphibian habitats and hiding places. The major concern with heavy metals lies with their acute toxicity and their ability to bioaccumulate in biological systems (Otitoloju and Don-Pedro, 2002a), resulting in a number of deleterious effects such as immunosuppression (Carey and Bryant, 1995), induction of stress proteins (Piano et al., 2004), oxidative stress (Farombi et al., 2007; Soundararajan et al., 2000) histopathological damage (Kothari et al., 1990; Andhale

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et al., 2011; Tarasub et al., 2011), disruption of reproductive potential and endocrine disruption (Drevnickand and Sandheinrich 2003; Kasperczyk et al., 2008) and mortality/acute toxicity (Kurdland, 1960; Otitoloju and Don-Pedro, 2002b). Metal are often in ionic forms and their ligands interact with hydrocarbons which makes them bioavailable and able to penetrate body surfaces where they readily pass through phospholipids cell membranes, causing harm due to the inability to metabolize them (Walker et al., 2001). Cooper and Manalis (1983) linked lead, cadmium and mercury with the impairment of pre-synaptic mechanisms such as acetylcholine inhibition in amphibians.

Amphibians living in the fringes of populated cities may come under pressure from a number of anthropogenic factors including pollution from heavy metals. Frogs have a wide variety of diet and live in rugged terrains such as swamps, tree, thickets (Hickman et al., 2008), as well as unkempt lawns and human habitations thus exposing them to polluting activities. A semi-permeable and highly vascularized skin allows cutenous respiration in amphibians (Noble, 1925) and therefore, may confer them with high a propensity to accumulate environmental pollutants in their tissues directly from water and moist surroundings (Willens et al., 2006). It is now commonly speculated that amphibian species may be globally on decline (Wake and Vredenburg, 2008). Their decline and loss of viable populations has been attributed to habitat destruction, introduction of invasive species, over exploitation, emerging diseases, pathogens, climate change and environmental contamination (Becker et al., 2007; Smith et al., 2009; Hayes et al., 2010). Their biology and peculiar habitat selection makes them candi-dates for heavy metal accumulation (Hayes et al., 2010; Hsu et al., 2006). A number of pollutants including heavy metals have been linked with the presence of free radicals which may induce oxidative stress in biological systems (Osuala, 2012). Certain biosynthetic mechanisms, such as induction of low molecular weight proteins exists which have been attributed to the ability to inhibit metal activity and possibly their absorption into the bloodstream of *Rana ridibunda* (Loumbourdis et al., 2007). The presence of these proteins can be a protective mechanism for managing oxidative stress and can confer tolerance to heavy metal pollution.

Some heavy metals are hepatotoxic agents causing liver disorders, largely due to their active metabolites and free radicals (Lygren et al., 1999). These activated radicals bind covalently to macromolecules and induce peroxidative degeneration of the endoplasmic reticulum (ER) lipid membrane, which is rich in polyunsaturated fatty acids resulting in cell damage (Sies, 1985). The release of lipid peroxidation products such as malondialdehyde (MDA), has been established as a useful biomarker in monitoring effects of pollutants such as polycyclic aromatic hydrocarbons, petroleum products as well as heavy metals (Otitoloju and Olagoke, 2011; King et al., 2012; Osuala, 2012). The lipid peroxidative degradation of biomembrane is one of the principal causes of heapotoxicity of heavy metals (Timbrell, 2000). Antioxidants defense system such as superoxide dismutase (SOD), glutathione-S-transferase (GST), reduced glutathione (GSH) and catalase are mobilized to reduce organism susceptibility to the damaging effects of reactive oxygen species (ROS) and free radicals that have been generated by the biodegradation of membranes and biotransformation of metallic moieties (Soundararajan et al., 2009; Sasaki et al., 1997; Azqueta et al., 2009). One of the major classes of antioxidant enzymes characterized in eukaryotic cells is SOD, a family of metalloenzymes which catalyzes the spontaneous dismutation of superoxide anion to hydrogen peroxide and molecular oxygen. SOD is widely distributed in aerobic organisms and plays an important role in the control of radical superoxide levels in the cellular compartments (Sasaki et al., 1997). Glutathione is believed to be the most important protective mechanism, occurring in most cells, especially the liver (Timbrell, 2000). This mechanism detoxify substances by conjugation with GST, conjugation with reactive metabolites or donation of proton or hydrogen atom to free radicals to bring about reduction and hence stop the damaging process of oxidative stress (Otitoloju and Olagoke, 2011). These enzymes are often employed in combinations as biomarkers of stress from free radicals in disturbed and polluted environments (Regoli et al., 2002).

