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Toxicology impacts of heavy metal pollution of Ogun and Lagos fishing waters by sewages on the antioxidant status and nutritional value of Prawn (*Macrobrachium macrobrachion*)

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Abstract

Most fishing waters in Nigeria are polluted via sewages thereby posing threats to the well-being of organisms depending on them as a water source. In this study, to ascertain the toxicology impacts of heavy metal pollution via sewages on aquatic organisms, prawn (Macrobrachium macrobrachion), one of the aquatic foods found in Ogun River and Epe Lagoon, Nigeria, was studied. Sixty M. macrobrachion (meanweight; 23 g and mean length; 13 cm) were obtained, with 10 samples from each sampled site; Ajegunle (control), Alaapa and Quarry (test sites) along Ogun River in Abeokuta, Ebute-Alashe (control), Oluwo and Marina (test sites) along Epe Lagoon in Epe. Physicochemical parameters included biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and were above the WHO safe limitfor the studied test waterbodies and proximate composition of the prawn samples collected from each site along the studied waterbodies showed varying significant($p < 0.05$) differences between the test sites and the respective control groups. Significant ($p < 0.05$) increases were observed for the heavy metal concentrations in the test fishing waters and hemolymph of M. macrobrachion upon comparing with the control groups. Antioxidant assays showed significant($p < 0.05$) increases in superoxide dismutase, catalase, and glutathione-S-transferase activities in the hemolymph of M. macrobrachion from Quarry and Marina sites. The glutathione concentration significantly ($p < 0.05$)decreased in hemolymph of M. macrobrachion from Alaapa (0.21 folds), Quarry (0.25 folds), Oluwo (0.22 folds) and Marina (0.53 folds) sites, while malondialdehyde concentrations in the hemolymph of M. macrobrachion from all the test sites showed no significant difference when compared with the respective controls. Proximate analysis of M. macrobrachion from all the test sites along Ogun River and Epe Lagoon showed significant ($p < 0.05$) increase in the moisture content while significant ($p < 0.05$) decrease was observed in the fat, ash and protein contents when compared with their respective controls. The results of this study therefore showed that heavy metal pollution of fishing waters via sewages, induced oxidative stress and diminished the nutritional value of M. macrobrachion from Ogun river and Epe lagoon. This study also suggests potential health threat to consumption of M. macrobrachion procured from these waterbodies.

Keywords: Sewage pollution, Ogun River, Epe lagoon, oxidative stress, antioxidants, proximate composition, *macrobrachiumn macrobrachion*

Impacts toxicologiques de la pollution aux métaux lourds des eaux de pêche d'Ogun et de Lagos par les eaux usées sur le statut antioxydant et la valeur nutritionnelle de la crevette (Macrobrachium macrobrachion)

Résumé

La plupart des eaux de pêche au Nigéria sont polluées par les eaux usées, ce qui constitue une *menace pour le bien-être des organismes qui en dépendent comme source d'eau. Dans cette étude, pour déterminer les impacts toxicologiques de la pollution par les métaux lourds via les eaux usées sur les organismes aquatiques, la crevette (Macrobrachium macrobrachion), l'un des aliments aquatiques trouvés dans la rivière Ogun et la lagune d'Epe, au Nigeria, a été étudiée. Soixante macrobrachion (poids moyen ; 23 g et longueur moyenne ; 13 cm) ont été obtenus, avec 10 échantillons de chaque site échantillonné ; Ajegunle (témoin), Alaapa et Quarry (sites d'essai) le long de la rivière Ogun à Abeokuta, Ebute-Alashe (témoin), Oluwo et Marina (sites d'essai) le long de la lagune d'Epe à Epe. Les paramètres physicochimiques étaient la demande biochimique en oxygène (DBO) et la demande chimique en oxygène (DCO), et étaient supérieurs à la limite de sécurité de l'OMS pour les plans d'eau d'essai étudiés et la composition approximative des échantillons de crevettes prélevés sur chaque site le long des plans d'eau étudiés a montré une variation significative ($p < 0,05$) différences entre les sites de test et les groupes de contrôle respectifs. Des augmentations significatives ($p < 0,05$) ont été observées pour les concentrations de métaux lourds dans les eaux de pêche d'essai et l'hémolymphe de M. macrobrachion lors de la comparaison avec les groupes témoins. Les dosages d'antioxydants ont montré des augmentations significatives ($p < 0,05$) des activités de lasuperoxydedismutase, de la catalase et de la glutathion-S-transférase dans l'hémolymphe de M. macrobrachion des sites Quarry et Marina. La concentration de glutathion a diminué de manière significative ($p < 0,05$) dans l'hémolymphe de M. macrobrachion des sites Alaapa (0,21 fois), Quarry (0,25 fois), Oluwo (0,22 fois) et Marina (0,53 fois), tandis que les concentrations de malondialdéhyde dans l'hémolymphe de M Le macrobrachion de tous les sites de test n'a montré aucune différence significative par rapport aux témoins respectifs. L'analyse immédiate de M. macrobrachion de tous les sites d'essai le long de la rivière Ogun et de la lagune d'Epe a montré une augmentation significative ($p <0,05$) de la teneur en humidité tandis qu'une diminution significative ($p <0,05$) a été observée dans la teneur en matières grasses, en cendres et en protéines par rapport à leurs contrôles respectifs. Les résultats de cette étude ont donc montré que la pollution par les métaux lourds des eaux de pêche via les eaux usées, induisait un stress oxydatif et diminuait la valeur nutritionnelle de M. macrobrachion de la rivière Ogun et de la lagune d'Epe. Cette étude suggère également une menace potentielle pour la santé liée à la consommation de M. macrobrachion provenant de ces plans d'eau.*

Mots-clés: Pollution par les eaux usées, rivière Ogun, lagune d'Epe, stress oxydatif, antioxydants, Macrobrachiumnmacrobrachion

Introduction

Waterbodies traditionally serve as means of waste disposal of sewage and other toxic wastes from different sources (Nouri *et al*., 2014). All living organisms on earth depend on water to survive which is undoubtedly not enough or mostly of low quality and have had serious effects on sustainable development (Taiwo *et al.,* 2014). Increase in domestic, industrial, agricultural and commercial wastes discharged into the aquatic environment has deleterious effects on aquatic organisms (Patil *et al., 2012).* Pollution of waterbodies via anthropogenic activities and sewage release directly into waterbodies are mostly responsible for low water quality and scarcity of freshwater (Wang *et al.,* 2010). Prawn (*Macrobrachium macrobrachion),* is one of the most widely distributed organisms in the aquatic environment which belongs to a group known as crustaceans. The nutritive and economic value of prawn is attributed to its being a good source of protein, vitamins (vitamin A, C and D), minerals (Ca, Mg, P, Mn and Cl) and richness in nonsaturated fatty acids (Kharat *et al.,* 2009). Prawn *is a commonly consumed aquatic food in the south-western part of Nigeria* and can be used as a biosensor of environmental pollution. Aquatic ecosystems have become polluted with inorganic pollutants such as heavy metals which exerts severe toxic effects because of their toxicokinetics, bioaccumulation, and biomagnification by aquatic organisms, most especially prawns (Shovon *et al.,* 2017). Defensive mechanisms to counteract the impact of reactive oxygen species (ROS) are found in many aquatic animals such as prawn. These systems include various antioxidant defense enzymes such as superoxide dismutases which catalyze the dismutation of superoxide radical to hydrogen peroxide, catalase acting on hydrogen peroxide, glutathione S-transferase family possessing detoxifying activities towards lipid hydroperoxides generated by organic pollutants such as heavy metal (Sharma *et al.,* 2014; Arojojoye and Adeosun, 2016).When antioxidant defence mechanisms are impaired or overwhelmed, oxidative stress occurs leading to deleterious consequences which may include damage to cellular macromolecules (DNA, RNA, proteins etc.), enzymatic inactivation and peroxidation of cell constituents (Bhagat *et al.,* 2016). Ogun River and Epe Lagoon, located in Ogun and Lagos states respectively, the industrial hubs of Nigeria, are waters depended on by humans living within their proximities for their livelihood, and has recently been of particular interest and concern owing to the continual release of untreated wastes from local industries and indiscriminate disposal of sewage wastewaters into the fishing waters, leaving the river polluted with numerous toxicants most especially heavy metals (Fafioye *et al.,* 2017). Thus, in this study, heavy metal concentration, antioxidant profiles, and the nutritional composition of *M. macrobrachion* (via proximate analysis), a commercial aquatic food from Ogun River and Epe Lagoon, Nigeria, were evaluated to know the toxicological impacts of sewage pollution on aquatic organisms and the surrounding ecosystem.

