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BIOCHEMICAL ENZYMES BIOMARKERS AND HISTOPATHOLOGY IN *CALLINECTES AMNICOLA* FROM POLYCHLORINATED BIPHENYLS (PCBS) CONTAMINATED AREAS OF LAGOS LAGOON, NIGERIA

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ABSTRACT

Oxidative stress induced by Polychlorinated biphenyls (PCBs) and its occurrence was investigated in sediment and biological samples collected from Abule-eledu and Atlantic Ocean in Lagos lagoon, Nigeria. Physicochemical parameters of the water were analysed with the aid of Horiba U-12 multi-sampler. The occurrence of PCBs in sediments and biological samples were analysed using Gas Chromatography coupled with Electron Capture Detector. Biomarkers of oxidative stress; Glutathione transferase, Superioxidase, Catalase coupled with Lipid perioxidation and histopathological biomarkers responses in the gill and muscle of Callinectes amnicola were investigated adopting standard and established methods. The turbidity (31.83 ± 4.32 NTU and 19.50 ± 4.48 NTU) in both stations was higher than the NESREA limit (10NTU) while PCBs 156 and 81 were the predominant congeners detected in the sediment. Glutathione transferase level in the gills and muscles of the C. amnicola collected from Abule-eledu (0.61 ± 0.06; 0.95 ± 0.07 µmol/mL/mg/ pro) was significantly ($p < 0.05$) lower in crab collected from the Atlantic Ocean (1.24 ± 0.08; 1.22 ± 0.09 µmol/mL/mg/ pro.) respectively. There were no significant ($p > 0.05$) differences in the levels of Superioxidase and Catalase of gill and muscle from both stations. The highest Lipid perioxidation detected in the gills (6.23 ± 0.27 and 7.73 ± 1.54 µmol/mL/mg/ pro) and muscle (6.63 ± 0.87 and 6.85±1.28 µmol/mL/mg/pro) from Abule-eledu and Atlantic Ocean stations respectively were not significantly ($p > 0.05$) different. Histological sections of the gills showed intermellar space, enlargement of secondary gill lamellae, disruption of pillar cells and swelling of secondary lamellae while generally the muscle displayed normal architecture with very rare feature of

muscle rupture. This implies that PCBs in the sediments of Abule-eledu are more biologically available than those of the Atlantic Ocean. This is an indication of a stressed environment that needs monitoring in order to protect the organisms inhabiting these areas.

KEY WORDS: *polychlorinated biphenyls, biomarkers, histopathology, lipid peroxidation, turbidity*

INTRODUCTION

Nigeria's vast water resources especially the Lagos lagoon are among those most affected by environmental stress imposed by human population growth, urbanization and industrialization as wells as pollutants such as PCBs. In the Lagos lagoon ecosystem, benthic organisms live on or just beneath the bottom of the lagoon or in the intertidal zone (mainly mudflats). They crawl over, burrow into, or are attached to the sediments or anything else on the bottom. Benthic organisms are important links in the estuarine food chains, providing an important food source for fishes, birds, and mammals. The blue crab *Callinectes amnicola* that inhabit the Lagos lagoon are ubiquitous, sedentary, benthic and able are able to survive in polluted sites thus their way of life makes them suitable as bioindicator species. The blue crab, *Callinectes* is an ecologically and economically relevant crustacean with a biogeographic range. In the aquatic environment PCBs re-dissolve at the water-sediment interface and then adsorb unto the sediments (Karvonen *et al.*, 2013; Huang *et al.* 2015) that are taken up by sediment-dwelling organisms (McLeod *et al.* 2008) such as the blue crab and bioaccumulate. Hence, the concentration of PCBs accumulated in crab may be used as a good tool to assess the degree of PCB pollution of the environment (Brázová *et al*., 2012b). Organism response could be tissue-specific and/or xenobiotic thus it is important to assay several endpoints related to oxidative stress. There are a various combinations of potentially toxic organic pollutant mostly contributed by anthropogenic activities that generate harmful reactive oxygen radicals or their by-products that illicit cell and tissue injury on living organism resident in the marine or other aquatic habitat resulting in oxidative stress (Winston 1991; Kelly *et al*., 1998). The exposure to a broad range of environmental chemicals such as aliphatic hydrocarbons, polynuclear aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs), polychlorinated dibenzo-p-dioxins, dibenzofurans, tributyl tins, nitroaromatics, phthalate esters, pesticides and heavy metals increase the levels of cellular oxidative stress in aquatic organisms (Farombi *et al*., 2007; Obanya *et al*., 2019). Antioxidant defense enzymes such as superoxide dismutase (SOD), catalase (CAT) glutathione peroxidases and small molecule antioxidants

(glutathione) are contained in aquatic organisms. Elevated antioxidant enzyme parameters associated with increased lipid peroxidation have been observed in the fish, *Fundulus* sp. and bivalves (Bacanskas *et al*., 2014; Martínez-Alvarezm *et al.*, 2005; Samuel, 2012) exposed to complex mixtures of organic chemicals (Bacanskas *et al.* 2004; Banni *et al.*, 2005). Lipid peroxidation, in particular, has been routinely measured in field studies to reflect chemical-induced oxidative damage (Barni *et al*., 2014).

Exposure to environmental contaminants can quantitatively alter these complements of antioxidant defenses thus exploited as biomarkers in the field (Livingstone 2001; Pandey *et al*., 2003; Banni *et al*., 2005). Biomarkers are very important early warning signaling tools of environmental degradation (Lam and Gray, 2003) which serves as an integrated measure of exposure and or effects of aquatic environmental pollutants (Miranda *et al.*, 2008). To address multi-contamination exposure context, the use of an array of complementary biomarkers such as morphological, histological and biochemical markers at different organizational levels has been widely utilized (Wang *et al*., 2010; Pereira *et al*., 2007; Boboescu et al, 2020). This serves as a toxicologically-relevant integrated assessment of environmental stress-induced alteration at the (sub) individual level (Van Der Oost *et al*., 2003). Many molecular and biochemical biomarkers are developed and applied in field studies which necessitates documentation of responses in tissues or individual level. A repression of antioxidant defense activities was reported by Ghedira *et al.* (2011) in gills and digestive gland of the crab *Carcinus maenas* collected from the contaminated area of Bizerte lagoon (Tunisia) associated with metals.

Environmental contaminants that cause alterations on tissue structure or organization as it affects crucial physiological functions in essential organs can be addressed using Histological biomarkers. Histological responses manifest as severe alteration or cell damage. This could be adaptive to an altered environment induced by pollutants which is more realistic and ecologically relevant (Au, 2004; Van Der Oost *et al*., 2003). In field studies the association between specific types of lesions and exposure to chemical pollutants has been observed by several authors (Bernet *et al*., 1999; Feist *et al*., 2004) that has shown that histological biomarkers are reliable and sensitive biomarkers of exposure to marine contaminants.

Blue Crab, *Callinectes amnicola* are inshore, demersal estuarine crab species. They occupy a variety of estuarine habitats from the lower reaches of freshwater rivers, estuaries and coastal marine waters and are highly mobile,making it possible for them to move between areas and to select habitats (Ryer *et al*., 1997; Micheli and Peterson, 1999). Blue Crab, *Callinectes amnicola* are inshore, demersal estuarine crab

species. They occupy a variety of estuarine habitats from the lower reachesof freshwater rivers, estuaries and coastal marine waters and are highly mobile, making it possible for them to move between areas and to select habitats (Ryer *et al.*, 1997; Micheli and Peterson, 1999).

The study established the occurrence of PCBs in sediment collected from Abule-eledu and Atlantic Ocean in Lagos lagoon and evaluated their environmental health using a suite of biomarker responses (CAT, SOD, GSH, LPO) as well as histopathology of the gills and muscle of *Callinectes amnicola* to measure oxidative stress in a PCB polluted area of the Lagos lagoon.