In furtherance of understanding the effects of human activities on animal populations around urban areas, this study seeks to assess the levels of some heavy metals in waters and sediments and their accumulation in the tissues of *Hoplobatrachus occipitalis* within the Lagos metropolis and to determine levels of oxidative stress enzymes with the goal to evaluate their suitability in monitoring the presence of stressors in the urban environment.

MATERIALS AND METHODS

Study design

Five Local Government/Local Council Development Areas (LGA/LCDAs): Amuwo-odofin, Ojo, Ikorodu, Badagry and Yaba (Figure 1), spanning the breadth of Lagos State, the commercial capital of Nigeria were selected as sampling locations for this study on a semi-random basis to cover areas of high and low commercial activities and population densities. African Tiger frog (*H. occipitalis*) were sampled once between March and July 2010, a period spanning between late dry season and mid rainy reason depending on accessibility of the area and availability of the frogs, and five individuals were trapped in each occasion for toxicological evaluation.

Classification of the study sites

The dominant anthropogenic activities observed in the five Local Government Areas are as follows:

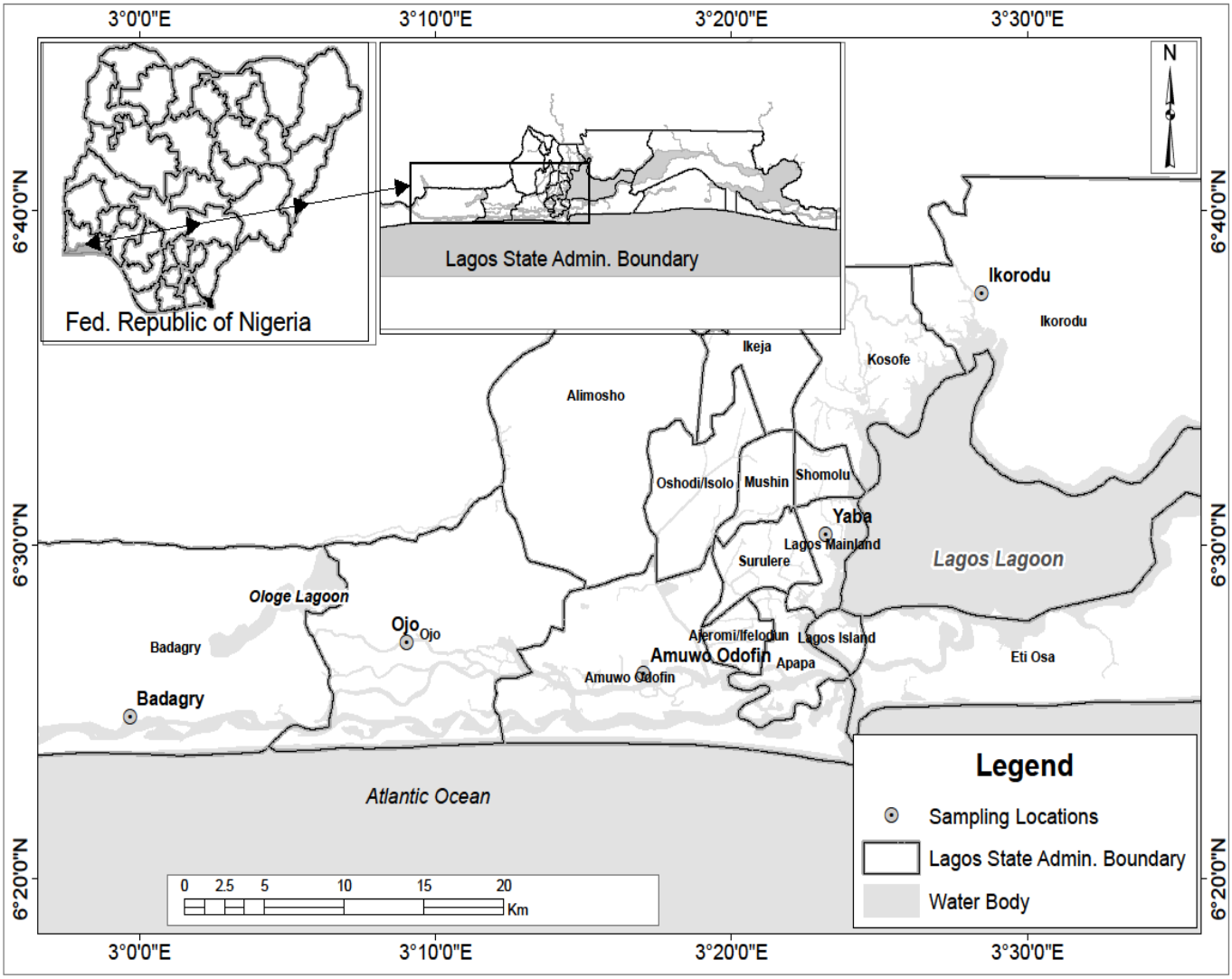

Figure 1. Map of sampling locations in Lagos, Nigeria.

Amuwo-Odofin: High population density, high vehicular traffic and large markets. Badagry- Low population density, low level of industrial activities and small markets. Ikorodu- Sub-urban/Rural with moderate population density, low industrial activities, moderate vehicular traffic and medium sized markets.Ojo- High population density, large electronics market, high vehicular traffic, numerous auto-mechanic workshops and open burning of worn-out electronics. Yaba- Highly urbanized, high population density, high vehicular traffic and numerous auto-mechanic workshops.

Efforts were made to select permanent or semi permanent ponds which are far from areas of reach of automobile tires and pedestrians. The ponds were often close to thickets and generally shallow (less than 50 cm).

Sampling technique

Soil and water were obtained once within the study period from sediment and ponds in each sampling sites, in triplicates and mixed together for each location so as to portray the average conditions in the respective areas. Soil samples were collected using a 10 kg auger in an area of 10 m^2 radius while water samples were scooped using new 1 L plastic cans which have been previously unused. Both samples were kept in flasks lagged with ice packs until transferred to the laboratory where they were stored at 4°C prior to analysis.