Materials and methods

Chemicals and reagents

Glutathione, 1-chloro- 2,4, dinitrobenzene, 5,5'-dithiobis (2-nitrobenzoic acid), Tris, hydrogen peroxide, pyrogallol, thiobarbituric acid, trichloroacetic acid were products from Sigma chemicals, USA and Randox kits were procured from Randoxlaboratories-US limited, USA. All chemicals and reagents used for this study wereof analytical grade.

Study area selection

Ogun River and Epe Lagoon serve as major sources of water for those inhabiting Abeokuta, Ogun state and Epe, Lagos state respectively, and other villages situated along their banks. The Ogun River and Epe Lagoon are used for many purposes such as agriculture, transportation, fishing, industrial activities, refuse dumpsite, irrigation, drinking and other domestic purposes. Along their watercourse, Ogun River and Epe Lagoon constantly receive effluents from abattoirs, saw mills, breweries, dyeing industries, sewages, refuse, prawn carcasses and domestic and industrial wastewaters. The sampled sites were selected based on evident human and industrial activities. The sites were marked with the aid of a global positioning system (GPS) and the coordinates of the sites were recorded. Ogun River is one of the major rivers in the south-western part of Nigeria having coordinates of 3°28'E and 8°41'N with a total area of 22.4 km^2 (Dimowo, 2013). It flows from Shaki, Oyo State through Abeokuta, Ogun State (Ayanda *et al.,* 2019). Two sewage-polluted sites (Alaapa and Quarry) and the control site (Ajegunle) along the Ogun River watercourse were selected and sampled during the rainy season between June to July, 2019 (Figure 1a). Epe Lagoon is one of the fishing waters located in Lagos state, Nigeria having coordinates between longitudes 6°33.710′N, 4°03′710′ E and latitudes 6°31.893′ N, 3°31.912′ E. It has a surface area of about 243 km^2 and a maximum depth of about 2.8 m (Edokpayi *et al.,* 2008). The Epe Lagoon is sandwiched between two lagoons; Lekki lagoon (freshwater) and Lagos lagoon (brackish). Epe Lagoon connects to the sea via the Lagos harbor (Lawal *et al.,* 2010). Two sewage-polluted sites (Oluwo and Marina) and the control site (Ebute-Alashe) along the Epe Lagoon watercourse were selected and sampled during the rainy season beween June to July, 2019 (figure 1b).

Sampling and sample collection

Water sampling was done following the procedure of Ndimele and Kumolu-Johnson (2012). Water samples were collected from the sampled sites in duplicates using 500 mL sampling bottles for physicochemical analysis and heavy metals analysis. Sixty live samples of adult prawn with average weight**:**23 g and length:13 cm were sampled; 10 samples each from Alaapa and Quarry in Abeokuta, Ogun state, along the Ogun River and 10 samples each from Marina and Oluwo in Epe, Lagos state along the Epe Lagoon. 10 samples each were also sampled from Ajegunle in Ogun state and Ebute Alashe in Epe, Lagos state, to serve as control. Prawns were procured from each site with the help of professional prawnermen residing within the proximity of the sampled areas along the fishing waters in the morning and were transported quickly in a cooler box containing the sampled site water to the laboratory. The prawns' weights and full lengths were measured and recorded. Hemolymph samples (3mL) were collected from the ventral side of the cephalothorax region of the prawns using a needle and syringe that has been pre-rinsed with heparin and immediately transferred into lithium-heparin tubeswhich were subsequently refrigerated. Aliquots of hemolymph samples were separated for heavy metal analysis, superoxide dismutase, catalase, glutathione-s-transferase activities, reduced glutathione concentrationsdetermination and lipid peroxidation assay. Fillets were prepared from properly rinsed prawns following beheading and gutting. Aliquots of edible tissues were taken for analysis ofprawns' proximate composition and stored at -20°C till further analyses.

Determination of physicochemical properties of collected water samples

The physicochemical analyses of collected water samples were performed following standard protocols (Onunkwor *et al*, 2019).The temperature (ºC) of the water from the four sampled river sites were determined using glass mercury thermometer which was pre- and post-rinsed with distilled water upon each use. The pH of the water from the sampled sites was determined using microprocessor-based pocket pH meter (waterproof pH scan WPI). Modified alkaline-azide method of Winkler (1888) was used to determine the dissolved oxygen (DO) and biochemical oxygen demand (BOD).Closed reflux titrimetric method was used for the determination of chemical oxygen demand (COD) of water samples as described in APHA (2005).

Heavy metals analyses of hemolymph and site water samples

The concentrations of heavy metals (arsenic, lead and cadmium) in the water and in the hemolymph of *M. macrobrachion* from the sampled sites were determined using atomic absorption spectrometry (AAS) (Perkin-Elmer 3110, USA) as described in APHA (2005). The principle of the AAS is based on the fact that in the process of excitation and decay of atoms to ground state, energy is absorbed or emitted. 200 mL of the water sample and 1 mL of hemolymph were separately digested with concentrated nitric acid and perchloric acid and brought to a constant volume.

Antioxidants profiling, lipid peroxidation and total protein content estimation of prawn's hemolymph

The hemolymph activity of superoxide dismutase was determined at 420 nm according to the Li (2012) modified procedure of Marklund and Marklund (1974). This method is based on the inhibition of pyrogallol (1, 2, 3–benzenetriol) by superoxide dismutase. One unit of superoxide dismutase activity is equal to one micromole of pyrogallol inhibited per minute under specified conditions at 25°C. To determine hemolymph catalase activity, the disappearance of H_2O_2 was monitored spectrophotometrically at 240nm following the protocol described by Beers and Sizer (1952). One unit of catalase activity is equal to one micromole of H_2O_2 decomposed per minute under specified condition at 25°C. Reduced glutathione concentration in hemolymph was determined at 420nm using the method described by Ellman (1959), in which 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) complexes with reduced glutathione at 25°C. Glutathione-S-transferase activity in hemolymph was determined at 340nm using the method described by Habig and Jakoby (1981) by measuring the formation of the 1-chloro-2, 4-dinitrobenzene and glutathione conjugate at 25°C and pH 7.0. One unit of Glutathione-S-transferase activity is equal to one micromole of product formed per minute under specified conditions at 25°C. Lipid peroxidation in hemolymph was estimated colorimetrically at 535nm following the method of Draper *et al.*(1993) involving the monitoring the formation of thiobarbituric acid reactive substances (TBARS). Total protein content in hemolymphwas determined using Randox kits (TP 245) from Randox laboratories-US, USA. The principle involves the interaction of cupric ionswith protein peptide bonds in an alkaline medium, resulting in the formation of a colored complex which is then monitored spectrophotometrically at 546nm.

