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Heavy metal bioaccumulation and oxidative stress in *Austroaeschna inermis* (Dragon fly) of the Lagos Urban ecosystem

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Urban ecosystems are often characterized by the receipt of pollutants, especially heavy metals from diverse anthropogenic activities. To better understand the distribution of heavy metals (Cd, Cu, Pb, Mn and Zn), *Austroaeschna inermis* from five different sites (Unilag, Mile 12, Olushosun Dump site, Imoshe and Badagry) in Lagos, sediments from the respective sites were assessed. This was followed by assessment of lipid peroxidation product; Malondialdehyde (MDA) and antioxidative stress enzymes; superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and reduced glutathione (GSH) in *A. inermis*. The results indicate widespread heavy metal distribution with Mn and Zn having the highest concentrations of 13.369±0.800 mg/kg and 21.473±2.001 mg/kg in sediment samples from Mile 12 and Olushosun Dump site respectively. Only Cd was bioaccumulated at two sites (Unilag and Badagry) with biota to soil accumulation factor (BSAF) of approximately 2. The oxidative stress biomarkers assessment in the insects did not indicate any trend to link heavy metal concentrations with respective sites. However there was strong ($r \geq 0.5 < 0.7$) to very strong ($r \geq 0.7$) positive correlation between Pb concentrations in *A. inermis* and most biomarkers. All enzymes and MDA showed negative correlation with the other heavy metals with values mostly between strong ($r \geq -0.5 < -0.7$) to very strong ($r \geq -0.7$) negative. The findings from this study reaffirms the ubiquity of heavy metals in the City of Lagos and the relevance of the insects as pollution indicators were discussed.

Key words: Biomonitoring, urban ecology, pollution, biomagnification.

INTRODUCTION

Heavy metals are among the most problematic causes of water and soil pollution, a situation heightened by their ubiquity and evolving knowledge of the biological effects. Although most metals occur naturally in rocks, ores, soil, water, and air, their levels are usually low and widely dispersed (Otitoloju, 2000). Metals that are of environmental concern fall into three classes: suspected carcinogens, those that are readily in soil and those that move through the food chain (Hodgson, 2011). Anthropogenic activities are the major culprits in the

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release of heavy metals in recent times.

Abiotic indices such as soil, air and water analysis alone are inadequate for the assessment of the availability and potential toxicity of contaminants to humans and wildlife (Talmage and Walton, 1991). Metal accumulation in the soil can cause harm to biota, by altering physiological activities and causing many genotoxic effects (Sanita di Toppi and Gabbrieli, 1999; Panda and Panda, 2002), disruption of reproductive potential and endocrine system (Drevnick and Sandheinrich 2003; Kasperczyk et al., 2008), immunosuppression (Carey and Bryant, 1995), induction of stress proteins (Piano et al., 2004) and oxidative stress (Soundararajan et al., 2009; Farombi et al., 2007).

Health risk due to heavy metal contamination of soil has been reported (Eriyamremu et al., 2005; Muchuweti et al., 2006). But the biotoxic effects of heavy metals depend upon the concentrations and oxidation states of heavy metals, kind of sources and mode of deposition (Duruibe et al., 2007). Most studies on metal accumulation and toxicity have focused on vertebrates, but recent works has revealed both negative and positive effects of heavy metals on the host defense response systems of marine invertebrates (Oweson and Hernroth, 2009; Vijayavel et al., 2009) and terrestrial insects (Sorvari et al., 2007; Van Ooik et al., 2008).

Insects are strategic to the welfare of man (Ewuim, 2004) and constitute a major component of the earth's biodiversity with their species richness or diversity exceeding that of any group of extant organisms. They account for 20,000 species (90.54%), many of which contribute significantly to the maintenance of life support systems, with 99.90% of the insect species being beneficial or neutral to man (Ivbijaro, 2003). They are abundant in a wide range of habitats including both terrestrial and aquatic ecosystems (especially fresh water) and including wetlands, either as aquatic or sub-aquatic species, even though they have never adapted to a typical marine environment (Cheng, 1976).

Insects inhabiting coastal areas receiving multiple pollution sources by urban, industrial, and agricultural activities are exposed to complex mixtures of different types of contaminants; while some are found in moist soil littered with dead organic matters (Fukul, 1996).

Dragonflies are amphibious in nature, with adults which fly about on land while their larvae are aquatic. Odonates are very sensitive to changes in habitat quality and as such they are used for monitoring impairments resulting from anthropogenic activities and long term climatic changes (Corbet, 1999; Oertli et al., 2002; Dijkstra, 2007).

The dispersal capabilities of dragonflies correspond to their ecological requirements (Adu and Ogunjobi 2014). Due to the sensitivity of these insects to environmental changes, both the larvae and adults may be used as bio-indicators of environmental conditions (USEPA, 2012).

In keeping with the recent trend in the use of arthropods for biomonitoring, we investigated heavy metal burden of dragonfly (*Austroaeschna inermis*) - ubiquitous in the City of Lagos as well as the correlation between oxidative stress markers and heavy metal levels in dragonfly (the most ubiquitous of the three) so as to draw conclusions which may provide vital insights for future investigations.