H. occipitalis from each sampling location were caught using sweep nets and transferred to properly aerated cages made from wire gauze, with floors lined with leaves. The frogs were treated humanely according to local ethical standards (University of Lagos) and dissection was carried out in the laboratory to collect liver and muscles for analysis after immobilizing them with cotton wool soaked in chloroform.

Determination of physico-chemical characteristics of sediment and water

Physico-chemical characteristics of the water and sediment samples were determined *ex situ* in the laboratory at least 48 h after sampling. The pH, electrical conductivity (EC), total dissolved solid (TDS) and dissolved oxygen (DO) were measured with a

Metler Toledo (Model In lab 730), and a Jenway 9720 DO_2. Turbidity was determined *ex situ* using HACH DR 2000 direct spectrophotometer method 8237 and then estimated against deionized water as blank at 450 nm. Colour, total suspended solids (TSS), nitrate (NO_3-N), phosphate (PO_4^{3-} -P) and sulphate (SO_4^{2}) were determined using HACH DR 2000 spectrophotometer. Acidity, alkalinity, chloride ion (Cl^-) and biological oxygen demand (BOD) were analyzed volumetrically according to the method described by APHA-AWWA-WEF (2005).

Water samples were incubated in the dark for 5 days at 20°C in BOD bottles so as to determine the BOD in mg/l.

Chemical oxygen demand (COD) was also analyzed *ex situ* by Winkler's titrimetry method (APHA-AWWA-WEF, 2005). Briefly, 5 ml of water samples were measured in a test tube and then followed by addition of 1 ml mercury sulphate, 5 ml H_2SO_4 and then 25 ml of $KMnO_4$. The solution was titrated against aqueous ammonium sulphate after being refluxed for 2 h and allowed to cool. Ferroin, an iron complex was used as an indicator of the end point.

Determination of heavy metal levels in sediment, water and tissues

Metal levels were evaluated in sediment, water and tissues using techniques employed by Don-Pedro et al. (2004). Fleshy portions of the muscles of frogs were oven dried at 70°C for 1 h and ground to powder in ceramic mortars and 5 g of each sample were made into paste by adding double distilled water. This was followed by digestion using 10 ml of 1 M HNO_3 and mild heat until brown fumes appeared. The samples were cooled off, made up to 50 ml in a standard volumetric flask which have been subjected to acid wash to remove any trace of residual metals and then filtered prior to analysis.