Proximate analysis of whole M. macrobrachion samples

The proximate composition of the prawn samples was determined using standard methods described in AOAC (2005). The total moisture content was determined by oven drying the muscle samples at 105°C (for about 10hr) until a constant weight was attained, then cooled in a desiccator, and reweighed. The total moisture content was taken to be the difference between the fresh and dry weights of prawn samples.

The percentage difference due to loss of water was calculated according to the equation:

% Moisture = <u>weight of initial sample - weight of sample after drying x 100</u>
Weight of initial sample

Determination of the total fat content was achieved by continuous and exhaustive extraction of fats from 2.0 g of dried prawn samples using petroleum ether as the extractant, in a Soxhlet apparatus. The extracted crude fats left after the solvent had evaporated were weighed, and the fat content calculated.

The crude protein content of samples (□ × 6.25) was estimated using Kjeldahl digestion procedure (Kjeldahl, 1883) as described in AOAC, 2005. The procedure involves the digestion of samples, distillation, and titration. Heating in concentrated sulphuric acid turned the digests to colorless, signaling the end of digestion. From the nitrogen-containing organic molecules, ammonium sulphate is formed. The amount of ammonia (distillate) was estimated by distillation followed by titration. The percentage of crude protein in the samples was then estimated by multiplying total percent nitrogen by 6.25.

To estimate the crude fibre in the prawn samples, 100 mL of trichloroacetic acid reagent was added to 1 g of ether defatted sample extract and then digested by refluxing for 40 minutes. The digest was filtered using Whatmann 1 filter paper and the residue was washed with boiling distilled water 6 times and finally with 10 mL of ethanol. The washed residue was dried and ashed in a muffle furnace at 550°C for 2 hours. The weight of the crude fibre is taken as the difference between the weight of dried insoluble residue and ash from which percentage crude fibre was calculated.

The Nitrogen-free extract (Soluble carbohydrate)was calculated by adding together the percentages of crude protein(CP), fat extract, crude fibre(CF) and ash on dry matter basis. i.e.

%NFE=100-[Y], where [Y]= %CP + %FE + %CF + %Ash.

To estimate the ash content in the prawn samples, pre-dried homogenized prawn samples obtained from moisture content analysis were ashed in a muffle furnace at 550°C overnight. The percentage of total ash was calculated from the difference between the weight of ash and the initial weight of the dried sample.

$$\%\text{Total ash} = \frac{(\text{Weight of crucible + Total ash after cooling}) - \text{Weight of crucible}}{(\text{Weight of crucible + Total as + Dry sample before ashing}) - \text{Weight of crucible}} \times 100$$

Statistical analysis

All results were expressed as Mean ± S.E.M.Data were analyzed using one-way analysis of variance (ANOVA) followed by post-hoc Dunnett's multiple comparisons test by Statistical Package ofGraphPad prism version 7.0. Differences were considered significant at $p < 0.05$. Correlational analysis was also done using statistical software package SPSS 20.0.

Results

Sewage pollution alters the physicochemical parameters of Ogun and Lagos fishing waters

Various physicochemical parameters of water have been observed to affect aquatic lives when they undergo a change, thereby making water unsafe and causing harm to aquatic organisms.To ascertain the impacts of sewage pollution on the fishing waters from the sites selected in this study, the physicochemical parameters were assayed. As shown in table 1, it was observed that with the exception of the biochemical oxygen demand (BOD) value of sewage-polluted Quarry site along the Ogun River and the mean chemical oxygen demand (COD) values of all the test sites along Ogun River and Epe Lagoon which were above the WHO (2008) safe limit, the results obtained for other physicochemical parameters (the pH, temperature (T), dissolved oxygen (DO)and total dissolved solids (TDS) for all the control and test sites along Ogun River and Epe Lagoon fell within the safe limit recommended by WHO (2008) for drinking water. There was no significant ($p < 0.05$) difference observed in the pH of the water sampled from Alaapa site along Ogun River while there was a significant ($p < 0.05$) decrease in the pH of the water sampled from Quarry site along Ogun River, Oluwo and Marina sites along the Epe Lagoon compared with their respective controls.

As regards temperature,a significant ($p < 0.05$) increase was observed in the temperature of the water sampled from Alaapa and Quarry sites along the Ogun River and Marina site along Epe Lagoon while no significant ($p < 0.05$) difference was observed in the temperature of the water sampled from Oluwo site along Epe Lagoon compared with theirrespective controls. The dissolved oxygen level in the water sampled from Quarry site along Ogun River as well as Oluwo and Marina sites along Epe Lagoon, significantly ($p < 0.05$)

decreased while no significant ($p < 0.05$) difference was observed in the DO level of the water sampled from Alaapa site along Ogun River compared with their respective controls. The biochemical oxygen demand was significantly($p < 0.05$) increased in the water sampled from Alaapa and Quarry sites along Ogun River and Marina site along Epe Lagoon while no significant ($p < 0.05$) difference was observed in the biochemical oxygen demand in the water sampled from Oluwo site along Epe Lagoon compared with their respective controls. A significant ($p < 0.05$) increase was observed in the chemical oxygen demand in the water sampled from Alaapa and Quarry sites along Ogun River and Marina site along Epe Lagoon while no significant ($p < 0.05$)difference was observed in the chemical oxygen demand in the water sampled from Oluwo site along Epe Lagooncompared with theirrespective controls. The total dissolved solids concentration were significantly($p < 0.05$) increased in the water from all sampled test sites along Ogun River and Epe Lagoon compared respective controls.

Table 1. Physicochemical analysis of water sampled from Ogun River and Epe Lagoon sites

Parameters	Ogun River Control	Alaapa	Quarry	Epe Lagoon Control	Oluwo	Marina	WHO limit
Temperature (°C)	28.20±0.10^{a}	28.70±0.10^{b}	29.30±0.10^{c}	28.30±0.10^{a}	28.45±0.05^{ab}	28.70±0.10^{b}	< 40 °C
pH	7.15±0.05^{b}	7.10±0.10^{b}	6.75±0.05^{a}	7.25±0.05^{b}	6.85±0.05^{a}	6.85±0.05^{a}	6.5-8.5
DO (mg/L)	8.06±0.04^{b}	7.90±0.02^{ab}	7.59±0.09^{a}	8.58±0.04^{c}	8.23±0.02^{b}	8.10±0.02^{a}	-
BOD (mg/L)	4.79±0.02^{a}	5.22±0.02^{b}	7.85±0.03^{c}	5.24±0.02^{a}	5.30±0.02^{ab}	5.42±0.04^{b}	< 6
COD (mg/L)	18.37±0.07^{a}	20.85±0.05^{b}	30.15±0.05^{c}	17.32±0.22^{a}	20.66±0.06^{a}	31.96±3.44^{b}	20
TDS (mg/L)	108.25±1.95^{a}	897.32±7.30^{c}	558.68±1.77^{b}	131.60±0.80^{a}	588.50±13.50^{b}	957.00±17.00^{c}	1000

All values are expressed as mean ± SEM. Values with different superscripts are significantly($p < 0.05$) differentacross the row.