MATERIAL AND METHODS

Description of study area. Lagos Lagoon is a major lagoon sharing its name with the city of Lagos, Nigeria, the largest city in Africa. The Lagos Lagoon empties into the Atlantic via Lagos Harbor. The principal ocean port of Lagos is located at Apapa (western branch) off the main channel of the harbor. North-east of the lagoon is connected by a channel passing south of the town of Epe to the Lekki Lagoon. Narrow winding channels connects the Lagoon through a broad band of coastal swamps and rivers, that extends to Sapele; 250 km to the east.

Abule eledu and the Atlantic Ocean were selected for sampling based on heavy traffic of anthropogenic activities dictating the pollution dynamics of the Lagos Lagoon. Abule eledu is geographically located between latitude 6° 31' N and longitude 3° 24' E while Atlantic is located latitude of 6° 25' N, longitude of 3° 21' E (Fig. 1).

Animal sampling. *Callinectes amnicola* (blue crab) were collected from the selected sample stations for three months from September to December, 2018. The organism was chosen based on its abundance and availability along the Lagos lagoon from the selected stations. Samples were collected using a stainless steel Van Veen Grab of 0.1 m^2. Animals were sieved with a 2 mm mesh size stainless sieve and kept in aluminum foil to avoid contamination. It was stored in a cooler at 4°C until transported to the laboratory. All the scoops, sieves and buckets used in the collection of the samples were made from stainless steel.

Sediment sampling. Sediment samples were collected in three (3) replicates from both selected stations of the Lagoon in the months of September, October and December 2018 and homogenized to produce a single composite sample for each station. The homogenized samples were kept in aluminum foil and stored in a cooler at 4^oC, until conveyed to the laboratory.

FIG. 1. Lagos Lagoon showing the sampled sites

Physicochemical Analysis of Water. Physicochemical parameters such as temperature (T^oC), Hydrogen potency (pH), turbidity, conductivity, dissolved oxygen (DO), Total Dissolved Solid (TDS) and salinity were taken *in situ*, using a hand-held probe water Sampler (Horiba U-50 model) on the surface of the water.

Analytical procedures Determination of PCBs in environmental samples. The determination of PCBs in the samples were in three (3) steps:

(i) extraction,
(ii) purification/fractionation of the extract
(iii) chromatographic separation, identification/ quantification.

Extraction for Polychlorinated Biphenyls and Clean Up. Five grammes (5 g) of *Callinectes amnicola* and sediment samples were respectively mixed with sodium sulphate anhydrous, in ratio 1:1, to remove any residual moisture. The polychlorinated biphenyls (PCBs) was extracted from the dry samples using 200 mL n-Hexane as a solvent. The solution was shaken rigorously for 1 hour with the aid of an electronic

shaker, stored overnight for complete separation of hexanic phases and evaporation of the solvent. The extracts were cleaned up using solvent-rinsed chromatographic columns (15 mm - 250 mm), packed with a plug of glass wool followed by 3 g deactivated silica gel and topped up with sodium tetraoxosulphate (VI). The columns were pre-rinsed with 15 mL hexane after which 2 mL of the extract was added to the column and eluted with 60 mL hexane. The extracts were then concentrated to approximately 2 mL using a rotary evaporator and kept in a sample vial for gas chromatographic analysis (USEPA. 1999).

Detection and Quantification of PCBs Content. Detection, identification and quantification of Polychorinated Biphenyls (PCB) congeners in cleaned-up extracts were done using a gas chromatograph coupled electron capture detector (GC-ECD). Polychorinated Biphenyls recovery standards at known concentrations were analysed, after which, the samples were also analysed. Target analytes in samples were tentatively identified and semi-quantitation made. Identifications were made by comparison of retention times, peak shapes and peak patterns of the samples with those of the recovery standards. Quantitation was based on sample peak areas or peak heights relative to standard peak areas or peak heights.

Quality assurance. All of the procedures were controlled strictly by the analysis of procedural blank samples as well as the recoveries of surrogate standard in each analysed sample.

Total Organic Carbon Analysis of Sediments. TOC content of the sediment samples was determined by adopting the Walkley-Black procedure with slight modification. Potassium dichromate ($K_2Cr_2O_2$) and concentrated H_2SO_4 was added to 1 g of sediment. The solution was swirled and allowed to cool as a result of the exothermic reaction of potassium dichromate and sulfuric acids. The solution was thereafter boiled gently at 150^{o}C for 30 minutes to enable complete digestion (Mebius, 1960). Water was then added to halt the reaction. After sample digestion, the solution was centrifuged and filtered to remove any suspended particles and then placed in a calorimeter set to measure the light absorbance at a wavelength of 601 nm. Quantitation of TOC was determined colorimetrically at 601 nm against a reagent blank. Measurement was based on the colour change that results from the presence of Cr^{3+} in solution.

Biomarker Analysis. Oxidative stress was investigated in the gills and muscles of *Callinectes amnicola* by the measurement of selected antioxidant biomarkers; Reduced Glutathione (GSH) Superoxide dismutase (SOD), Catalase (CAT) and Lipid Peroxidation (malonaldehyde, MDA).

Homogenization of Gill and Muscle samples. The gill (0.5 g) and muscle (0.5 g) of *Callinectes amnicola* were homogenized with 5 mL of 0.4 M Phosphate buffer

using pestle and mortal. The homogenate was centrifuged at 3000 r.p.m for 15 minutes and the supernatant samples were stored at -20^{0}C for biomarker analysis.

Catalase Enzyme Assay. CAT 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 H_2O_2. The reaction mixture (3 mL) contained 0.3 mL of crude extract and 2.7 mL of 30 mM H_2O_2 in phosphate buffer pH 7.0. Phosphate buffer was used as reagent blank, absorbance was read at 60 seconds interval for 5 minutes. An extinction coefficient for H_2O_2 at 240 nm of 40.0 $M^{-1}cm^{-1}$ was used for the enzyme activity calculation. The specific activity of catalase was expressed as moles of H_2O_2 reduced per minute per milligram protein.

Superoxide Dismutase Enzyme Assay. SOD activity was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 at 30^{0}C as described by McCord and Fridovish, (1989). Three (3.0 mL) of Na_2CO_3 buffer was added to 0.02 mL of tissue homogenate (Tris-Hcl buffer, pH 7.5) and treated with 0.03mL epinephrine reagent and centrifuge at room temperature for 10 minutes at 3,000 rpm. The clear supernatant was transfer into 1.5 mL cuvette and the absorbance was measured against reference blank at 480 nm using spectrophotometer.

Reduced Glutathione Assay. GSH activity was determined by the method of Ellman (1959). Three (3 mL) of the 10% TCA was added to 3 mL of homogenate and centrifuge at 3000 rpm for 10min. Then, 1.0 mL of supernatant was treated with 0.5 mL of Ellman's reagent and 3.0 mL of phosphate buffer (0.2 M, pH 8.0), before the absorbance was read at 412 nm using spectrophotometer.

Lipid peroxidation Assay. LPO level was measured as malondialdehyde (MDA) concentration according to method of Niehaus and Samuelsson (1968). Exactly 0.1 mL of tissue homogenate (Tris-Hcl buffer, pH 7.5) was treated with 2 mL of (1:1:1 ratio) TBA-TCA-HCl reagent and place on water bath for 15 minutes, cooled and centrifuge at room temperature for 10 minutes at 3,000 rpm. The clear supernatant was transfer into 1.5 mL cuvette and the absorbance was measured against reference blank at 535 nm using spectrophotometer.

Total Protein was measured by the method of Lowry *et al*. (1951). The diluted biuret reagent was added to 0.02 mL of the samples**,** while blank reagent was used for the preparation of the protein standard and left at room temperature for 10 minutes. The clear supernatant was transferred into 1.5 mL cuvette and the absorbance was measured against reference blank at 546 nm using spectrophotometer.