MATERIALS AND METHODS

Study design

Five sites in the urbanized western section of Lagos mainland, including Unilag, Imoshe, Olushosun Dumpsite, Mile 12 and Bagdagry (Figure 1) were selected for this study on the basis of the spread in the kind and level of anthropogenic influence in the area. The simple random sampling method was further used to pick triplicate insect samples from the sample sites.

Classification of study sites

The detailed description of the five study sites are as follows:

(i) Owode in Mile 12 (06° 36. 252'N and 003° 24.457'E) characterized by very high human and vehicular density as well as automobile emissions;
(ii) Imoshe (06° 32.348'N and 003° 12.632'E), a relatively less dense area characterized by the presence of a flowing stream where transportation and fishing activities occur;
(iii) Bagdagry (06° 30.664'N and 002° 57.543'E) characterized by farming activities with low industrial and transportation activities;
(iv) Olusosun Dumpsite (06° 35.791'N and 003° 22.766'E), established in 1991 and became operational in 1992, believed to be the largest landfill in Lagos State and Nigeria and managed by Lagos state waste management authority (LAWMA). The site is about 42 hectares of land and in terms of capacity it receives between 40-45% of solid waste generated in Lagos State (9000metric tons of waste per day);
(v) Unilag (University of Lagos) - (06° 32.352'N and 003° 23.854'E), busy campus with high population and vehicular density.

Sampling operations

Sediment samples from fresh and dried ponds from the five sites were collected using a 10 kg soil auger in triplicates and pulled into one for each of the sites making a total of five samples. Samples were kept in flasks lagged with ice packs until they were transferred to the laboratory where they were stored at 4°C prior to analysis.

The insects were caught using sweep nets and transferred in properly aerated cages made with wire gauze, with floors lined with moist soil samples from different locations. The insects were collected by sweeping across vegetations at three different locations within each site in the early hours of the day. They were transported to the laboratory in plastic bottles containing soil from collection sites. Within 24 h of collection, they were frozen at -20°C for preservation prior to analysis.

Heavy metal analysis for sediment samples

The sediment samples were allowed to dry at room temperature and passed through a 2 mm sieve. 5 g of the sieved sample were

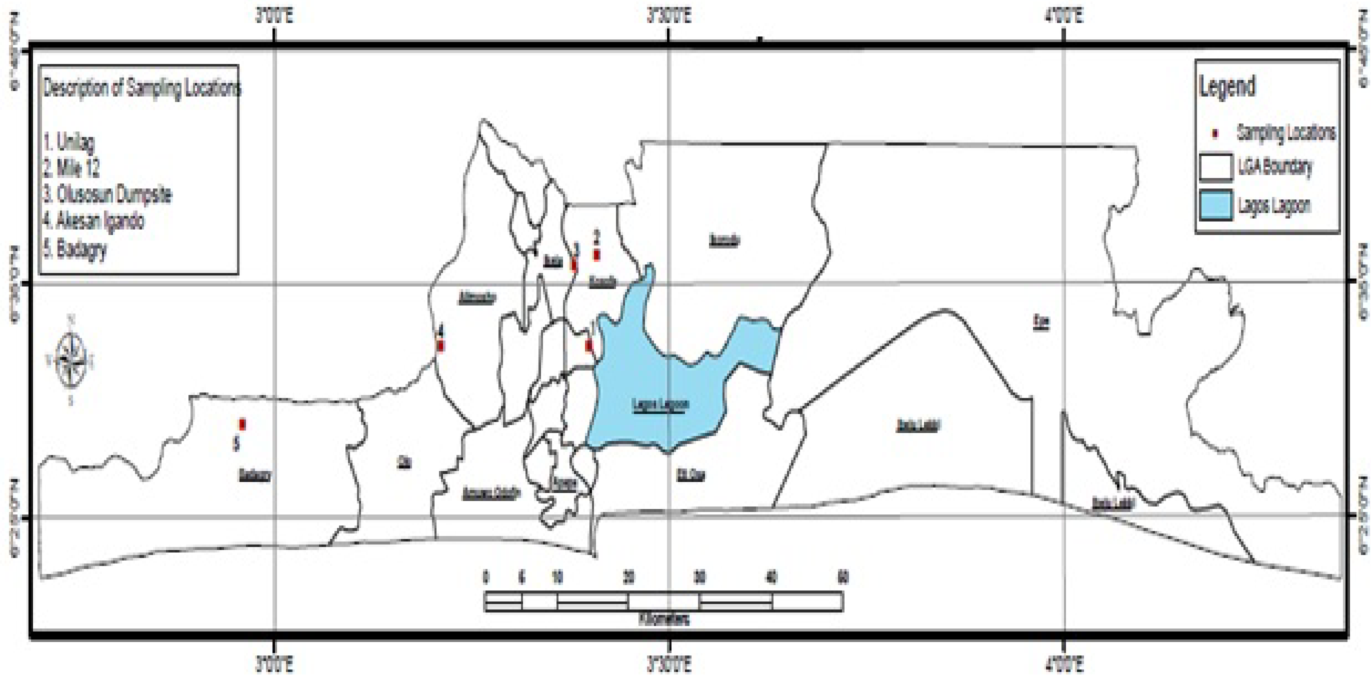

Figure 1. The distribution of sampling sites for the study in Lagos State.

weighed and 10 ml concentrated nitric acid added. The mixture in a beaker was covered with a watch glass and refluxed for 45 min. The watch glass was then removed and the content in the beaker evaporated to dryness. 5 ml aqua regia was added and evaporated to dryness after which 10 ml, 1 M nitric acid was added and the suspension filtered. The filtrate was then diluted to volume with distilled water in a 50 ml volumetric flask. The Concentrations of heavy metals - Cd, Cu, Mn, Zn and Pb - were determined using a Perkin Elmer 403 Atomic Absorption Spectrophotometer (AAS) at wavelengths specific to each metal based on the method reported by Don-Pedro et al. (2004).