Heavy metal concentrations in Ogun and Lagos fishing waters and M. macrobrachion hemolymph samples are elevated by sewage pollution

Heavy metals have been reported to be highly persistent, toxic in trace amounts, and can also potentially induce elevated levels of oxidative stress in aquatic organisms.Heavy metals accumulated in the tissues of prawn may catalyze reactions that generate reactive oxygen species (ROS) which may lead to oxidative stress (Akhiromen and Ogbonne, 2018). To determine the effect of sewage pollution on the concentration of heavy metals on the selected fishing water sites selected in this study as well as *M. macrobrachion hemolymph samples*, the concentration of heavy metals were estimated. Arsenic, lead and cadmium concentrations in the water from the sampled sites are shown in figure2a. The arsenic concentrations in the water sampled from the control sites and Oluwo site along the Ogun River and Epe Lagoon, fell within the permissible limit (0.01 mg/L) of WHO (2008) while the arsenic concentrations in the water sampled from Alaapa and Quarry sites along Ogun River and Marina sites along Epe Lagoon, were higher than the permissible limits (0.01 mg/L) of WHO (2008). A significant ($p < 0.05$)increase was observed in the arsenic concentrations in the water sampled from Alaapa and Quarry sites along the Ogun River while there was no significant($p < 0.05$)difference in the arsenic concentrations in the water sampled from both Oluwo and Marina sites along Epe Lagoon when compared with their respective controls. Lead concentrations in the water sampled from the control sites, Alaapa and Oluwo sites along the Ogun River and Epe Lagoon fell within the permissible limit (0.01 mg/L) of WHO (2008) while lead concentrations in the water sampled from Quarry and Marina sites along Ogun River and Epe Lagoon respectively were above the permissible limit (0.01 mg/L) of WHO (2008) for safe drinking water. A significant($p < 0.05$) increase was observed in the lead concentration in the water sampled from Quarry and Marina sites along Ogun River and Epe Lagoon respectively compared with the respective controls (Figure 2b). As depicted in Figure 2c, cadmium concentrations in the water sampled from the control sites and Alaapa site along Ogun River and Epe Lagoon were within WHO (2008) permissible limit (0.003 mg/L) while cadmium concentrations in the water sampled from Quarry site along Ogun River, and Oluwo and Marina sites along Epe Lagoon, were above the permissible limit of WHO (2008). Significant($p < 0.05$) increase was observed in the cadmium concentration in the water sampled from Quarry along Ogun River while no significant ($p < 0.05$) difference was observed in the cadmium concentrations in the water sampled from Alaapa, Oluwo and Marina sites along Ogun River and Epe Lagooncompared with theirrespective controls. Figures 3a and 3b show the concentration of arsenic and lead in the hemolymph of *M. macrobrachion* from the sampled sites along Ogun River and Epe Lagoon respectively. A significant($p < 0.05$) increase was observed in the concentration of arsenic in the hemolymph of *M. macrobrachion* sampled from all the test sites along Ogun River and Epe Lagoonexcept Alaapa site which showed no significant ($p < 0.05$) difference while for lead, significant($p < 0.05$) increase was observed only in the hemolymph of *M. macrobrachion* sampled from Quarry and Marina sites along Ogun River and Epe Lagoon respectively compared with the controls. No significant difference was observed in the lead concentrations in the hemolymph of *M. macrobrachion* sampled from Alaapa and Oluwo sites along Ogun River and Epe Lagoon respectivelycompared with the controls. As shown in Figure 3c, a significant ($p < 0.05$)increasewas observed in the cadmium concentration in the hemolymph of *M. macrobrachion* sampled from Marina site along Epe Lagoon while no significant ($p < 0.05$) difference was observed in the cadmium concentration in the hemolymph of *M. macrobrachion* sampled from Alaapa and Quarry sites along Ogun River and Oluwo site along Epe Lagoon compared with the controls.

Antioxidants profiles in M. Macrobrachionhemolymph samples are modified by sewage pollution of Ogun and Lagos fishing waters

As illustrated in Figures 4a,4b and 4c, superoxide dismutase, catalase and glutathione–S–transferase activities in the hemolymph of *M. macrobrachion* sampled from Quarry and Marina sites along Ogun River and Epe Lagoon respectively showed significant ($p < 0.05$) increases compared with the respective controls, along Ogun River and Epe Lagoon. From Figure 5a, a significant ($p < 0.05$) decrease was observed in the glutathione concentration in the hemolymphsamples of *M. macrobrachion* sampled from all the test sites along Ogun River and Epe Lagoon compared with the respective controls. In Figure 5b, the malondialdehyde concentrations in the plasma of *M. macrobrachion* sampled from all the test sites along Ogun Riverand Epe Lagoon showed no significant difference ($p < 0.05$) compared with the respective controls.

Sewage pollution reduces the nutritive value of M. macrobrachion from polluted Ogun and Lagos fishing waters

M. macrobrachion is a delicacy in Nigeria and is of dietary, health and economic importance to man being a good source of healthy aquatic food and income for prawn marketers. To evaluate the impacts of sewage pollution of the natural habitat of prawn on its nutritive value, the proximate composition of the prawn samples was analyzed. As shown in Table 2, a significant($p < 0.05$) increase was observed in the moisture content of *M. macrobrachion* from all the sampled test sites along Ogun River and Epe Lagoonwhen compared with the controls. The ash content, fat content and crude protein of *M. macrobrachion* from all sampled test sites along Ogun River and Epe Lagoon were significantly ($p < 0.05$)decreased while the crude fibre content and nitrogen-free extract of *M. macrobrachion* from all the sampled sites along Ogun River and Epe Lagoon showed no significant ($p < 0.05$) difference when compared with the respective controls.

Table 2: Proximate composition of *M. macrobrachion* sampled from Ogun River and Epe Lagoon sites

	Ogun River			Epe Lagoon		
Parameters (%)	Control	Alaapa	Quarry	Control	Oluwo	Marina
Moisture	69.55±0.22[a]	73.40±0.80[b]	77.59±0.72[c]	68.61±0.17[a]	71.97±0.84[b]	75.96±1.13[c]
Ash content	0.28±0.05[b]	0.21±0.04[ab]	0.15±0.003[a]	0.30±0.07[b]	0.23±0.05[ab]	0.14±0.00[a]
Fat content	2.63±0.09[b]	1.69±0.21[a]	1.67±0.25[a]	2.59±0.04[b]	1.74±0.19[a]	1.44±0.24[a]
Crude fibre	0.05±0.00[a]	0.13±0.15[a]	0.15±0.05[a]	0.05±0.00[a]	0.16±0.05[a]	0.16±0.06[a]
Crude protein	26.07±0.12[b]	16.72±1.30[a]	17.98±1.06[a]	25.71±0.30[b]	18.87±1.82[a]	17.02±1.62[a]
Nitrogen-free extract	1.56±0.23[a]	7.85±1.92[b]	2.34±1.49[a]	3.76±1.15[a]	5.82±2.35[a]	8.29±2.78[a]

All values are expressed as Mean ± S.E.M. Different superscript letters represent significant differences ($p < 0.05$) across the rows.