Histopathology of the Gill and muscles of *Callinectes amnicola*. The gills and muscle of Callinectes amnicola was harvested from the sacrificed animals. Samples were

fixed in Bouin's solution for 6 hours to ensure hardening of the tissues. The tissues were then transferred into phosphate buffered formalin (10%) for preservation. Gills and muscles were dehydrated with ethyl alcohol 95% and 100%, and embedded in paraffin. The longitudinal sections of 5µm were stained with Delafield haematoxylin/eosin (H&E) and observed with optical microscope (Munro and Roberts, 1989).

Statistical Analysis. All analysis was carried out in triplicate. Data analysis was done using Statistical Package for Social Sciences (SPSS) version 16.0 and Excel Statistical Tool pack. The physicochemical parameters for each sample station were subjected to one-way Analysis of Variance (ANOVA) and Duncan Multiple Range Test. The PCBs concentration for each sample station and sample type was subjected to two-way analysis of variance (ANOVA) and Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Mean Concentrations of PCBs in *Callinectes amnicola.* The mean total PCBs in the *C. amnicola* from Abule-eledu and Atlantic Ocean were 1.98 ± 1.81 mg/kg and 1.26 ± 0.72 mg/kg respectively (Fig. 2). Twenty-six (26) PCB congeners were detected in the *C. amnicola* collected from Abule-eledu while 24 congeners were detected in that from the Atlantic Ocean out of which PCBs 81 and 18 were predominant.

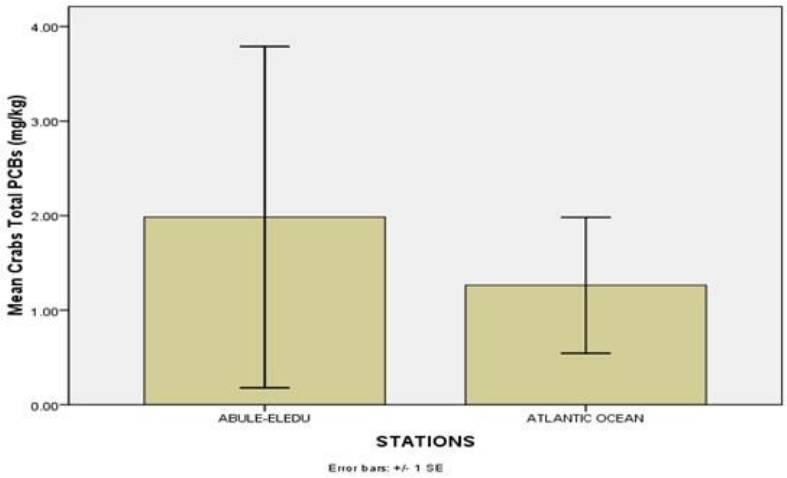


FIG. 2. Concentrations of PCBS in *Callinectes amnicola*

Mean Concentrations of PCBs in the Sediments. Twenty-two (22) and 26 PCB congeners were detected in the sediments collected from Abule-eledu and Atlantic Ocean respectively out of which PCBs 156 and 81 were the predominant congeners. The mean total PCBs in the sediments collected from Abule-eledu and Atlantic Ocean were 0.57 ± 0.45 mg/kg and 4.68 ± 4.58 mg/kg respectively, this was significantly ($p < 0.05$) different between the two stations (Fig. 3).

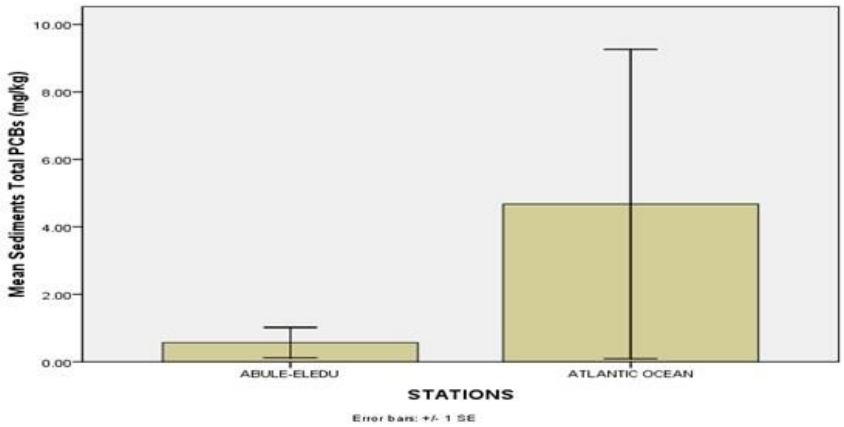


FIG. 3: Concentrations of PCBS in the sediments

Physico-chemical Parameters of water. The results of the Physico-chemical parameters of water samples collected from Abule-eledu and Atlantic Ocean showed that the mean temperature (25.95 ± 1.70^0C and 29.07 ± 0.54^0C) and turbidity (31.83±4.32 NTU and 19.50±4.48 NTU) were significantly ($p < 0.05$) different between the two stations whereas the turbidity was above the National Environmental Standards and Regulations Enforcement Agency (NESREA) maximum limit (Table 1). The conductivity (0.38±0.04 mS/cm and 13.79±6.31), Dissolved Oxygen (2.68 ± 0.54 mg/L and 6.61 ± 0.23 mg/L), pH (7.30 ± 0.04 and 7.90 ± 0.07), salinity (0.17 ± 0.03 ppt and 3.67 ± 0.03 ppt) and TDS (0.26 ± 0.02 g/L and 2.39 ± 1.17 g/L) were significantly ($p < 0.05$) different between the two stations but below the NESREA maximum limit (Table 1).

TABLE 1: Physico-chemical properties of water

Water physicochemical parameters	Abule-eledu	Atlantic Ocean	NESREA Limits
Temperature (^{0}C)	25.95 ± 1.70	29.07 ± 0.54	< 40
pH	7.30 ± 0.04^a	7.90 ± 0.07^a	6 – 9
Conductivity (mS/cm)	0.38 ± 0.04 ^{a}	13.79 ± 6.31^b	NA
Turbidity (NTU)	31.83 ± 4.32^a	19.50 ± 4.48^b	10
Dissolved Oxygen (mg/L)	2.68 ± 0.54^a	6.61 ± 0.23^b	5.0
Salinity (ppt)	0.17 ± 0.03^a	3.67 ± 0.03^b	NA
TDS(g/L)	0.26 ± 0.02 ^{a}	2.39 ± 1.17^b	0.2

Different letters (superscript) in lower case means significantly different at $p < 0.05$ across rows

Antioxidant status of Callinectes amnicola gills. The mean CAT levels (8.63±2.95 µmol/mL/mg/pro and 8.94±2.12 µmol/mL/mg/ pro) and SOD levels (2.31 ± 0.12 µmol/mL/mg/pro and 2.34 ± 0.08 µmol/mL/mg/ pro) measured in the gills of *C. amnicola* showed that there was no significant ($P > 0.05$) difference between the two stations respectively (Figs. 4 and 5). Whereas the mean GSH level in samples collected from Abule-eledu (0.61 ± 0.06 µmol/mL/mg/ pro) was significantly ($p < 0.05$) lower than that detected in crab collected from the Atlantic Ocean (1.24 ± 0.08 µmol/mL/mg/ pro.) (Fig. 6). However, the highest mean level of MDA in the gills of the *C. amnicola* collected from the two stations were 6.23 ± 0.27 µmol/mL/mg/pro and 7.73 ± 1.54 µmol/mL/mg/ pro respectively which was not significantly ($P > 0.05$) different (Fig. 7).

Antioxidant status of *Callinectes amnicola* muscles. The mean CAT levels (7.83±1.47 µmol/mL/mg/pro and 9.85±2.18 µmol/mL/mg/pro) measured in the muscle of *C. amnicola* showed a significant ($P < 0.05$) difference between the Abule-eledu and Atlantic Ocean stations respectively (Figure 8). The results of SOD levels (2.26±0.10 µmol/mL/mg/ pro and 2.37±0.15 µmol/mL/mg/ pro) showed no significant ($P > 0.05$) difference between the two stations respectively (Figure 9). The mean GSH level in the muscle of the *C. amnicola* from Abule-eledu 0.95±0.07 µmol/ml/mg/ pro and 1.22±0.09 µmol/ml/mg/ pro was significantly ($P < 0.05$) lower than that of the crab from the Atlantic Ocean (1.24 ± 0.08 µmol/ml/mg/ pro.) (Figure 10).