Determination of heavy metal content in these insects

About 0.5 g of the insects were weighed into a Teflon bomb and 5ml of aqua-regia (1:3 of HNO_3: HCl) was added and then 5ml of Hydrogen fluoride. The Teflon bomb was sealed and heated in an oven for 6 h at 165°C. After the digestion, 10 ml of saturated boric acid solution was added to the mixture and allowed to cool at room temperature about 2 h. The resulting solution was properly mixed and transferred into a 50 ml standard flask and made up to the mark with distilled water. The digest solutions were used to analyze for the metals in an AAS as in sediment analysis

Determination of Lipid peroxidation levels and Antioxidative stress enzyme activities

Dragonflies collected for biochemical assays were removed from the refrigerator and allowed to defrost. They were first homogenized as whole insects and then the protein content determined by Biuret method (Gonall et al., 1949). The level of lipid peroxidation was determined based on the thiobarbituric acid (TBA) reactivity assay (Yagi, 1998).

Superoxide dismutase was determined as described by Sun and Zigma (1978) at absorbance at 480 nm for 5 min. The catalase activity was determined according to the method of Beers and Sizer as described by Usoh et al. (2005) by measuring the decrease in absorbance at 240 nm due to the decomposition of Hydrogen peroxide (H_2O_2) in a UV recording spectrophotometer with the specific activity of catalase was expressed as moles of H_2O_2 reduced per minute per mg protein.

Reduced glutathione (GSH) was determined as non-protein sulphydryls estimated according to the method of Sedlak and Lindsay (1968) while glutahthione-s-transferase (GST) activity was measured using the method of Habig et al. (1974) using 1-Chloro-2, 4-Dinitrobenzene (CDNB) as substrate.

Data analysis

The Data obtained for assessment of heavy metal concentrations were presented as mean ± SD. Statistical analysis was performed using the SPSS statistical package, with significance level determined at $p<0.05$. Two-way analysis of variance (two-way ANOVA) was applied to determine differences between sites and sampling periods. Kolmogorov- Smirnoff test and Levene's test were applied to test normal distribution and homogeneity of variance, respectively.Data were log-transformed where necessary. Correlations between biomarkers and heavy metals were examined by Pearson's correlation coefficient. Determination of Biota to soil accumulation factor (BSAF) was as reported in Idowu et al. (2014).

$$BSAF = \frac{\text{Concentration of heavy metal in insect tissue}}{\text{Concentration of heavy metal in sediment sample}}$$

Table 1. Heavy metal concentration in soil samples (mean ± S.D) and *Austroaeschna inermis* across the five sampling sites in Lagos State.

Sampling sites	Heavy metals (mg/kg)				
	Cd	Cu	Mn	Pb	Zn
Unilag	-	-	-	-	-
Sediment	0.027±0.007	0.997±0.225	8.395±0.696	0.227±0.139	20.743±1.686
A. inermis	0.050[a]	0.181[a]	2.929 [a]	ND	3.893[a]
Mile 12					
Sediment	0.109±0.061	1.278±0.216	13.369±0.800	2.820±0.370	18.327±2.210
A. inermis	0.028[b]	0.113[a]	1.914[b]	0.007[a]	1.383[a]
Olushosun Dump site	-	-	-	-	-
Sediment	0.1±0.078	1.038±0.085	10.081±1.260	4.723±0.409	21.473±2.001
A. inermis	0.011	0.014	3.100	0.004	0.448
Imoshe					
Sediment	0.02±0.014	0.165±0.153	4.73±0.812	0.0153±0.010	5.39±0.848
A. inermis	0.008[a]	0.011[a]	0.757[a]	ND	0.230[b]
Badagry	-	-	-	-	-
Sediment	0.012±0.006	0.859±0.203	5.923±0.794	3.497±0.634	17.73±1.323
A. inermis	0.024[b]	0.036[b]	1.376[a]	0.007[a]	0.759[a]

*ND: metal not detected, different alphabets indicate significant difference ($p<0.05$).

RESULTS

Heavy metal accumulation

Heavy metals were ubiquitous occurring at varying concentrations in the sediments and insects at varying sampling sites (Table 1). The heavy metal with the least concentration in the sediments was Cd, was the second lowest, with its highest concentration recorded at Mile 12 while the least at Badagry sampling site. With respect to Cu, Mile 12 had the highest sediment concentration while Imoshe had the least.

Among the insect, the least concentration was also detected at Imoshe while the highest concentration of Cu as well at Mile 12. Overall, Mn had the second highest concentration in sediments and the highest concentration in the insect. Specifically, the highest Mn concentration was detected at sediments from Mile 12, followed by Olushosun Dump site while the least was recorded at Imoshe. The least insect Mn burden was measured at Imoshe. Lead although detected in all sediment samples was not detected in most insects caught. Its concentration was highest in sediments from Olushosun Dump site, followed by Badagry and Mile 12 while Imoshe had the least concentration. Zn had the highest concentration of heavy metals among the sediments analyzed, with the highest recorded at Olushosun Dump site and the lowest at Imoshe.