Water samples were also digested and prepared using 1 M HNO_3 after being evaporated in a heated sand bath (70-80°C) according to the method of APHA-AWWA-WEF (2005). Sediment samples were sieved using a 200 µm sieve and the uniform particles were then digested using established technique (Agemian and Chau, 1976). All three samples were each subjected to Atomic Absorption Spectrophotometer (AAS) using Perkin Elmer series AAS to determine levels of selected heavy metals against known standards of mixed heavy metal elements (SIGMA Aldrich) by comparing absorbance. The samples were run twice and mean was recorded as the concentration of heavy metals in mg/kg in the respective tissue, water and sediment.

Determination of heavy metal accumulation

The biota to soil accumulation factor (BSAF) and bio-concentration factor (BCF) were determined as ratio of heavy metals in the bullfrogs to that in the soil and water samples as follows:

$$BSAF = \frac{Concentration\ of\ heavy\ metal\ in\ animal\ tissue}{Concentration\ of\ heavy\ metal\ in\ soil\ sample}$$

$$BCF = \frac{Concentration\ of\ heavy\ metal\ in\ animal\ tissue}{Concentration\ of\ heavy\ metal\ in\ water\ sample}$$

Measurement of oxidative stress markers

Following dissection, liver samples were stored in the freezer at -20°C prior to biochemical analysis within 48 h. Measurement of oxidative stress markers were based on established methodologies. Superoxide dismutase was determined as described by Sun and Zigma (1978). Reduced glutathione (GSH) was determined according to Sedlak and Lindsay (1968) and lipid peroxidation based on the method of Buege and Aust (1978). The Biuret method was used in determining the total protein levels in liver samples (Gonall et al., 1949).

Data analysis

The obtained data for physicochemical properties of water samples and heavy metal concentrations were analyzed using one way analysis of variance (ANOVA) performed with GraphPad Prism 5 software (GraphPad Prism software Inc. La Jolla, CA, USA)®. Significantly different results were established at $P<0.05$.

RESULTS

Physico-chemical characteristics of water samples

The results of the physico-chemical assessment of water samples from all the study locations indicates that conditions differ widely between sites, thus reflecting the ecological diversity and differences in anthropogenic pressure in different parts of the City. The physico-chemical characteristics of the sampling ponds are presented with detail in Table 1. The yellowish colouration of the water was due to the muddy and shallow nature of the ponds which are typical of anuran habitats in this area. Turbidity ranged from 0.25 FTU at Ikorodu to 5.0 FTU at Amuwo. Conductivity, TSS and TDS were highest in the ponds around highly urbanized areas such as Amuwo-Odofin, Ojo and Yaba where human activities tend to be high and ponds are located close to human dwellings, markets and industrial sites. This trend was also reflected in the high acidity, COD, sulphate and low pH recorded in most areas particularly around the industrial town of Amuwo. The pond water pH was generally low across the stations, with Amuwo-Odofin and Ojo having particularly low vales of 4.10 and 4.20 respectively while the highest value of 6.10 was recorded at Badagry. This was also expressed in the high level of total acidity across all sampling locations. TSS was above the Nigerian Federal Environmental Protection Agency (FEPA) acceptable limit of 30 mg/l at the three most polluted sites (Amuwo-Odofin, Ojo and Yaba). Ojo and Yaba had the highest nitrate levels: 4.80 and 5.90 mg/l, respectively. Phosphate levels remained <1 mg/L across sampling locations with less urbanized locations of Badagry and Ikorodu having the least values. Incidentally these sites also had high BOD, values which are higher than the FEPA limit of 50 mg/L.

The nitrate levels, however, fell within the national approved limit for water. Measured total alkalinity of the surface water was >20 mg/L in most stations except at Yaba. Dissolved oxygen level was generally low and remained at the <5 mg/L set limit in most cases. Except for DO and BOD, all physiochemical characteristics of the water in the frog habitats, varied significantly ($P<0.05$).

Table 1. Levels of some physical-chemical parameters in water from five Local Government Areas of Lagos State (values are mixed triplicates of water samples collected once).