Associations between investigated parameters

Table 3shows the correlation between the hemolymph heavy metals of *M. macrobrachion* and the heavy metals in the water from the sampled sites along Ogun River and Epe Lagoon.Hemolymph As had significant positive associations with all the water metals:**As ($r = 0.828$; $p = 0.000$), Pb ($r = 0.716$; $p = 0.001$) and Cd ($r = 0.577$; $p = 0.010$)**. There were significant positive associations between hemolymph Pb and all the water heavy metals:**As ($r = 0.740$; $p = 0.000$), Pb ($r = 0.756$; $p = 0.000$) and Cd ($r = 0.617$; $p = 0.005$)**. Hemolymph Cd had no significant associations with all the water metals.

Table 4shows the correlation between the antioxidant parameters and heavy metals in thehemolymph of *M. macrobrachion*from the sampled sites along Ogun River and Epe Lagoon.The activity of SOD in the hemolymph of *M. macrobrachion* showed significant positive associationswith all the hemolymph heavy metals:As ($r = 0.558$; $p = 0.011$), Pb ($r = 0.588$; $p = 0.006$) and Cd ($r = 0.689$; $p = 0.001$).The activity of CAT in the hemolymph of *M. macrobrachion*showed significant positive associations with As ($r = 0.652$; $p = 0.006$) and Pb ($r = 0.707$; $p = 0.000$) andsignificant negative associations with hemolymph Cd ($r = -0.556$; $p = 0.011$). There were significant negative associations between GSH concentrations in the hemolymph*of M. macrobrachion*and all the hemolymph heavy metals:As ($r = -0.775$; $p = 0.000$), Pb ($r = -0.730$; $p = 0.000$) and Cd ($r = -0.556$; $p = 0.011$). There were significant positive associations between GST activity in the hemolymph of *M. macrobrachion* and all the hemolymph heavy metals: As ($r = 0.720$; $p = 0.000$), Pb ($r = 0.782$; $p = 0.000$) and Cd ($r = 0.589$; $p = 0.006$). No significant association was observed between MDA concentrations and all the hemolymph heavy metals. Table 5shows the correlation between the proximate composition of *M. macrobrachion* and heavy metals in thewaterfrom the sampled sites along Ogun River and Epe Lagoon.Ash had significant positive association with all the water heavy metals: As (r = 0.644; p = 0.002), Pb (r = 0.619; p = 0.004) and Cd (r = 0.498; p = 0.025). Crude fibre had a significant positive association with water As (r = 0.477; p = 0.033) and no significant associations with Pb and Cd. Crude fat had significant negative associations withwater As (r = 0.794; p = 0.000), Cd (r = 0.721; p = 0.000) and no significant associations with Pb. The crude protein content of *M. macrobrachion* hadsignificant negative associations with all the water heavy metals:As (r = -0.655; p = 0.002), Pb (r = -0.647; p = 0.002) and Cd (r = -0.497; p = 0.026). The moisture content of *M. macrobrachion* had significant positive associations with all the water heavy metals: As (r = 0.764; p = 0.000), Pb (r = 0.910; p = 0.000) and Cd (r = 0.788; p = 0.000).

Table 3: The correlation between the heavy metals in the water and the hemolymph of *M. macrobrachion* from the sampled sites along Ogun River and Epe Lagoon

		Water As	Water Pb	Water Cd
Hemolymph As	Pearson Correlation	.828**	.716**	.577**
Hemolymph Pb	Pearson Correlation	.740**	.756**	.617**
Hemolymph Cd	Pearson Correlation	0.428	0.292	0.275

** means correlation is significant at the 0.01 level (2 -tailed). * means correlation is significant at the 0.05 level (2-tailed).

Table 4: The correlation between the heavy metals and antioxidants parameters of *M. macrobrachion* sampled from Ogun River and Epe Lagoon.

		Hemolymph As	Hemolymph Pb	Hemolymph Cd
SOD	Pearson Correlation	.558*	.588**	.689**
CAT	Pearson Correlation	.656**	.707**	0.205
GSH	Pearson Correlation	-.775**	-.730**	-.556*
GST	Pearson Correlation	.720**	.782**	.589**
MDA	Pearson Correlation	0.273	0.372	0.227

**means correlation is significant at the 0.01 level (2 -tailed). * means correlation is significant at the 0.05 level (2-tailed).

Table 5: The correlation between the heavy metals in the sampled sites water and the proximate composition of *M. macrobrachion* sampled from Ogun River and Epe Lagoon

		Water As	Water Pb	Water Cd
Ash Content	Pearson Correlation	.644**	.619**	.498*
Crude Fibre	Pearson Correlation	.477*	0.236	0.335
Crude Fat	Pearson Correlation	-.794**	-.710**	-.721**
Protein	Pearson Correlation	-.655**	-.647**	-.497*
Moisture Content	Pearson Correlation	.764**	.910**	.788**
NFE	Pearson Correlation	0.184	0.027	-0.042

** means correlation is significant at the 0.01 level (2 -tailed). * means correlation is significant at the 0.05 level (2-tailed).

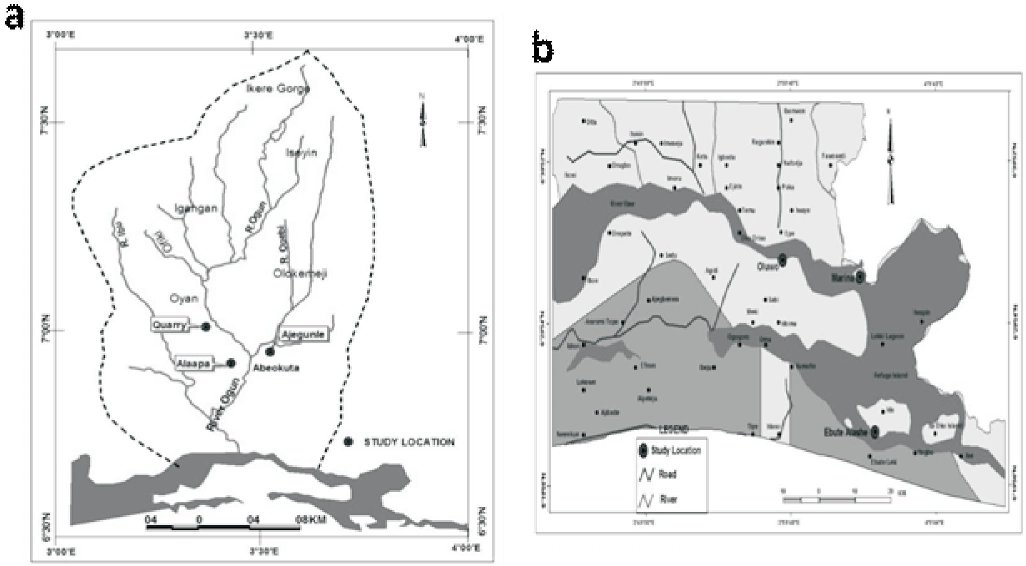
Table 6 shows the correlation between the proximate composition and heavy metals in the hemolymph of *M. macrobrachion* from the sampled sites along Ogun River and Epe Lagoon. No significant association was observed between the crude fibre, crude protein and NFE content of *M. Macrobrachion* and all the hemolymph heavy metals investigated. Ash content of *M. macrobrachion* had a significant positive association with the hemolymph As (r = 0.614; p = 0.004) and no significant associations with hemolymph Pb and Cd.There were significant negative associations between the crude fat content of *M. macrobrachion* and the hemolymph

As (r = -0.595; p = 0.006) and hemolymph Pb (r = -0.685; p = 0.001). The moisture content of *M. macrobrachion* had significant positive associations with hemolymph As (r = 0.570; p = 0.009), Pb (r = 0.719; p = 0.000).