The heavy metal analysis in the insect clearly showed a higher mean concentration of Mn and Zn in all the insects across all the sites. The trend entails Mg>Zn>Cu>Cd>Pb in most of the sampling sites. The analysis of variance test carried out on metals found on the insect for the various sites considered does not show significant differences (Table 1). Cd was the only heavy metal that was bioaccumulated and this occurred Unilag and Badagry, having BSAF values of 1.85 and 2.00 respectively (Table 2).

Oxidative stress biomarkers in Dragonflies

The relative levels of oxidative stress enzyme activities as well as the levels of lipid peroxidation product, MDA in the insects are presented in Table 3. MDA levels in the dragonflies was highest at Imoshe and least in those caught around the Olushosun Dump site. With respect to the antioxidative stress enzymes, dragonflies at Olushosun dumpsite had the least activity of SOD while those at Badagry recorded the highest. Catalase activity was however least in those caught at Unilag and highest in those around Imoshe site. The highest level of the reduced glutathione (GSH) was recorded in grasshoppers from Imoshe, followed by Badagry and least in those caught at Olushosun Dump site. Glutathione-s transferase (GST) activity was also highest at Imoshe, followed by Badagry and least Unilag.

Correlation of heavy metal burden with Oxidative stress biomarkers in Dragonflies

The assessment of the overall relationship between mean biochemical biomarker levels with heavy metal

Table 2. The biota to soil accumulation factor (BSAF) for the heavy metals in *Austroaeschna inermis* at the sampling sites

Sampling sites	Heavy metals				
	Cd	Cu	Mn	Pb	Zn
Unilag	1.85	0.18	0.35	ND	0.19
Mile 12	0.026	0.09	0.14	0.00	0.08
Olushosun Dump site	0.11	0.01	0.31	0.00	0.02
Imoshe	0.4	0.07	0.16	*	0.04
Badagry	2.00	0.04	0.23	0.00	0.04

* No data for BSAF calculation.

Table 3. Mean antioxidant enzyme activity and level of lipid peroxidation product, MDA (u/mg pro) in *Austroaeschna inermis* across the sampling sites in Lagos

Sampling sites	SOD[b]	CAT[b]	GSH[b]	GST[b]	MDA[b]
Unilag	2.65	3.34	0.21	26.31	0.028
Mile 12	5.85	4.05	0.36	38.67	0.026
Olushosun dumpsite	0.64	4.15	0.17	40.20	0.020
Imoshe	3.02	7.15	0.95	49.96	0.067
Badagry	9.51	6.14	0.55	44.09	0.034

*Similar alphabets (b) imply no significant difference across sampling sites using Chi squre analysis.

concentrations in the *A. inermis* showed that most had weak to very strong negative correlation (Figure 2). Except for SOD activity, Cd was negatively correlated with all other biomarkers. Lead was positively correlated with CAT and GST activities and very strongly and positively correlated ($r \geq 0.7$) with MDA, SOD and GSH. Except for MDA levels, anti oxidative enzyme activities correlated either strongly negatively ($r \geq -0.5 < -0.7$) or very strongly and negatively ($r \geq -0.7$) with Cu in the dragonflies. Mn was at least strongly negatively correlated with all biomarkers. Zn showed very strong negative correlation with GST and strong negative correlation with CAT and GSH.

DISCUSSION

The ubiquity of heavy metals and their relative importance as pollutants of concern is once again brought to the fore by findings from this study. The mean metal concentration of the sediment samples across the sites was generally less than the limits for heavy metals in the soil (USEPA, 2012). Diverse human activities continually increase environmental concentrations of these toxicants to levels where widespread threat to human and animal health can result (Kurdland, 1960; Pereira et al., 2006).

The concentrations of Zn and Mn were particularly high across the sediments and together with Pb were highest at Unilag, Mile 12 and Olushosun Dump site, three areas that are highly impacted by human activities. Lead was particularly highest at Olushosun dump site, followed by Mile 12. While the former may be associated with leachates from the assorted wastes which its collects, the latter may be associated with the high vehicular density and deposits from diver activities. Of all the dumpsites in Lagos state, Olusosun is the most active in terms of traffic and quantity of waste recovery daily at the dumpsite (Odunaiya, 2002).

The soil is the primary recipient of solid wastes (Nyle and Ray, 1999) as well as tons of wastes from a variety of sources; industrial, domestic and agricultural find their way into the soil. Apart from interacting with the soil system thereby changing the physical and chemical properties (Piccolo and Mbagwu, 1997) as well as productivity, an important cause for concern is the possibility of bioaccumulation of these metals.

The accumulation of contaminants is aided by the capacity of soil to bind with clay minerals and organic substances. Their accumulation has multiple effects on the usability and functions of soil in the ecosystem. The stable nature of soils enables the metals to remain for long periods, enhancing changes of uptake.