Parameter	Study location					FEPA limit
	Amowu-Odofin	Ojo	Yaba	Badagry	Ikorodu	
Colour	Yellow	Yellow	Yellow	Yellow	Yellow	NS
Turbidity (FTU)	5.0	4.0	4.96	4.34	0.25	10
Conductivity (µS/cm)	1560	1780	1476	225	196	NS
Total suspended solid (mg/l)	110	100	120	85	3	30
Total dissolved solids (mg/l)	746	570	640	125	96	200
pH	4.1	4.2	6.8	6.1	6.6	6.0-9.0
Total acidity (mg/l)	56	26	38	54	20	NS
Total alkalinity (mg/l)	24	30	16	24	36	20
Chloride (mg/l)	544	610	512	198	44	NS
Nitrate (mg/l)	2.6	4.8	5.9	3.7	2.4	10
Phosphate (mg/l)	0.16	0.45	0.15	0.53	0.91	5.0
Dissolved oxygen (DO) (mg/l)	20.0	4.0	4.2	4.2	4.0	5.0
Biological oxygen demand (BOD) (mg/l)	86.0	76.0	80.0	36.0	15.0	50
Chemical oxygen demand (COD)	172.0	184.0	96.0	135.0	32.0	NS
Sulphate (mg/l)	198.0	240.0	113.0	150.0	0.3	NS

NS = Not stated; FEPA = Federal Environmental Protection Agency.

Heavy metal concentrations and bioaccumulation in the tissues of *H. occipitalis*

Heavy metals were ubiquitous, occurring at varying concentrations across the study sites (Table 2). Cadmium (Cd) was detected in the soil, water and sediment at two of the sampling locations (Ojo and Badagry), which lie closest where electronic are sold and worn out parts are dumped openly. The concentration of Cd in the sediment samples from the different sites differed significantly ($P<0.05$), having a highest concentration of 0.60 mg/kg around the Ojo electronics market; as well as biota to soil accumulation factor (BSAF) of 0.35. Copper (Cu), iron (Fe) zinc (Zn) and nickel (Ni) were detected in the water, sediment and frogs in all the five sample locations. Cu was highest in water at Ojo and lowest at Amuwo-Odofin, while in the sediment it was highest at Ikorodu and lowest at Badagry. The sediment concentrations of Cu, unlike in water and tissue samples was significantly varied ($P<0.05$) between sampling sites across the Lagos metropolis. Bio-concentration factor (BCF) of Cu was highest at Amuwo-Odofin (236.67) while the highest BSAF of 12 was obtained in the tissues of *H. occipitalis* at Badagry. Fe concentration in water, sediment and tissue also varied significantly ($P<0.05$) between sampling sites. It was highest in sediments from Amuwo-Odofin and lowest at Yaba, while in the water samples, Fe was highest at Badagry and least at Ikorodu, with BCF of 19.38 at Yaba as the highest and 4.11 at Ikorodu as the lowest.

Zn levels in water did not vary significantly ($P>0.05$) between sampling sites and overall, it was highest in water samples at Amuwo-Odofin and lowest at Ikorodu, while in the sediment, it was highest at Yaba and least at Badagry. The sediment and tissue concentrations of Zn however, were significantly different ($P<0.05$) among the sampling sites. High BCF of 121.04 and 149.42 were recorded at Ojo and Yaba, respectively for this heavy metal. BASF with respect to Zn was highest at Badagry (171.49) and lowest at Ikorodu (0.55). Lead (Pb) was heavy metal with the lowest concentration recorded in water, sediment and muscles of *H. occipitalis* and values were not significantly different across sampling sites. It was not detected in all three components at Badagry while at Amuwo-Odofin it was only detected in the muscle tissues at a concentration of 0.27 mg/kg. Overall, Ni recorded the highest level of BCF as compared to the other metals with a value of 514 at Ikorodu. Also, its concentrations in sediments and the frog tissues was significantly different across sampling sites.

Assessment of oxidative stress

H. occipitalis were found to express significantly different ($P<0.05$) anti-oxidative stress enzyme activities and lipid peroxidation and across sites (Table 3). Reduced glutathione was induced highest in *H. occipitalis* collected at Amuwo (145.60 ± 50.38 units), while it was lowest at the sub-urban/rural town of Ikorodu (27.43 ± 4.02 units). GSH levels interestingly exhibited strong positive correlation with concentrations of Cu, Fe and Zn in the *H.*

Table 2. Water (n=3), sediments (n=3) and *H. occipitalis* muscle tissue (n=5) heavy metal concentrations (mg/kg), biota to soil accumulation factor (BSAF) and bio-concentration factor (BCF) in sites of Lagos State.