Table 6: The correlation between the heavy metals and the proximate composition of *M. macrobrachion*sampled from Ogun River and Epe Lagoon.

		Hemolymph As	Hemolymph Pb	Hemolymph Cd
Ash Content	Pearson Correlation	.614**	0.249	0.041
Crude Fibre	Pearson Correlation	0.185	0.106	0.177
Crude Fat	Pearson Correlation	-.595**	-.685**	-0.409
Protein	Pearson Correlation	-0.352	-0.366	-0.132
Moisture Content	Pearson Correlation	.570**	.719**	0.202
NFE	Pearson Correlation	-0.056	-0.17	0.022

** means correlation is significant at the 0.01 level (2 -tailed). * means correlation is significant at the 0.05 level (2-tailed).



Cartographic maps showing the Ogun river and Epe lagoon sampled sites. **a. Cartographic map sowing Ogun River sampled sites.b. Cartographic map showing Epe Lagoon sampled site.**

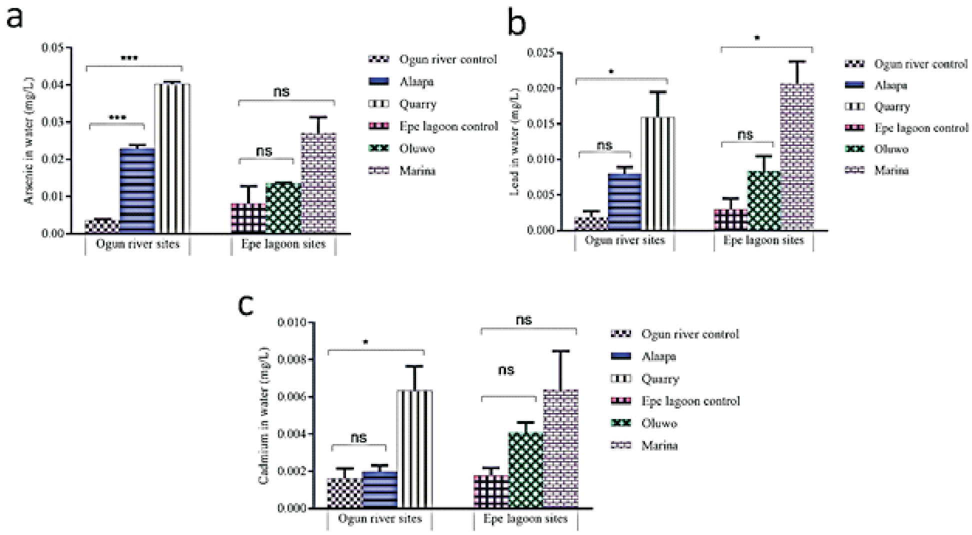


Figure 2. Heavy metal concentrations in sampled sewage-contaminated fishing waters. a. Arsenic concentrations in water from sewage-polluted Ogun River and Epe Lagoon sampled sites.b. Lead concentrations in water from sewage-polluted Ogun River and Epe Lagoon sampled sites.c. Cadmium concentrations in water from sewage-polluted Ogun River and Epe Lagoon sampled sites.All values are expressed as mean ± SEM of independent triplicate measurements. Asterisks denote significant difference at $p < 0.05$, ns; no significant difference.

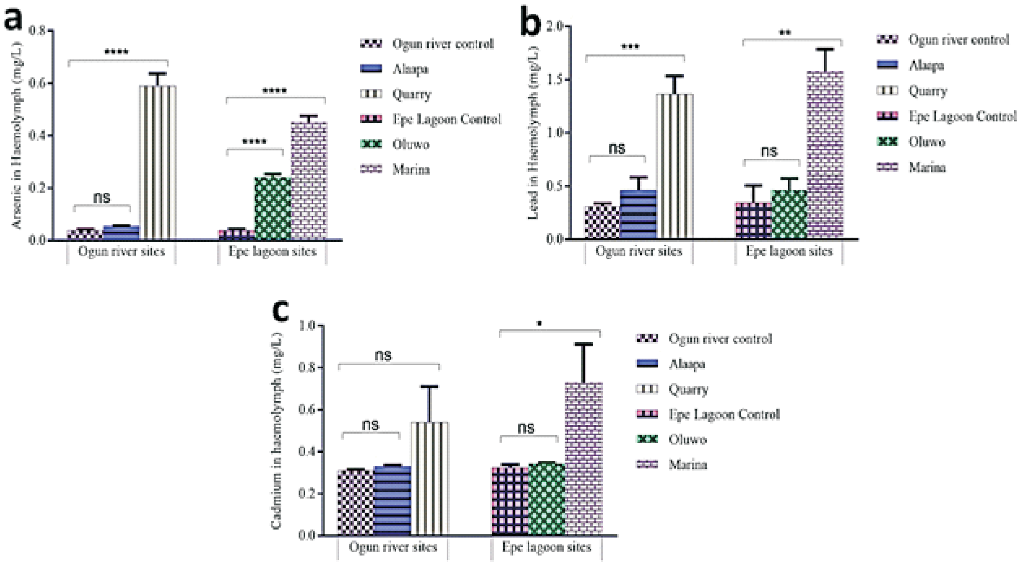


Figure 3. Heavy metal concentrations in *hemolymph samplesof M. macrobrachionfrom* sewage-contaminated fishing waters. a. Arsenic concentrations in *M. macrobrachionhemolymph samples* from sewage-polluted Ogun River and Epe Lagoon sampled sites.b. Lead concentrations in *M. macrobrachionhemolymph samples* from sewage-polluted Ogun River and Epe Lagoon sampled sites.c. Cadmium concentrations in *M. macrobrachionhemolymph samples* from sewage-polluted Ogun River and Epe Lagoon sampled sites.All values are expressed as mean ± SEM of independent triplicate measurements. Asterisks denote significant difference at $p < 0.05$, ns; no significant difference.