Mile 12 recorded the highest level of Cd, Cu and Mn while appreciable amounts of Pb and Zn were also found. This high heavy metal concentration may be due to the emission of atmospheric pollutants by vehicles. Mile 12 is a major junction community and the most important route

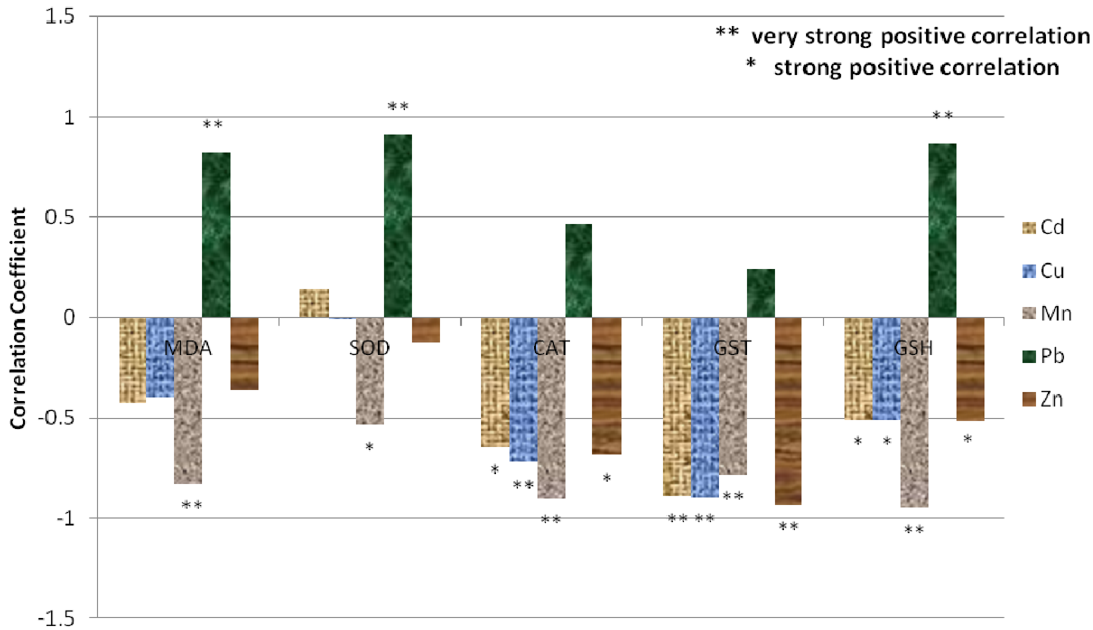

Figure 2. Relationship between heavy metal burden and oxidative stress markers in *A. inermis.*

conveying traffic to and from the Ikorodu axis of Lagos. Road transports contaminate the atmosphere, water and soil near the highway via atmospheric fallout containing potentially toxic metals like lead, cadmium and zinc (ATSDR, 1994, 1999). The least heavy metal concentration was recorded at Imoshe and this could be attributed to the relatively lower level of anthropogenic activity in the area. Imoshe is a coastal community whose primary preoccupation is fishing and crop cultivation. Thus there is limited level of polluting activities in the area. Considerable amount of heavy metals were detected in the insects especially Mn and Zn.

The findings from this study indicates that heavy metal burden were often higher in insects collected at more polluted sites. However, only Cd was bioaccumulated and this can be inferred from the fact that Cd being a very toxic metal as categorized by Walker et al. (2001) is often not found in high concentrations in the environment. These insects acquire heavy metals mostly in ingested food, via water or such as leaf litter, plant material and captured prey, or rarely through dermal absorption (Heliövaara and Väisänen, 1993).

Although comparism is made with the sediments in this study as environmental store of these metals, they are not the most important source of the heavy metals to the insects. Feeding is a much more important route of entry into their system. This therefore may imply that the true BSAF levels is likely to be higher than was recorded in this study because most other sources of the metal are likely to contain lower concentrations than the sediment.

The findings from this study indicate that there was no significant difference between oxidative stress markers in dragonflies across the sampling sites. However there were overall strong positive or negative correlation between heavy metals concentrations in the dragonflies and respective oxidative stress markers in them.

Metals might increase the production of reactive oxygen species, and directly or indirectly cause oxidative damage by inhibiting antioxidant activity (Migula et al., 2004). High concentrations of Cu acts directly, causing an increase in reactive oxygen species, while Cd acts indirectly leading to an increase in cellular iron levels or directly inhibiting the antioxidant activity of glutathione-related enzymes and deplete cellular glutathione (Kang, 1997). Lead recorded strong positive correlation with MDA levels implying that increases in its concentration may be linked with increased cell membrane damage in the insect and subsequently oxidative stress. Metals can enhance oxidative stress and lipid peroxidation in insects especially when other per-oxidant constituents are present in their diet (Ahmad, 1995; Felton and Summers, 1995; Chrascina et al., 1996).

Lead also had strong positive correlation with SOD and GSH, implying that the concentration in the insects was not high enough to inhibit SOD activities or that its mechanism of action does not relate directly with SOD activities. Mn on the other hand had negative correlation with the antioxidative stress enzymes including MDA levels. This also raises questions about their mechanism of action in the insect, the threshold for toxic action and the possibility of an inherent detoxifying mechanism in the insects. Both enhancement and inhibition have been reported for the activity of antioxidant enzymes such as SOD, CAT or GST, depending on the metal levels, form and period of exposure, and insect species (Zaman et al., 1994; Migula and Glowacka, 1996).