However, the highest mean level of MDA in the gills of the *C. amnicola* collected from the two stations were 6.23 ± 0.27 µmol/mL/mg/pro and 7.73 ± 1.54 µmol/mL/mg/ pro respectively which was not significantly ($P > 0.05$) different (Figure 11).

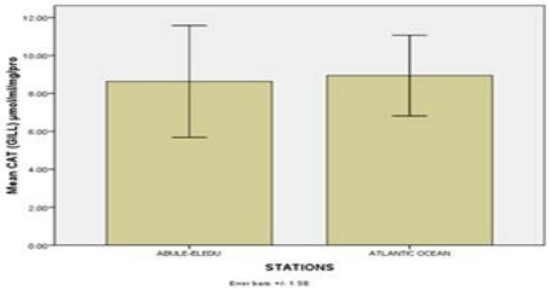


FIG. 4: CAT Levels in the Gills of *C. amnicola*

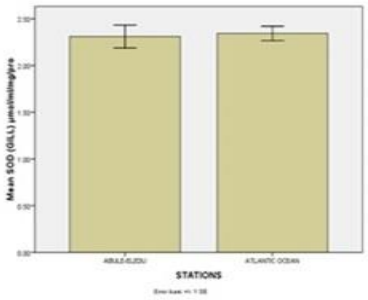


FIG. 5: SOD Levels in the Gills of *C. amnicola*

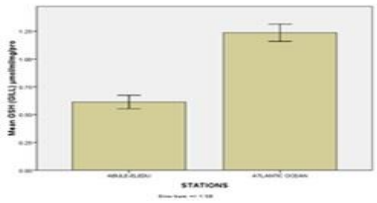


FIG. 6: GSH Levels in the Gills of *C. amnicola*

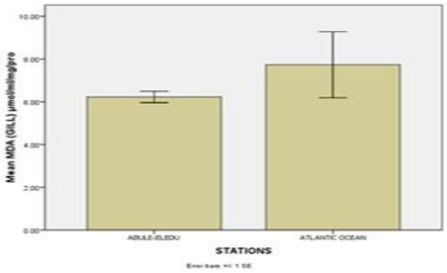
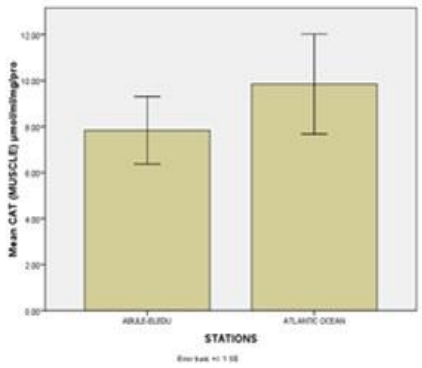


FIG. 7: MDA Levels in the Gills of *C. amnicola*



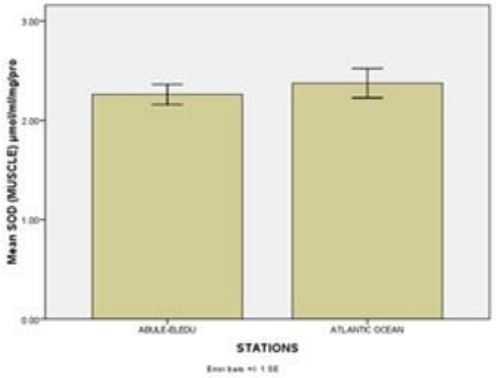
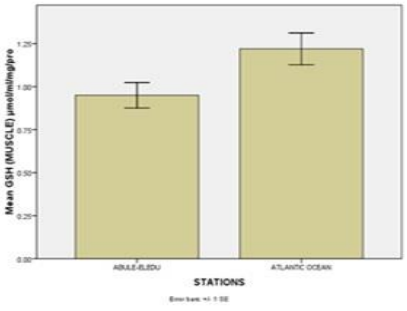


FIG. 8: CAT Levels in the Muscle Samples of *C. amnicola*

FIG. 9: SOD Levels in the Muscle Samples of *C. amnicola*



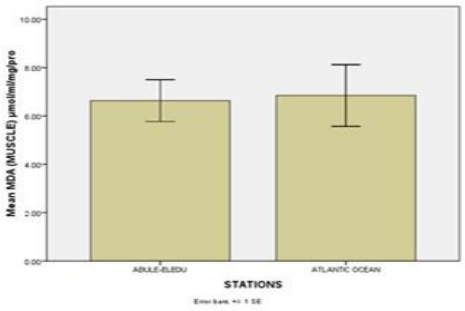


FIG. 10: GSH Levels in the Muscle Samples of *C. amnicola*

FIG. 11: MDA Levels in the Muscle Samples of *C. amnicola*

Histopathology of gills and muscle samples of *Callinectes amnicola*. Histological section of gill of *C. amnicola* from both stations showed alterations such as intermellar space, enlargement of secondary gill lamellae (ESGL); observed in all the gill samples, disruption of pillar cells (DPC) and swelling of secondary lamellae (SSL) (Plates 1, 2 and 3). However, only one of the gill sample of from the Atlantic Ocean had hypertrophy (Plate 4). While others had normal gill architecture with the primary gill lamellae, secondary lamellae and pillar cells intact (Plates 5 and 6).

Histological section of muscle samples of *C. amnicola* from Abule-eledu and the Atlantic Ocean had normal muscle architecture. The striated muscle fibers were tightly packed and the nuclei were arranged along the margins of the muscle bundles (Plates 7, 8, 10, 11 and 12). Although, one muscle sample of *C. amnicola* from Abule-eledu showed rupture of muscle bundle (RMB) (Plate 9).

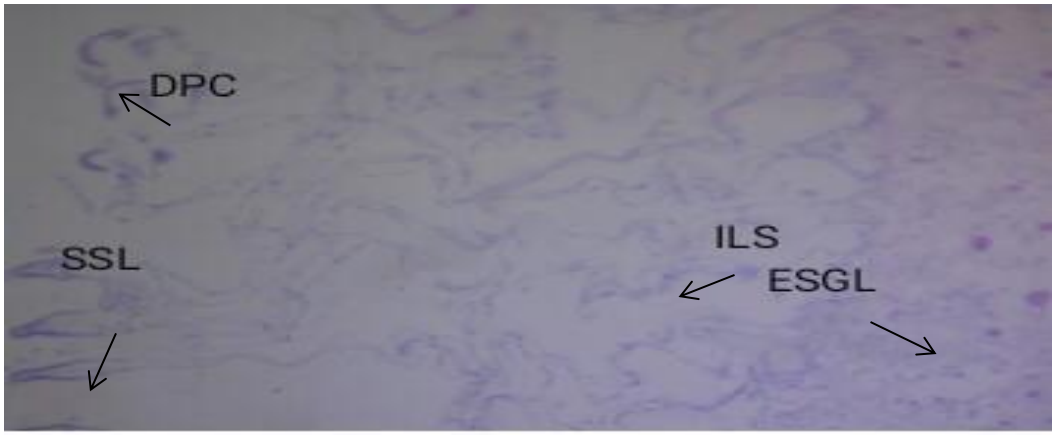


PLATE 1. Histological section of gill of *C. amnicola* collected from Abule-eledu showing alterations such as inter lamellar space (ILS), enlargement of secondary gill lamellae (ESGL), disruption of pillar cells (DPC) and swelling of secondary lamellae (SSL) (H &E stain; X40)