Sampling location	Heavy metals					
	Cd	Cu	Fe	Zn	Pb	Ni
Amuwo-Odofin						
Water	ND	0.03[a]	5.88[a]	6.67[a]	ND	0.03[a]
Sediment	0.61[a]	0.98[a]	42.03[a]	15.21[a]	ND	1.09[a]
Tissue	0.61±0.01[a]	7.10±0.11[a]	109.10±3.68[a]	120.20±1.49[a]	0.27±0.03[a]	3.26±0.09[a]
BSAF	1.00	7.24	2.60	7.90	*	2.99
BCF	*	236.7	18.6	18.0	*	125.4
Ojo						
Water	0.01[a]	3.03[a]	4.47[b]	0.96[a]	0.06[a]	0.34[a]
Sediment	0.60[b]	1.06[b]	31.40[b]	12.68[b]	0.08[a]	1.22[b]
Tissue	0.21±0.11[a]	5.33±0.17[a]	48.67±0.36[b]	116.20±2.12[b]	0.12±0.02[a]	3.58±0.01[b]
BSAF	0.35	5.03	1.55	9.16	1.5	2.93
BCF	21	1.76	10.9	121.0	2.0	10.5
Yaba						
Water	ND	1.08[a]	5.80[c]	0.86[a]	0.83[a]	0.47[a]
Sediment	0.14[c]	1.19[c]	7.33[c]	20.46[c]	8.35[a]	0.98[c]
Tissue	ND	13.40±0.19[a]	112.40±2.03[c]	128.50±1.69[c]	18.24±0.89[a]	7.28±0.37[c]
BSAF	*	11.26	15.33	6.28	2.18	8.14
BCF	*	12.4	19.38	149.4	22.0	15.5
Badagry						
Water	0.23[a]	0.44[a]	6.08[d]	2.72[a]	ND	0.94[a]
Sediment	0.42[d]	0.07[d]	8.33[d]	0.67[d]	ND	0.01[d]
Tissue	5.03±0.14[a]	0.84±0.02[a]	50.70±0.43[d]	114.90±0.78[d]	ND	3.20±0.15[d]
BSAF	11.98	12	6.09	171.49	*	320
BCF	21.9	1.9	8.3	42.2	*	3.4
Ikorodu						
Water	ND	0.08[a]	0.26[e]	0.22[a]	0.20[a]	0.01[a]
Sediment	0.26[e]	1.34[e]	13.14[e]	6.77[e]	ND	0.79[e]
Tissue	ND	0.74±0.02[a]	3.19±0.11[e]	3.70±0.07[e]	4.90±0.17[a]	5.14±0.16[e]
BSAF	*	0.55	0.24	0.55	*	6.51
BCF	*	9.3	4.11	16.8	24.5	514.0

ND = Not detected; * = Data not available; dissimilar alphabets (a, b, c, d, e) implies significant differences per metal.

Table 3. Antioxidant enzyme activity in liver tissues of African tiger frogs *(H. occipitalis)* collected from five local government areas of Lagos State (mean ± SD; n= 5).

Biomarker	Sampling location (LGAs)					
	Amwuo-Odofin	Ojo	Yaba	Badagry	Ikorodu	F Cal
MDA (units)	3.31 ± 1.62	5.60 ± 3.65	4.21 ± 4.22	8.60 ± 2.69	5.87 ± 2.44	[b]4.32
GSH (Units)	145.60 ± 50.38	126.80 ± 16.70	121.50 ± 44.93	68.38 ± 8.65	27.43 ± 4.02	[c]24.11
SOD (U/mg pro)	1.09 ± 0.82	1.86 ± 2.21	1.18 ± 0.33	1.25 ± 0.71	2.59 ± 0.98	[a]2.85
Protein (mg)	77.43 ± 7.21	68.68 ± 32.79	90.38 ± 14.45	113.30 ± 9.19	34.38 ± 7.42	[c]28.64

The letters a, b, c represent how significantly different each parameter is across the different locations.

occipitalis muscle tissues. There was however a negative correlation (r = -0.5721) with tissue Ni levels and a weakly positive (r = 0.3458) with Cd. With respect to SOD induction, the reverse was the case because the lowest value (1.09 ± 0.82 µ/mg protein) was obtained at Amuwo-Odofin, while the highest (2.59 ± 0.98 µ/mg protein) was detected in those from Ikorodu, indicating that those obtained at Amuwo-Odofin were under a much more intense oxidative stress. The SOD levels showed strong negative correlation with Cu (r =-0.5477), Fe (r =-0.8809) and Zn (r =-0.6993) across sampling sites but Cd and Ni showed weak negative correlation. Lipid peroxidation as expressed by the malondialdehyde levels (MDA) was inconsistent among the frogs across all sampling locations. It also showed negative correlation with metals in tissues of frogs from the various sites except for Ni which was strongly positively correlated (r = 0.9155) with the lipid peroxiidation product.