Insects waxy cuticle and fatty tissues may also hold these metals in inactive forms, thereby preventing metal penetration and toxic action. Earlier studies on the reactive oxygen species and antioxidant defense mechanism in insects suggested that there exists a regulatory mechanism for balancing pro-oxidants and antioxidants (Ahmad, 1992). Controversially, relatively higher MDA levels in dragonflies were recorded in Imoshe and Bagdagry where the lowest heavy metal values were recorded reflecting stress possibly due to factors other than metal intoxication.

The activity of the enzyme SOD was much lower in dragonflies found in the Dump site compared to the other sampling sites, implying some level of inhibition. The enzyme SOD is known to provide cyto-protection against free radical induced damage by converting superoxide radicals (O^{-2}) generated in peroxisomes and mitochondria to hydrogen peroxides. The hydrogen peroxide is then removed from the system by the enzyme CAT, which converts it to water and molecular oxygen (O_2). The inhibition of the enzyme SOD by the presence of pollutants will therefore lead to increased oxidative stress in the tissues as a result of the damaging effects of the superoxide radicals ($O^{-\ 2}$). Although CAT was not equally lowest at the Dump site, its link with SOD activities is well established. The inhibition of the enzyme SOD is believed to result in a reduction in the activity of the enzyme CAT, due to a decrease in H_2O_2 generation from SOD activities. Similar observation of a decrease in CAT activity following an inhibition of the activity of enzyme SOD has been reported by Fatima and Ahmad (2005).

GST response to toxic chemicals follows a similar bell-shaped trend as CAT (Viarengo et al., 2007) hence increased and decreased enzyme activities have been reported in polluted areas (Regoli et al., 2004). Thus describing a trend for the activities of these enzymes over large study areas and variables is often difficult as observed in this study. Glutathione transferases however plays an essential role in the overall fitness of insects exposed to potentially toxic exo- or endogenous substances and are induced by organic contaminants as part of the phase II biotransformation pathway (Sheehan and Power, 1999). It has been reported to respond differently to different compounds, for example, Hamed et al. (1999) reported that the enzyme was strongly inhibited by dimethoate, while Zhang et al. (2004) reported statistically significant enhancement in GST in animals exposed to oxidative stress of 2,4-dichlorophenol.

The highest levels of MDA in the dragonflies, the key indicator of lipid peroxidation damage was found in the least disturbed sites; Imoshe and Bagdagry. Increased or elevated levels of MDA is due to an inhibitory effect on mitochondrial electron transport system leading to stimulation in the production of intracellular ROS (Stohs et al., 2001).

Elevated ROS level in tissues leads to cellular damage when the rate of its generation surpasses the rate of its decomposition by antioxidant defense systems. The measurements of lipid peroxides levels in plants and animal tissues exposed to different pollutants have been recognized as reliable early warning signal of exposure to environmental stress and therefore often integrated to environmental monitoring programs (Avci et al., 2005; Fatima and Ahmad, 2005; Valavanidis et al., 2006).

Conclusion

The findings from this study reaffirm the varied and dispersed concentrations of heavy metals in Lagos as reported earlier by Idowu et al. (2014). The heavy metal concentrations in the insects with respect to the sediment samples did not reflect widespread bioaccumulation. This may imply that a feeding route of bioaccumulation assessment may be more important for terrestrial insects than absorption through their cuticle and other passive processes. Although there was either at least strong positive or negative correlation between heavy metal burdens and oxidative stress markers in the insects, further explanation is needed as to why these activities were not higher in areas with higher metal contamination. Thus the use of oxidative stress markers for biomonitoring of heavy metal contamination in field studies with insects appears a difficult and controversial subject given the numerous other environmental factors and contaminants that may interplay.

Conflict of Interest

The authors declare that there are no conflicts interest regarding this article.

REFERENCES

Adu BW, Ogunjobi JA (2014). Assessment of dragonflies and damselflies of Owena Forest Southwestern, Nigeria. Int. J. Agri. Sci. 4(3):153-159.

Ahmad S (1992). Biochemical defense of pro-oxidant plant allelochemicals by herbivorous insects. Biochem. Sys. Ecol. 20:269-296.

Ahmad S (1995). Oxidative stress from environmental pollutants. Arch. Insect Biochem. Physiol. 29:135-157.

ATSDR (1994). Toxicological profile for zinc. US Department of Health and Human Services, Public Health Service, Atlanta, GA.

ATSDR (1999). Toxicological profile for cadmium. US Department of Health and Human Services, Public Health Service, Atlanta, GA.

Avci A, Kaçmaz M, Durak I (2005). Peroxidation in muscle and liver tissues from fish in a contaminated river due to a petroleum refinery industry. Ecotoxicol. Environ. Saf. 60:101-105.

Carey C, Bryant CJ (1995). Possible interrelations among Environmental Toxicants, Amphibian Development and Decline of Amphibian Population. Environ. Health Perspect. 103 (4): 13-17.

Cheng L (1976). Marine insects. Scripps Institution of Oceanography Research, Scripps Institution of Oceanography.