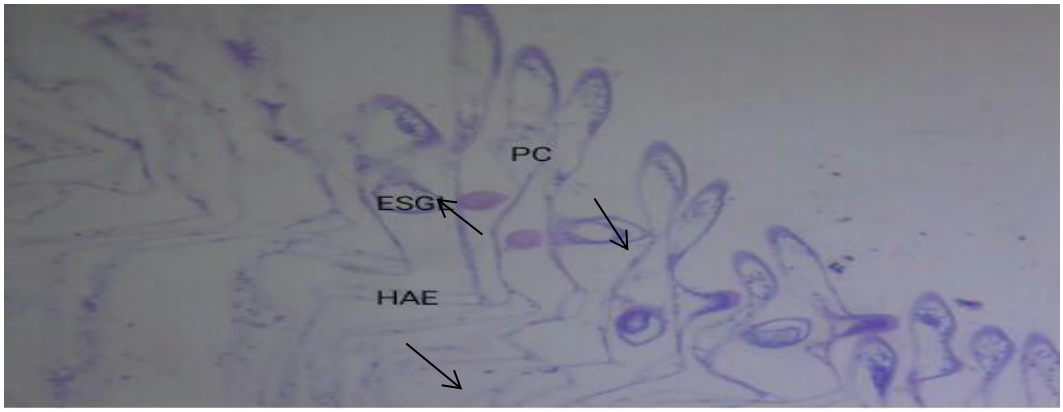


PLATE 2. Histological section of gill of *C. amnicola* collected from Abule-eledu showed pillar cells (PC) and haemocytes (HAE). Enlargement of secondary gill lamellae (ESGL) was also observed (H&E stain; X40)

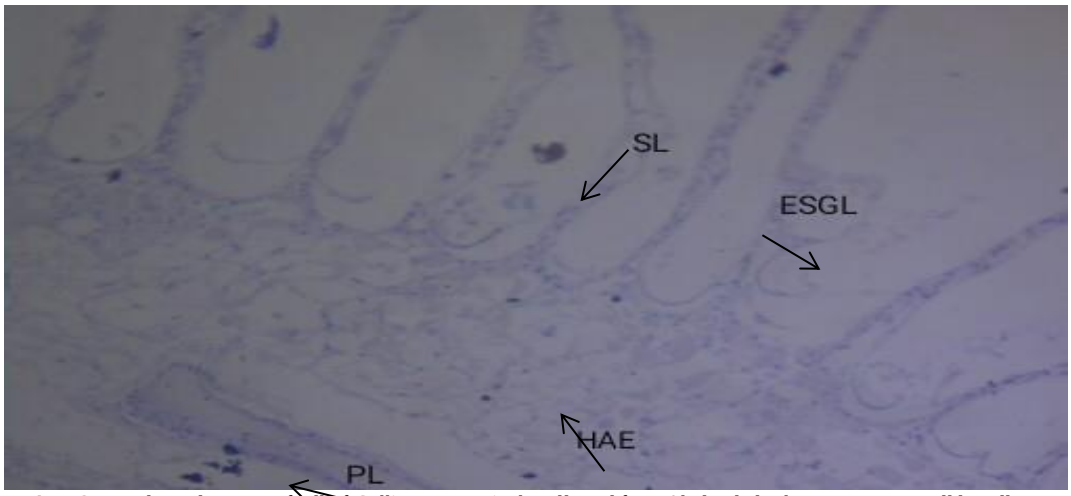


PLATE 3. Histological section of gill of *Callinectes amnicola* collected from Abule-eledu showing primary gill lamellae (PL), haemocytes (HAE) and secondary gill lamellae (SL). Also observed was ESGL – enlargement of secondary gill lamellae (H&E stain; X40)

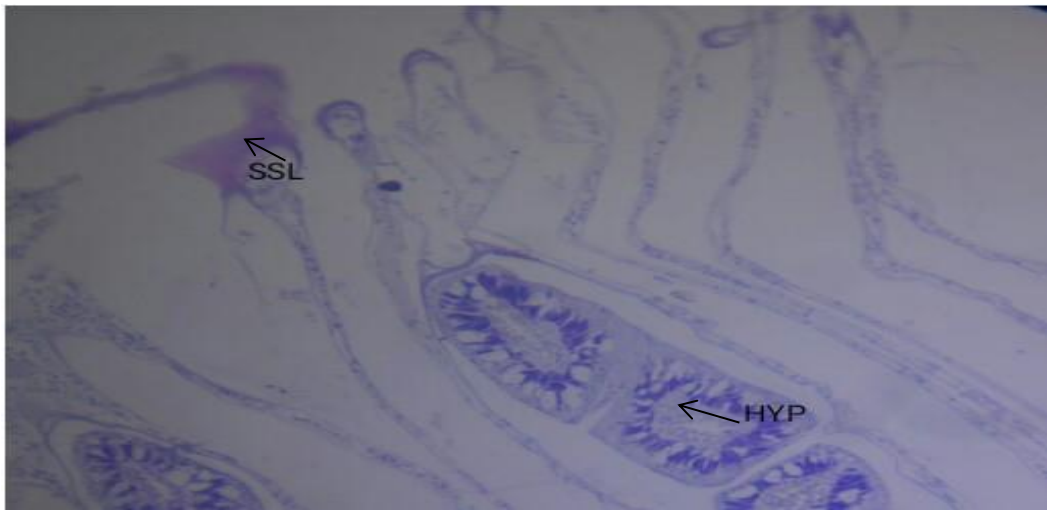


PLATE 4. Histological section of gill of *C. amnicola* collected from the Atlantic Ocean showed alterations such as swelling of secondary lamellae (SSL) and hypertrophy (HYP) (H&E stain; X40)

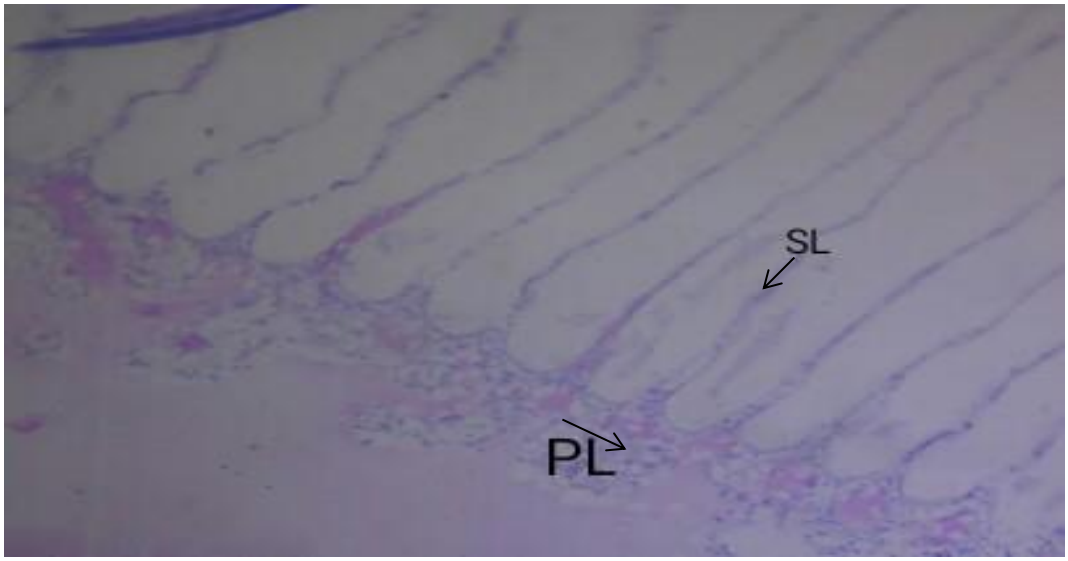


PLATE 5. Histological section of gill of *C. Amnicola* collected from the Atlantic Ocean showed a distinct primary gill lamella (PL) and secondary gill lamellae (SL). (H&E stain; X40)