DISCUSSION

The findings in this study points to widespread pollution which varies significantly depending on the predominant activities in the area and hence unsustainable utilization of the environment. Amphibians are physiologically sensitive animals, inhabiting some of the harshest and unpredictable habitats (Hickman et al., 2008) and hence are already living under adaptation stress. In the present study, the physico-chemistry of waters, levels of heavy metals and certain biomarkers of oxidative stress as surrogate bioindicators of aquatic pollution in *H. occipitalis* were evaluated.

Measured levels of physical and chemical characteristics of water bodies show clearly a large disparity between sampling locations. Nitrate and TDS levels in all sites remained lower than the Nigerian National set limit of the Federal Environmental Protection Agency (FEPA, 1991). Whereas high BOD and COD levels were recorded particularly in areas associated with high anthropogenic activities, such Amuwo, Ojo and Yaba LGA/LCDA. These high readings of chemical and biological oxygen demand were corroborated by the generally high sulphate, phosphate, suspended solids and most of the heavy metals assessed. Saliu and Ekpo (2006) have earlier reported high level of phosphate and nitrate levels in Ogbe creek which receives high input of sewage and other organic wastes in Lagos. Population density is often associated with high level of waste generation and hence pollution (Ajao et al., 1996). Areas around Ikorodu Local Government which is characterized by relatively moderated population density and low level of industrial activities either recorded the lowest values or generally low values of pollution indicating parameters. Low pH values indicative of high acidity were recorded in all sites with levels below 5.0 at Amowu-Odofin and Ojo, which are associated with high level of commercial and industrial activities. According to Freda (1986), acidity of ponds has been linked with reproductive effects in amphibians by causing direct mortality of embryo and larvae which are the most sensitive stages of their development.

Domestic sewage, combustion, emissions, mining operations, metallurgical activities and industrial effluents, all sources of anthropogenic metal inputs into the environment are prevalent in the sampling areas particularly at Amuwo-Odofin, Ojo and Yaba, and according to Chinni and Yallapragda (2000) most of metallic inputs into the environment exhibit some form of toxicity. Badagry and Ikorodu are less inhabited and disturbed, although they are the new choice areas for urban sprawl and unplanned human settlements. The levels of Pb were significantly higher in Yaba than in other locations, probably due to the high daily automobile traffic density and the preponderance of road side mechanic workshops from where Pb leaches into the environment. High sediment Cd and Ni levels recorded at Ojo could be linked to leachates from electronic and battery waste dumps which are common in the area.

The non-biodegradability of these metals makes them persistent and increases their environmental health impacts. The recent Pb poisoning in Gold mining communities in Zamfara State, North-Western Nigeria which lead to the death of hundreds of children and domestic animals upon chronic exposure in soil and water (WHO, 2010) raises important concern regarding the extent of potential harm possible from unregulated commercial practices prevalent in the country. Cd accumulates in the liver and kidney, leading to massive proteinuria as a result of breakdown of kidney cells when the metal levels exceed critical concentrations (Walker et al., 2001). The toxicity of cadmium, like few other metals lies in its high bioaccumulative tendency (Hopkins, 1989).

Generally, sediments accumulated more heavy metals than the water and these confirms earlier observations by Don-Pedro et al. (2004) in the Lagos lagoon. Given the static nature of sediments, they tend to accumulate more toxicant than water which may flow away, drain off or even evaporate. Iron and zinc were higher in both water and sediments samples when compared with other heavy metals in most sampling areas.

The high content of Fe and Zn in the sediment may be due to clayey material that forms the soil structure in the area sampled. Don-Pedro et al. (2004) also reported high Fe and Zn concentrations in the sediments of the Lagos lagoon. The high concentration of metals in sediment may also be attributed to human activities such as wastes from automobiles and generator fumes, burning of fossil fuel, discharge of untreated sewage and industrial effluents containing metals into water bodies, as well as the natural ability of the sediment to act as sink (Kakulu and Osibanjo, 1988). This is in consonance with Odiete (1999), who concluded that sediment is the major depository of metals, in some cases, holding more than 99% of the total amount of a metal present in the aquatic

system.