Chrascina M, Kafel A, Migula P (1996). Patterns of detoxifying enzymes in larval stage *Smerinthus ocellatus* L. exposed to cadmium, tocopherol or quercetin. St. Soc. Sci. Torun 4: 31-37.

Corbet PS (1999). Dragonflies: Behaviour and Ecology of Odonata. Harley Books, Colchester.

Dijkstra K-DB (2007).Demise and rise: The biogeography and taxonomy of the Odonata of tropical Africa. PhD Thesis, Leiden University.

Don-Pedro KN, Oyewo EO, Otitoloju AA (2004). Trend of heavy metal concentrations in Lagos lagoon ecosystem, Nigeria. WAJAE 5:103 - 114.

Drevnick PE, Sandheinrich MB (2003). Effects of dietary methyl mercury on reproductive endocrinology of Fathead minnows. Environ. Sci. Technol. 37: 4390-4396.

Duruibe JO, Ogwuegbu MDC, Egwurugwu JN (2007). Heavy metal pollution and human biotoxic effects. Int. J. Phys. Sci. 2: 112-118.

Eriyamremu GE, Asagba SO, Akpoborie A, Ojeaburu SI (2005). Evaluation of lead and cadmium levels in some commonly consumed vegetables in the Niger-Delta oil area of Nigeria. Bull. Environ. Contam. Toxicol. 75: 278- 283.

Ewuim SC (2004). A study of the insect fauna of the Permanent Site of Nnamdi Azikiwe University, Awka, Ph.D. Thesis. Nnamdi Azikiwe University Awka.

Farombi EO, Adelowo OA, Ajimoko YR (2007). Biomarkers of Oxidative Stress and Heavy Metal Levels as Indicators of Environmental Pollution in African Cat Fish (Clarias gariepinus) from Nigeria Ogun River. Int. J. Environ. Res. Public Health 4(2):158-165.

Fatima RA, Ahmad M (2005). Certain antioxidant enzymes of Allium cepa as biomarkers for the detection of toxic heavy metals in wastewater. Sci. Total. Environ. 346(1-3):256-73.

Felton GW, Summers CB (1995). Antioxidant systems in insects. Arch. Insect Biochem. Physiol. 29:187-197.

Fukul M (1996). Notes on the larval stages of *Oligoaeshna pryeri* Martin Aeshna 32:9-13.

Gonall AG, Bardawill CJ, Davd MM (1949). Glutathione transferases. The first step in mecapturic acid formation. J. Biol. Chem. 77:751-760.

Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Sci. 249:7130-7139.

Hamed RR, Elawa SE, Farid NM, Ataya FS (1999). Evaluation of detoxificationenzyme levels in Egyptian catfish, *Clarias lazera*, exposed to dimethoate. Bull. Environ. Contam. Toxicol. 63:789-796.

Heliövaara K, Väisänen R. (1993). Insects and Pollution. CRC Press, Ann Arbor, 393 pp.

Hodgson E (2011). A Textbook of Modern Toxicology. Wiley Publishers.

Idowu ET, Amaeze NH, Adie PI, Otubanjo OA (2014). Heavy metal bioaccumulation and biomarkers of oxidative stress in the wild African tiger frog, *Hoplobatrachus occipitalis.* AJEST 8(1):6-15.

Ivbijaro MFA (2003). Insect and the environment. A key note address presented at the 34th Annual Conference of the Entomological Society of Nigeria (ESN). University of Lagos.

Kang YJ (1997). Alteration of antioxidant system In: Masaro, E. (ed.) Handbook of human toxicology, CRC Press, Boca Raton, Florida, USA, pp. 275-284.

Kasperczyk A, Kasperczyk S, Horak S, Ostalowsk A, Grucka-Mamczar E, Romuk E, Olejek A, Birkner E (2008). Assessment of semen function and lipid per oxidation among lead exposed men. Toxicol. Appl. Pharmacol. 228:378-384.

Kurdland L (1960). Minamata disease. World Neurol. 1:370-385.

Migula P, Glowacka E (1996). Heavy metals as stressing factors in the red wood ants (*Formica polyctena*) from industrially polluted forests. Fresenius J. Anal. Chem. 354: 653-659.

Migula P, Laszczyca P, Augustyniak M, Wilczek G, Rozpedek K, Kafel A, Woloszyn M (2004). Antioxidative defence enzymes in beetles from a metal pollution gradient. Biol. Brat. 59: 645-654.

Muchuweti M, Birkett JW, Chinyanga E, Zvauya R, Scrimshaw MD, Lester JN, (2006). Heavy metal content of vegetables irrigated with mixture of waste water and sewage sludge in Zimbabwe: Implications for human health. Agric. Ecosystem aEnviron. 112: 41-48.

Nyle CB, Ray RN (1991). The Nature and Properties of soils. 12th Ed. United State of America. pp. 743-785.

Odunaiya CO (2002). Repositioning solid waste management practices in cosmopolitan Lagos state Polytechnic Inaugural Lecture Series No. 6 (26 th June 2002) P. 66.

Oertli B, Joye DA, Castella E, Juge R, Cambin D, Lachavanne JB (2002). Does size matter? The relationship between pond area and biodiversity. Biol. Cons.104:59-70.

Otitoloju AA (2000). Joint action toxicity of heavy metals and their bioaccumulation by benthic animals of the Lagos Lagoon. Ph.D Thesis, University of Lagos, Lagos, Nigeria. P. 231.