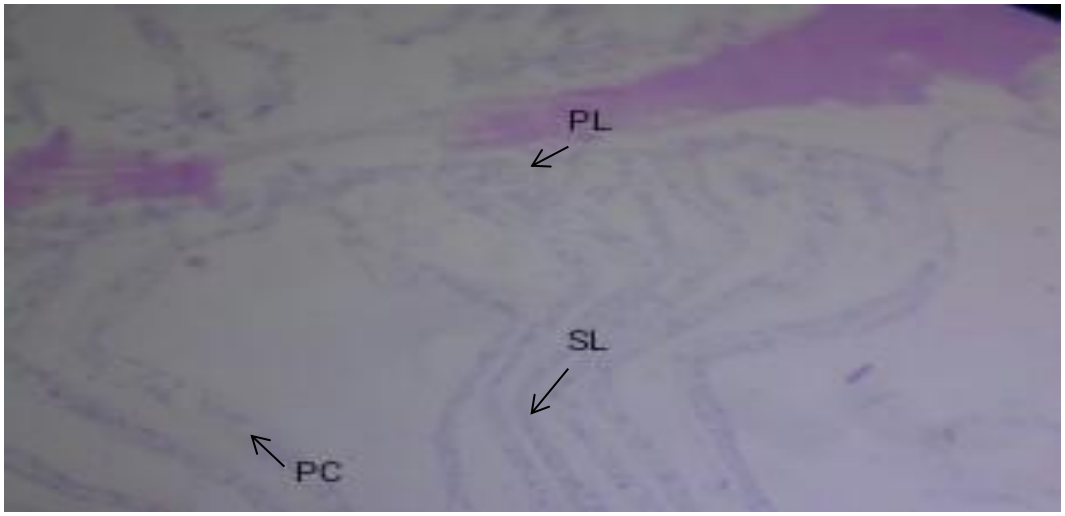


PLATE 6. Histological section of gill of *C. amnicola* collected from the Atlantic Ocean showed a distinct primary gill lamella (PL), secondary gill larmellae (SL) and pillar cells (PC). (H&E stain; X40)

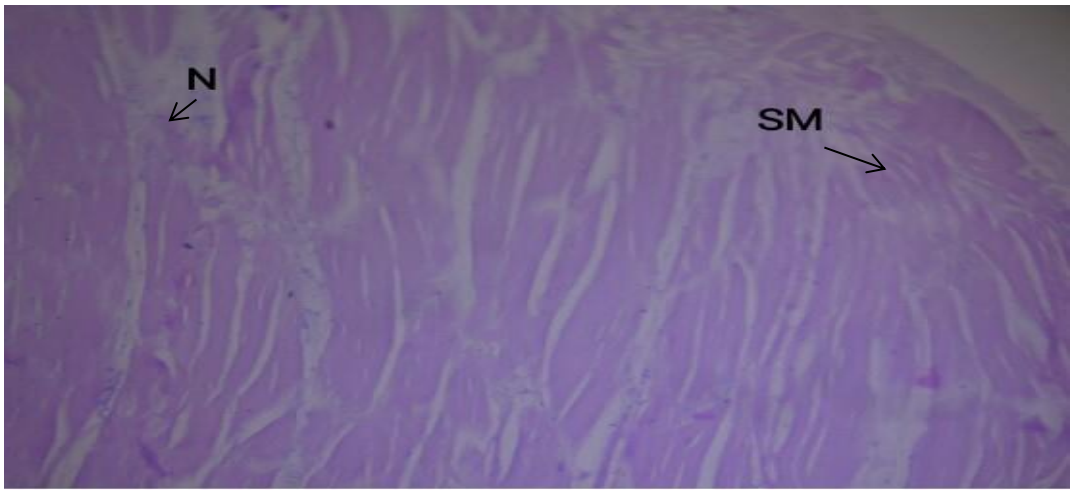


PLATE 7. Histological section of muscle of *C. amnicola* collected from Abule-eledu showed striated muscle fibers (SM) that were tightly packed, and nuclei (N) that were arranged along the margins of the muscle bundles (H&E stain; X40)

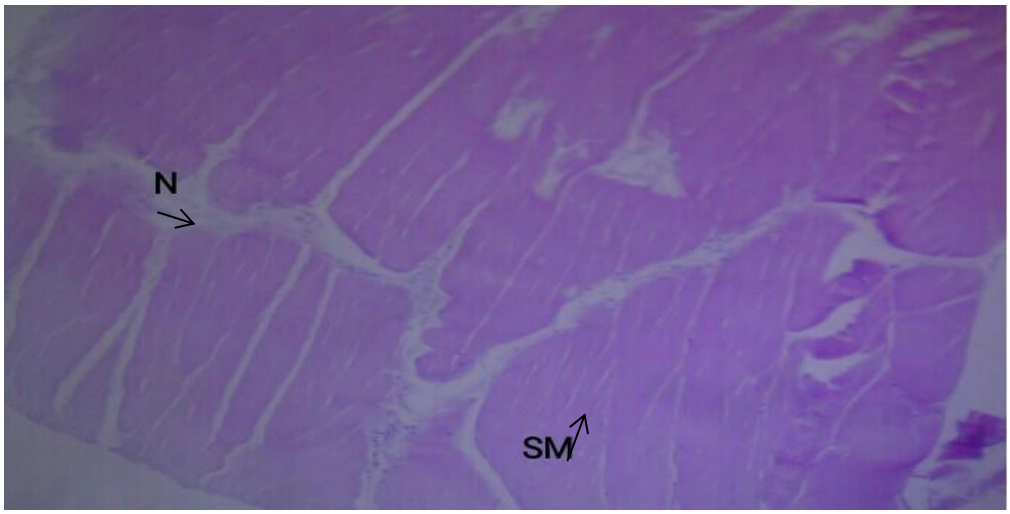


PLATE 8. Histological section of muscle of *C. amnicola* collected from Abule-eledu showed striated muscle fibers (SM) that were tightly packed, and nuclei (N) that were arranged along the margins of the muscle bundles (H&E stain; X40)

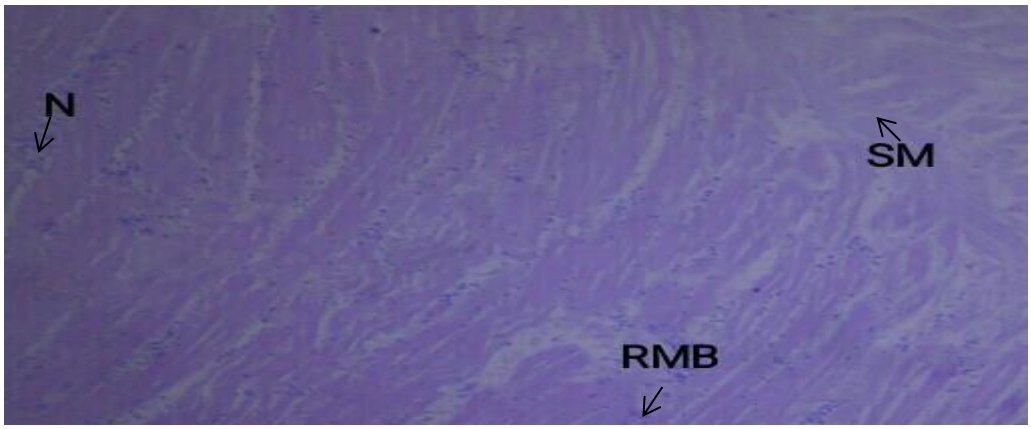


PLATE 9: Histological section of muscle of *C. amnicola* collected from Abule-eledu showing striated muscle fibers (SM) that were tightly packed, and nuclei (N) that were arranged along the margins of the muscle bundles. Rupture of muscle bundle (RMB) was also observed. (H&E stain; X40)