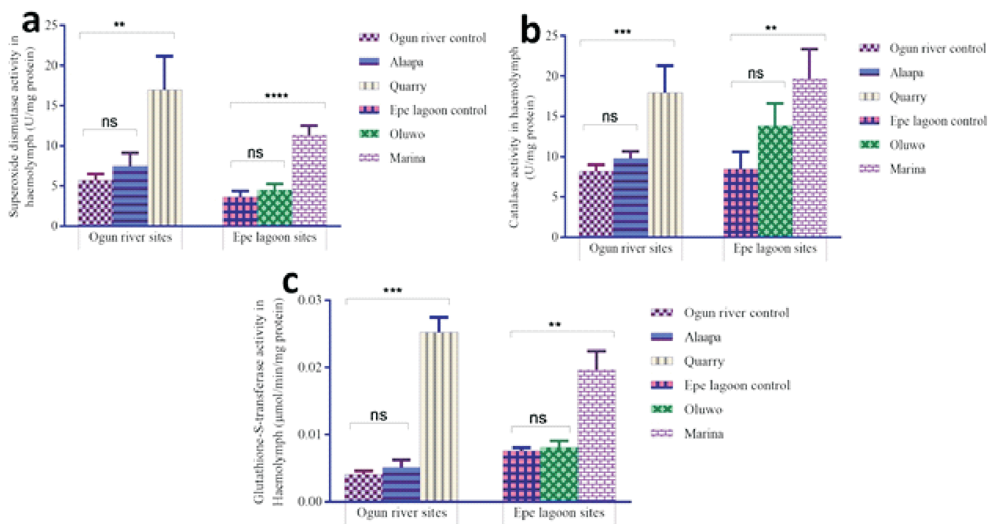


Figure 4. Antioxidant enzyme activities in *hemolymph samples of M. macrobrachionfrom* sewage-contaminated fishing waters. a. Superoxide dismutase activity in hemolymphsamples of *M. macrobrachion*from sewage-polluted Ogun River and Epe Lagoon sampled sites.b. Catalase activity in hemolymph of *M. macrobrachion*from sewage-polluted Ogun River and Epe Lagoon sampled sites.c. Glutathione-S-transferase activity in hemolymph of *M. macrobrachion*from sewage-polluted Ogun River and Epe Lagoon sampled sites.All values are expressed as mean ± SEM, n = 6. Asterisks denote significant difference at $p < 0.05$, ns; no significant difference.

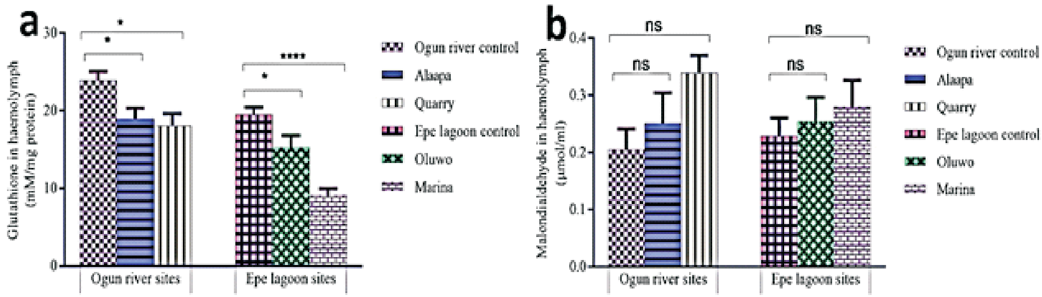


Figure 5. Non-enzymatic antioxidants concentration in *hemolymph samples of M. macrobrachionfrom* sewage-contaminated fishing waters. a. Glutathione concentrations in hemolymph of *M. macrobrachion*from sewage-polluted Ogun River and Epe Lagoon sampled sites.b. Malondialdehyde concentrations in plasma of *M. macrobrachion*from sewage-polluted Ogun River and Epe Lagoon sampled sites.All values are expressed as mean ± SEM, n = 6. Asterisks denote significant difference at $p < 0.05$, ns; no significant difference