The muscle burdens of Fe and Zn in *H. occipitalis* were significantly higher than those of other metals in all sites except for Ikorodu. The reason for this may be traced to dietary intake since frogs feed on herbivorous insects that may have accumulated these metals from aquatic plants which constantly absorb these metals from the soil Nummelin et al. (2007), as well as through direct uptake from contaminated water and sediments. The concentration of Pb was generally low, but in Yaba, it was significantly higher in tissues than the environment and as indicated by BCF, this could be as a result of high level of bioaccumulation directly from the environment from atmospheric depositions given the high vehicular traffic in this area. This is in line with the findings of Charles et al. (1986) who reported low bioaccumulation of Pb in bullfrog (*Rana catesbeian*) and green frog (*Rana clamitans*) tadpoles inhabiting highway drainages. High accumulation of Zn in the tissue of frog 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 (Jaffar and Pervaiz, 1989). Zinc also acts as a catalyst in metal biomolecules and sulphur ligands to form tetrahedral zinc metalloproteins.

Zinc (BSAF) was also highest in almost all sampling areas and this may also be linked with the natural tendencies of biota to take up Zn, for being an essential metal. However, it must be noted that the levels of metals detected in most sampling stations were significantly higher than the Nigerian national set limits (FEPA, 1991), thus threshold of tolerance may often be exceeded leading to oxidative stress and physiological imbalance, deformities and mortality. Wall (1999) had earlier published a thesis that confirmed the ability of heavy metals to induce abnormalities such as teratogenicity in the northern leopard frogs (*Rana pipens*) obtained from creeks and marsh lands in Vermont, USA.

MDA production did not follow a uniform pattern and was not consistent with metal levels in the different areas studied and this may be due to some other extraneous factors not captured within this study. Membrane damage and repair occur simultaneously in different cells leading to the formation of unsaturated fatty acids and the production of the lipid peroxidation product, MDA (Timbrell, 2000).

The simultaneous process of damage and repair could have accounted for the irregular levels of MDA detected in the sampled frogs irrespective of location. This contradicts the findings of King et al. (2012) who reported a consistent increase in the lipid pero-xidation product in catfishes, *Clarias gariepinus* exposed to sub lethal concentrations of petroleum products and crude oil. However, the definite trend of responses in the case of King et al. (2012) could be due to the stable conditions that a laboratory environment confers as compared to what is obtainable in field studies like this present one.

Hence, MDA did not appear to be suitable for use as biomarker of heavy metal pollution stress against *H. occipitalis* in non laboratory conditions. Frogs captured at Ikorodu area with the lowest levels of heavy metals, had the highest level of SOD activity. This may imply causal factors other than heavy metal pollution. SOD levels in this study appeared to be lower in frogs from areas with higher metal pollution, showing a somewhat inverse relationship. This was corroborated by the consistent negative correlation reported between the heavy metals accumulated in the frog tissues and SOD activities. This was however in contradiction with reduced glutathione (GSH) values estimated, because higher levels of GSH activity were detected in frogs from the most polluted areas, that is, Ojo, Yaba and Amuwo-Odofin. Interaction of toxic heavy metals with GSH metabolism is an essential part of the toxic response of many metals (Hultberg et al., 2001).

When GSH is depleted by metal, its synthesizing systems start generating more GSH form cysteine via r-glutamyl cycle. If GSH depletion continues because of chronic metal exposure, several enzymes in antioxidant defense systems may protect this imbalance (Hultberg et al., 2001; Stohs and Bagchi, 1995). This investigation revealed that the level of Pb in Yaba was highest and this corresponds with high level of GSH activity in frogs from this location. This is probably due to the fact that GSH received a boost by antioxidant enzymes and self re-synthesis after depletion as observed by King et al. (2012) and Hultberg et al. (2001). The apparent GSH activity in liver samples also suggests an adaptive and protective role of this biomolecule against oxidative stress induced by prevailing environmental stressors including heavy metals. The results are in agreement with the findings of Stohs and Bagchi, (1995) on fish from Painpat River in India.

Conclusion

This study has revealed that there is widespread heavy metal pollution in Lagos State emanating from diverse sources. The bioaccumulative potentials of measured heavy metals in *H. occipitalis* obtained around Lagos metropolis raises salient pollution management questions. The fact that oxidative stress markers (GSH and SOD) correlated with heavy metal accumulation in some locations makes a case for their use in monitoring environmental pollution. The findings also raise concerns regarding the effectiveness of lipid peroxidation product MDA as an effective biomarker of oxidative stress resulting to environmental pollution in anurans in the wild.

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