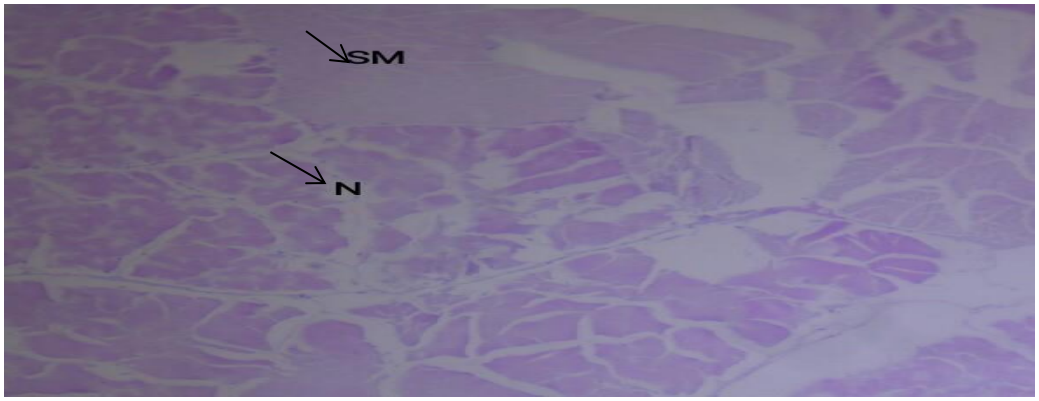


PLATE 10. Histological section of muscle of *Callinectes amnicola* collected from the Atlantic Ocean showed striated muscle fibers (SM) that were tightly packed, and nuclei (N) that were arranged along the margins of the muscle bundles (H&E stain; X40)

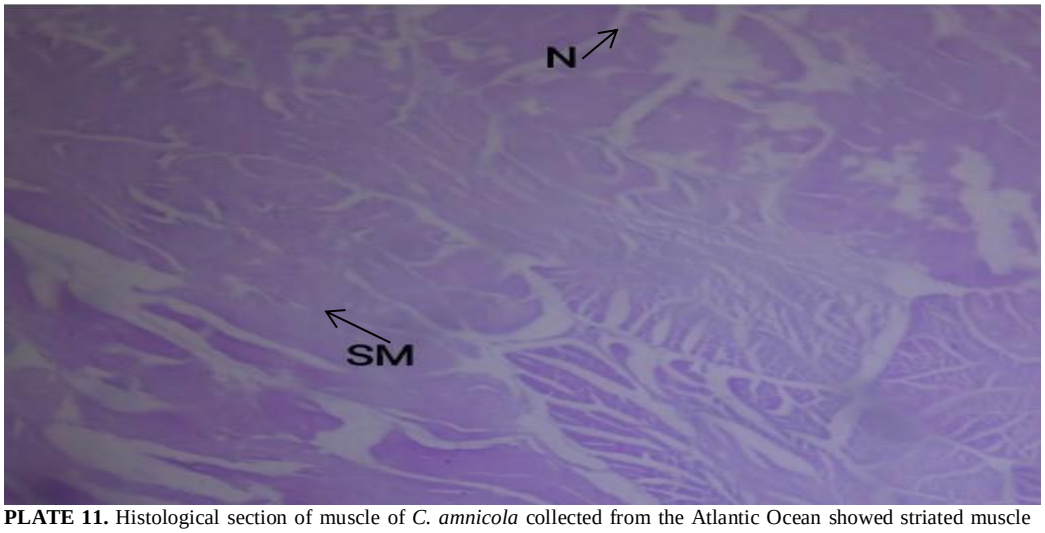


PLATE 11. Histological section of muscle of *C. amnicola* collected from the Atlantic Ocean showed striated muscle fibers (SM) that were tightly packed, and nuclei (N) that were arranged along the margins of the muscle bundles (H&E stain; X40)

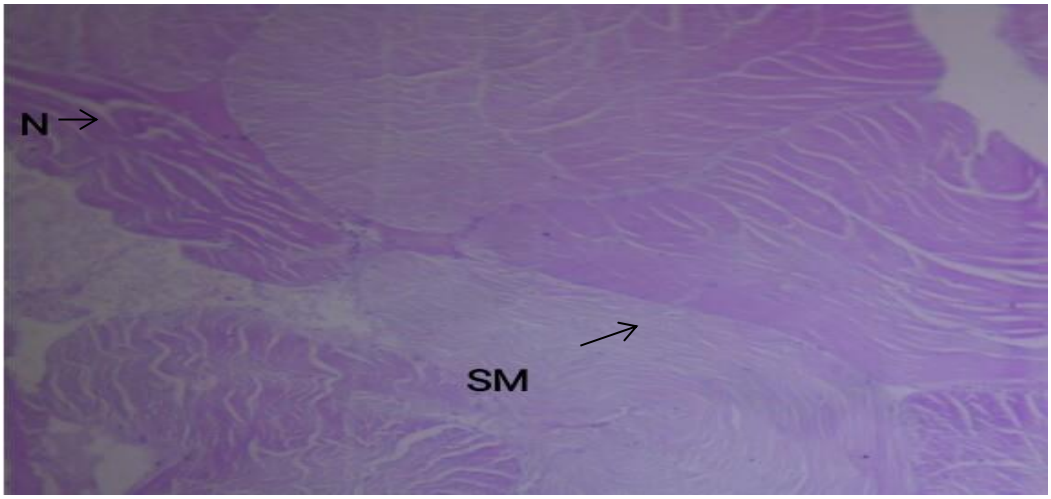


PLATE 12. Histological section of muscle of *C. amnicola* collected from the Atlantic Ocean showed striated muscle fibers (SM) that were tightly packed, and nuclei (N) that were arranged along the margins of the muscle bundles (H&E stain; X40)

The environmental health status of Abule-eledu and the Atlantic Ocean was investigated by evaluating the Polychlorinated biphenyls (PCBs) levels in sediments, and *Callinectes amnicola* collected from both stations. *Callinectes amnicola* was further assessed for oxidative stress using a battery of antioxidant defense enzyme markers, Lipid peroxidation and histopathology.

The mean water temperature levels of Abule-eledu and Atlantic Ocean were in tandem with temperatures between 28^{o}C and 32^{o}C recorded in large area of shallow water and seawater column in tropical ocean waters. However, the presence of PCBs in water as indicated from several studies showed that Temperature has marked effect in PCBs disposition *in vivo* (Andrea *et al*., 2007 and Paterson *et al*., 2007). The implication is that at lower temperatures there is the probability that temperature dependent processes will decrease elimination rates of PCBs in the animal system.

The pH values of the two stations that were within NESREA recommended range is an indication that the water body is slightly alkaline. This probably indicated that the waterbodies are suitable for biological productivity. Cavallo *et al.* (1999) and Kiely (1998) also reported a range of 7.96 to 8.14 in tropical Ocean waters. Abowei and George (2009) observed that a pH lower than 4 is harmful to aquatic organisms. This implies that the water body is conducive for the wellbeing of the aquatic organisms

inhabiting these stations. However, this singular parameter is not adequate to declare the health status of the Lagos lagoon under study.

The mean turbidity of the two stations which was observed to be higher than the NESREA maximum limit (10 NTU) could be attributed to particulate matter in flux, discharge of waste, run-off from urban sources, that stir up sediments stir up by bottom feeders, wave and current actions (especially in less deep areas). According to Nkwoji *et al.* (2010), the authors noted that particulate matters enter into water bodies by run-off that has the ability to increase the turbidity. This could inhibit the penetration of light and photosynthetic activities to the detriment of aquatic fauna.

The mean dissolved oxygen (DO) level recorded in Abule-eledu that was below the NESREA minimum limit (> 5 mg/L) portends an aquatic body deficient of sufficient oxygen. Oxygenation in aquatic ecosystems is as a result of an imbalance between the process of photosynthesis, degradation of organic matter, aeration (Aston, 1980) and oceanographic properties of water (Muller, 1988). Dissolved oxygen (DO) affects the solubility and the availability of nutrients (Lawson, 2011). Its low level as observed in Abule-eledu could probably result in damages to oxidation state of substances from the oxidized to the reduced form, thereby increasing the levels of toxic metabolites (Lawson, 2011). According to United States Department of Agriculture (USDA, 1992), the level of oxygen depletion depends primarily on the amount of waste added, the size, velocity, turbulence of the stream and the temperature of the water. The USDA further stated that frequent deaths of fish in water should not be attributed to the toxicity of matters, but rather from deficiency of consumed oxygen from biological decomposition of pollutants.