Discussion

Water quality is a primary principle that serves as a groundwork for the health and sustenance of aquatic bodies and hydrology (Olatunji and Osibanjo, 2012). The uncontrolled release of domestic sewages into waterbodies lead to bioaccumulation of heavy metals and other toxicities in aquatic environments with consequential toxicimpacts on biodiversity and health (Pisutpaisal and Sirisukpoca, 2014). *Macrobrachium macrobrachion,*a highly valued aquatic food of the Nigerian inland watershas recently been focused on due to its dietary, health and economic benefits. However, little is known of the toxic impacts of sewage pollution of the aquatic habitat on prawn metabolic system, nutritive value and the ecosystem, hence the focus of this study. The physicochemical parameters are considered as the most important factors in identification of the nature, quality and degree of pollution in an aquatic environment (Mohamed *et al*., 2015). Among these parameters, pH and temperature are vital properties of aquatic environment since all biochemical functions and retention of physicochemical attributes of water depend on them (Jalal and Sanalkumar, 2013). Although significant variations were observed in the water samples from some of the test sites along the Ogun River and Epe Lagoon, the observed pH, temperature and dissolved oxygen values of the water from all the sampled sites were within the WHO (2008) permissible limit (pH = 6.5-8.5; Temperature $\leq$40°C, DO$\geq$6 mg/L) (Table 1). These findings are similar to that reported by Hegazi *et al*. (2015) and Saidu and Musa (2012). The mean BOD values of all the sampled test sites along both Ogun River and Epe Lagoon were within the WHO (2008) permissible limit (< 6 mg/L) for good water quality required for prawns and other aquatic lives, except for Quarry site along the Ogun River which was higher than the permissible limit (Table 1). In agreement with a previous report that a BOD value higher than 5 mg/L is a direct indication of water pollution(Clerk, 1996),the mean BOD values of water from all the sampled test sites along Ogun River and Epe Lagoon(> 5 mg/L) indicates pollution of the waters, which could be attributed to the release of sewage drainages and dumping of domestic wastes as observed during the initial survey of the sampled test sites. Meanwhile, the mean COD values of water from all sampled test sites along Ogun River and Epe Lagoon were higher than the WHO (2008) permissible limit (< 20 mg/L). The significant increase ($p < 0.05$) in the COD observed in the water sampled from Quarry and Marina sites along Ogun River and Epe Lagoon (Table 1) is consistent with the findings of Ojekunle *et al*. (2014),who reported a significantly increased COD in polluted Lagos coastal waters in Nigeria.The TDS values observed from all sampled sites along Ogun River and Epe Lagoon were below 1000 mg/L which is within the permissible limit set by WHO (2008) (Table 1). Taken together, although some of the physicochemical parameters (pH, temperature and BOD) of the sampled test sites along Ogun River and Epe Lagoon fell within the WHO (2008) recommended safe limits, thus implying that the waters were safe for aquatic life habitation as well as for a variety of utility purposes, the high COD and TDS values signifies gross pollution of the waters, with inherent detrimental effects on aquatic life and the ecosystem. Arsenic is one of the most important heavy metals in the world which has several adverse effects on the environment and humans as it exerts its toxic effect through the impairment of cellular respiration by inhibiting several mitochondrial enzymes and uncoupling oxidative phosphorylation(Hughes *et al.,* 2011). Lead is a persistent hazardous heavy metal, as it competes with essential metallic cations for binding sites and inhibitthe activity of antioxidant enzymes, induces cell death, oxidative stress, DNA damage and transcriptional activation of stress genes (Sfakianakis *et al.,* 2015). Cadmium on the other hand, is an endocrine disrupter, a severe irritant of the pulmonary and gastrointestinal system, and can also damage the kidneys, nervous system, lungs, bones, and the cardiovascular system if accumulated for prolonged periods in the human body (Zhang *et al.,* 2014). The elevated levels of arsenic, lead and cadmium observed in the water from the test sites as well as hemolymphof *M. macrobrachion* collected from the test sites along Ogun River and Epe Lagoon(Figs. 2 and 3)served as direct indicators of the high rate of pollution of the waters, which could be a consequence ofthe channeled sewage drainages and the different anthropogenic activities taking place at the embankments of the sampled sites, thus resulting in the accumulation of these heavy metals. This observation is corroborated by the findings of Farombi *et al.* (2007), Nsofor *et al*. (2014)andAdesiyan *et al*. (2018)where high concentrations of arsenic, lead and cadmium were observed in the water and *M. macrobrachion* from some sampled sites along Ogun River and Epe Lagoon.Bioaccumulation of arsenic, lead and cadmium is confirmed by the significant positive associations seen between the concentrations of arsenic, lead and cadmium in water and in the hemolymph of *M. macrobrachion* from the sampled sites (Table 3). The high concentrations of these heavy metals observed both in the fishing waters and hemolymph of *M. Macrobrachion may endanger the health of both aquatic and human lives as they become biomagnified along the food chain.* The assessment of oxidative damage and antioxidant defense in aquatic organisms can serve also as bio-indicators of heavy metal contamination of the aquatic habitat as heavy metals are wellknown to induce oxidative stress, which result in the oxidation of lipids and proteins, alteration of gene expression, and changes in cell redox status (Sheriff *et al.,* 2014). Aquatic organisms have antioxidant defense mechanisms such as catalase, superoxide dismutase, glutathione, and glutathione-S-transferase, which protect them from the oxidative effect of heavy metals (Palermo *et al.,* 2015). In the cell, superoxide dismutase is the first detoxification enzyme and most powerful antioxidant, as it is known to exhibit a cytoprotective ability against the damage induced by free radicals by converting superoxide radicals (O^{2-}) generated in the peroxisomes and mitochondria to hydrogen peroxides (Ighodaro and Akinloye, 2018). Catalase is the most sensitive enzymatic antioxidant as it secure cells from the lethal effects of hydrogen peroxide (H_2O_2) and other reactive oxygen species (ROS) (Raja and Puvaneswari, 2017). Decreased levels of cellular reduced glutathione levels is a potent indicator of the oxidizing state of the cell as it forms Glutathione-S-metal complexes with various heavy metals through its thiol group (Thirumavalavan, 2014). Glutathione-S-transferase aids the protection of cells against toxicants and pollutants through the conjugation of the thiol group of glutathione to electrophilic xenobiotics, and thus defends cells against the mutagenic, carcinogenic, and toxic effects of toxicants (Hegazi *et al.,* 2015).The observed increase in the activities of superoxide dismutase, catalase and glutathione-S-transferase (Fig. 4) in the hemolymph of *M. macrobrachion* from the sampled test sites along Ogun River and Epe Lagoon could be attributed to an increased free radical production consequent to exposure of the prawns to elevated levels of heavy metals observed in the water from the sampled test sites, indicating the polluted state of the waters. These observations agree with that of a previous study byValon *et al*. (2013) and Arojojoye and Adeosun (2016)where superoxide dismutase, catalase and glutathione-S-transferase activities significantly increased ($p < 0.05$) as a result of exposure to heavy metals and pesticides. The positive correlation observed between the activity of SOD, CAT and GST and the concentrations of arsenic, lead and cadmium in this study further supports the deduction that the increase in the activity of SOD, CAT and GST in the hemolymph of *M. macrobrachion* is as a result of increase in the concentration of arsenic, lead and cadmium in the hemolymph of the prawns sampled from Ogun river and Epe lagoon (Table 4). Furthermore, the significant decrease observed in the reduced glutathione concentration in the hemolymph of *M. macrobrachion* sampled from all the test sites along Ogun River and Epe Lagoon (Fig. 5a) depicts the oxidation state of the erythrocytes as a result of exposure to heavy metals. This observation is corroborated by the findings of Akhiromen and Ogbonne (2018). Heavy metals results in increase in production of reactive oxygen species (ROS), leading to oxidative damages such as lipid peroxidation and enzyme inactivation (Mahboob, 2013). Malondialdehyde (MDA) is an end-product of lipid peroxidation which serves as a good oxidative stress biomarker (Singh *et al.,* 2014). In the present study, no significant ($p < 0.05$) increase was observed in malondialdehyde concentration in the hemolymph of *M. macrobrachion* sampled from all the test sites (Figure5b) even though an increase was observed, indicating an increase in lipid peroxidation level which may be consequential of increased reactive oxygen species generation as a result of exposure to heavy metals such as arsenic, lead and cadmium, with an attendant significant decrease in glutathione concentration in the hemolymph of *M. macrobrachion from the sampled test sites*. This observation suggests that exposure to heavy metals activates lipid peroxidation in *M. macrobrachion* which is ultimately hazardous to the health of the prawn. A similar finding was previously reported byArojojoye and Adeosun (2016). As depicted in Table 2, the moisture content of

*M. macrobrachion*from all the sampled test sites along the Ogun River and Epe Lagoon,significantly($p < 0.05$) increased. This agrees with earlier observations byFafioye *et al*. (2017) and Ayanda *et al*. (2018).The observed increase is indicative of the susceptibility of the prawns to oxidative stress. A high moisture content will allow enzymatic reactions go on smoothly and thus, is good for living organisms. However,a high moisture content could also be of great disadvantage as it could make aquatic organisms more susceptible to spoilage by microbes, increase the oxidative degradation of polyunsaturated fatty acids (PUFAs) and consequently decrease their quality (Magami *et al.,* 2016). The significant ($p < 0.05$)decreaseobserved in the crude protein and ash content of *M. macrobrachion* from all test sites along Ogun Riverand Epe Lagoon may be attributed to the effect of the stressful condition necessitated by exposure to heavy metals. The significant ($p < 0.05$) decrease observed in the crude fat of *M. macrobrachion* from all the test sites indicates reduction in the nutritive value and occurrence of fat oxidation which might be attributed to the adaptation of the prawns to the energy requirement of the oxidative stress exerted by heavy metals. Ash content is a measure of the mineral composition in an organism as it is the inorganic residue left behind after organic matter has been burnt off (Adewumi *et al.,* 2014; Ayanda *et al.,* 2018). A significant ($p < 0.05$) decrease was observed in the ash content of *M. macrobrachion* sampled from Quarry and Marina sites along Ogun River and Epe Lagoon respectively. The total carbohydrate component is crude fiber and soluble carbohydrate (nitrogen-free extract (NFE). NFE is the source of energy since most prawn5s cannot digest fiber. No significant difference ($p < 0.05$) was observed in the crude fibre content and NFE of *M. macrobrachion* from all the sampled test sites along Ogun River and Epe Lagoon compared with the respective controls.These variations in the proximate composition of *M. macrobrachion* observed in this study suggesting alterations caused as a result of exposure to heavy metals including As, Pb and Cd is confirmed by the significant positive or negative associations between the proximate composition of the prawns and the concentrations of As, Pb, and Cd in the sampled sites water and the hemolymph of *M. macrobrachion.* Summarily, the various alterations observed in the activities and concentrations of the studied biomarkers of oxidative stress in the hemolymphand plasma of *M. macrobrachion* from Ogun Riverand Epe Lagoon as well asits decreased nutritional value is a resultant effect suggestive of exposure to heavy metals (arsenic, lead and cadmium) present in the fishing waters caused by sewage pollution at each of the sites.Hence, serves as warning for the deleterious effects associated with sewage pollution of aquatic habitats.

Conclusion

Sewage pollution of Ogun River and Epe Lagoon resulted in bioaccumulation of heavy metals leading to associated toxicities that induced oxidative stress in *M. macrobrachion* and significantly decreased the nutritional value and consequently the economic value of sampled *M. macrobrachion*. Hence, a potential risk to consumers of the latter from the sampled waterbodies.

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