Oweson C, Hernroth B (2009). A comparative study on the influence of manganese on the bactericidal response of marine invertebrates. Fish Shellfish Immunol. 27:500-–507.

Panda BB, Panda KK (2002). Genotoxicity and mutagenicity of metals in plants. Prasad, K.N.V. and Strzalka, K., (Ed.), Physiology and BioChemistry of Metal Toxicity and Tolerance in Plants, Kluwer Academic Publishers, Netherlands. pp. 395-414.

Pereira R, Pereira ML, Ribeiro R, Goncalves F (2006). Tissues and hair residues and histopathology in wild rats (*Rattus rattus* L.) and Algerian mice (*Mus spretus* Lataste) from an abandoned mine area (Southeast Portugal). Environ. Pollut. 139:561-575.

Piano A, Valbonesi P, Fabbri E (2004). Expression of cytoprotective proteins, heat shock protein 70and metallothioneins, in tissues of *Ostrea edulis* exposed to heat and heavy metals. Cell Stress Chaperon. l9(2):134-142.

Piccolo A, Mbagulu JSC (1997). Exogenuos humic substances as conditions for the rehabilitation of degraded soil agro Foods Industry Hi-Tech. March/April 21-28.

Regoli F, Frenzilli G, Bocchetti R, Annarumma F, Scarcelli V, Fattorini D, Nigro M (2004). Time-course variations of oxyradical metabolism, DNA integrity and lysosomal stability in mussels, *Mytilus galloprovincialis*, during a field translocation experiment. Aquat. Toxicol. 68: 167-178

Sanita di Toppi L, Gabbrieli R (1999). Responses to cadmium in higher plants. Environ. Exp. Botany 41(2):105-130.

Sedlak J, Lindsay RH (1968). Estimation of total protein bound and non-protein sulfhydryl groups in tissues with Ellman's reagent. Anal. Biochem. 25:1192-1205.

Sheehan D, Power A (1999). Effects of seasonality on xenobiotic and antioxidant defence mechanisms of bivalve molluscs Comp. Biochem. Physiol. C 123:193-199.

Sorvari J, Rantala LM, Rantala MJ, Hakkarainen H, Eeva T (2007). Heavy metal pollution disturbs immune response in wild ant populations. Environ. Pollut. 145:324-328.

Soundararajan M, Veeraiyan G, Samipillai SS (2000). Arsenic-induced oxidative stress in fresh water tilapia (*Tilapia mossambica*). J. Phytol. 1(4):267-276.

Stohs SJ, Bagchi D, Hassoun E, Bagchi M (2001). Oxidative mechanisms in the toxicity of chromium and cadmium ions. J. Environ. Pathol. Toxicol. Oncol., 20:77-88.

Sun, M, Zigma, S (1978). An improved spectophotometric assay of dismutase based on epinephrine antioxidation. Anal. Biochem. 90:81 - 89.

Talmage SS, Walton BT (1991). Small mammals as monitors of environmental contaminants. Rev. Environ. Contam. T. 119: 47-145.

USEPA (2012). Water: Monitoring and Assessment. Macro invertebrates and Habitats. United States Environmental Protection Agency. http://water.epa.gov/type/rsl/monitoring/vms40.cfm

Usoh FI, Akpan EJ, Etim EO, Farombi EO (2005). Antioxidant actions of dried flower of *Hibiscus sabidariffa* L. on sodium arsenite- induced oxidative stress. Pak. J. Nutr. 4: 135-141.

Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M (2006).

Molecular Biomarkers of Oxidative Stress in Aquatic Organisms in Relation to Toxic Environmental Pollutants. Ecotox. Environ. Saf. 64:178-189.
Van Ooik T, Pausio S, Rantala MJ (2008). Direct effects of heavy metal pollution on the immune function of a geometrid moth, *Epirrita autumnata*. Chemosphere 71: 1840-1844.
Vijayavel K, Gopalakrishnan S, Thiagarajan R, Thilagam H (2009). Immunotoxic effects of nickel in the mud crab *Scylla serrata*. Fish Shellfish Immunol. 26:133-139.
Viarengo A, Lowe D, Bolognesi C, Fabbri E, Koehler A (2007). The use of biomarkers in biomonitoring: A 2-tier approach assessing the level of pollutant induced stress syndrome in sentinel organisms. Comp. Biochem. Physiol. C 146:281- 300.
Walker CH, Hopkin RM, Sibly RM, Peakall DB (2001). Principles of Ecotoxicology. Taylor and Francis, New York.
Yagi K (1998). Simple procedure for specific enzyme lipid hydroperoxidases in serum or plasma. Methods Mol. Biol. 108:107-110.
Zaman K, Macgill RS, Johnson JE, Ahmad S, Pardini RS (1994). An insect model for assessing mercury toxicity: Effect of mercury on antioxidant enzyme activities of the housefly (Musca domestica) and the cabbage looper moth (*Trichoplusia ni*). Arch. Environ. Contam. Toxicol. 26:114-118.
Zhang J, Shen H, Wang X, Wu J, Xue Y (2004). Effects of chronic exposure of 2,4 dichlorophenol on the antioxidant system in liver of freshwater fish *Carassiu sauratus* . Chemosphere 55: 167-174.