The high values of TDS recorded in the two stations could be linked to the sampling period. Total suspended and dissolved solids affect metabolism and physiology of fish and other aquatic organisms (Parvez *et al.*, 2006). They are products of run offs that is heightened with increased rainfall thus affecting the levels of dissolved oxygen and carbon dioxide. Suspended solids in water are directly proportional to dissolved solids. Dissolved solids could directly influence water conductivity, thus the higher the dissolved solids, the higher the conductivity (Lawson, 2011) as evidenced with the data recorded in this study. Rain reaches the earth's surface as runoff, flows over and through the soil and rocks, dissolving and picking up other substances (Lee *et al.*, 2011). This implies that the health of organism such as *C. amnicola* could probably be at risk.

Higher level of conductivity recorded in Atlantic Ocean compared to Abule–eledu could have been as a result of high evaporation rate and its ability to conduct an electric current (Akinde and Obire, 2011) an attribute of an aqueous medium. The conductivity of in any season is determined by the presence of total ions concentration,

mobility, valence, relative concentrations and the temperature of the system (Akinde and Obire, 2011).

The higher salinity level detected in Atlantic Ocean could be attributed to the eroding of rocks by acids in the rainwater resulting in ions, or electrically charged atomic particles (NOAA, 2017). These ions are carried away in run off to streams, rivers and, ultimately, to the ocean (NOAA, 2017). Natural concentrations of salinity for open seas in the tropical regions are usually high, but could be higher where rainfall is low and evaporation is high. The value is lower where large rivers enter the sea (King, 1981; Lalli, 1997).

The elevated concentrations of high molecular weight PCB congeners in sediments and *C. amnicola* from both stations, compared to the low molecular weight congeners, reflected stability and persistence. Another probable explanation is that PCBs in the sediments of Abule-eledu are more biologically available than those observed in Atlantic Ocean. Anyasi and Atagana (2013) observed that lower chlorinated PCB congeners tend to be more volatile and soluble in water, while adsorption to organic materials, sediments, and soils tends to increase with chlorination of PCB and organic content of the substrate (Passatore *et al.,* 2014).

The relatively higher concentration of PCBs in *C. amnicola* is suggestive of enhanced availability due to industrial inputs from point and nonpoint sources. Environmental contamination with PCB is generally related to point sources (industrial discharges and sewage treatment plant effluents) or diffuse sources such as atmospheric transport and deposition (Health Canada, 2004). Persistent organic pollutants extend to depths of 2,500 m deep in the Atlantic Ocean, but at concentrations not acutely toxic to deep-sea organisms (Caoxin *et al*., 2016). *Polychlorinated biphenyls were detected in crustacean samples such as Callinectes ornatus* (blue crab), *Hepatus pudibundus* (box crab), *Libinia spinosa* and *Portunus spinimanus* in Brazil, with the highest level found in *Callinectes ornatus* where the total PCBs were 17,4 ng/g (Rosângela and Rolf, 2004). The authors stated that differences in PCBs distribution in different species such as *C. ornatus* and *H. pudibundus* are associated to different type of bottom sediments as well as differences in their feeding habits and probably metabolic differences in PCBs bio-transformations.

According to Voorspoels *et al.* (2004) different benthic invertebrates (flying crab, common shrimp, and red starfish) were collected in the Belgian North Sea and along the Scheldt Estuary, both representing areas impacted by various contaminants to different degrees and found that the sum of PCBs detected in benthic invertebrates ranged from 1.5 to 280 ng/g wet weight (ww).

GSH levels in the gill and muscle samples of *C. amnicola* collected from Abule-eledu that were statistically lower than that from the Atlantic Ocean suggested that Abule-eledu is under severe environmental stress when compared to the Atlantic Ocean. Reduced glutathione concentrations are used frequently as markers of effects in both field and laboratory situations. Experimental supplementation with GSH has been shown to reduce oxidative damage directly induced by some PCB congeners (Slim *et al*., 2000). In combination with other markers, GSH can serve as a useful indicator of early responses to oxidative challenge to cells. Rodriguez-Ariza *et al*. (2003) observed a decline in GST levels over a longer term of PCB exposure. According to the authors the activity continued to decline until 110 days' exposure, suggesting a slow overburdening of the system due to PCB accumulation while GSH levels increased and decreased with time (Rodriguez-Ariza *et al*., 2003). The production of reactive oxygen species is also prevalent in the world's oceans. Oxidative stress is an important component of the stress response in marine organisms exposed to a variety of stressors as a result of changes in environmental conditions such as thermal stress, exposure to ultraviolet radiation, or exposure to Pollution (Lesser, 2006). As in the clinical setting, reactive oxygen species are also important signal transduction molecules and mediators of damage in cellular processes, such as apoptosis and cell necrosis, for marine organisms (Lesser, 2006).

The SOD, CAT and MDA levels in the gills and muscle samples of *C. amnicola* from both stations that were not in significant variance probably had a similar effect on these antioxidant enzymes. Oxidative stress indicated by the production of reactive oxygen species (ROS) and lipid peroxidation occurred in a chronic exposure to PCBs (Hassoun *et al.,* 2000, 2002). Oxidative stress was also found to be associated with the inhibitory effects of Aroclor 1254 (Sredivi *et al.,* 2007). The antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) are part of the antioxidant defense mechanism of the cell, and play important roles in the protection against the induction of oxidative stress, in response to different xenobiotics in the environment (Davies, 1995; Josephy, *et al*., 1997). SOD and CAT levels were found to be suppressed in animals exposed to Aroclor 1254 for 30 days (Muthuvel *et al*., 2006; Venkataraman *et al.,* 2007). In an effort to adapt to oxidative stress induced by various xenobiotics in the environment, organisms are able to up-regulate their antioxidant defense mechanisms in response to low concentrations of those xenobiotics (Davies, 1995). In addition, studies by Schlezinger and Stegeman (2001) demonstrated significant increases in hepatic antioxidant enzyme activities of the marine fish scup, in response to a low concentration of PCBs. Lipid peroxidation, a manifestation of oxidative stress, has been reported as a major contributor to the loss of cell function under chemical stress and has been

frequently indicated by MDA levels (Liu *et al*., 2007; Almroth *et al*., 2008; Li *et al*., 2012). Mingbae *et al.* (2013) demonstrated that long-term exposure to organic compounds caused lipid peroxidation, with evidence of the elevated MDA levels mainly noted in the high-dose groups.

The histopathological changes of gill as observed in *C. amnicola* could result in hypoxia, respiratory failure problems with ionic and acid-base balance (Alazemi *et al.,* 1996). The animal's defense responses are excessive mucus secretion due to the stress caused by the environmental change and pathologic agents that induce the proliferation of mucus cells (Richmonds and Dutta, 1989; Cardoso *et al.,* 1996). Lifting of the epithelium, lamellar fusion and club shaped lamellae could be protective in that it diminishes the amount of vulnerable gill surface area (Richmonds and Dutta, 1989; Maharajan *et al.,* 2012; Maharajan *et al.,* 2013). Karlsson-Norrgren and Runn, (1985) reported that an increase in the volume of the secondary lamellae as observed in the present study results in closing of the interlamellar space and decrease of the diffusion capacity of the gill which could be detrimental to the fish.

One muscle sample of *C. amnicola* from Abule-eledu showed that the muscle bundles were ruptured. This corroborated with the findings of Rakhi *et al.* (2013). Exposure of *Labeo rohita* to hexachlorocyclohexane was found to induce separation of muscle bundles and intracellular oedema in the muscle tissues (Das and Mukherjee, 2000). Moreover, Mohamed (2009) observed degeneration of muscle bundles with aggregations of inflammatory cells and focal areas of necrosis in the muscle tissues of *Tilapia zilli* and *Solea vulgaris* exposed to heavy metal. Such observations were also made in muscle tissues of *Oreochromis mossambicus* on exposure to dimethoate (Parikh *et al.,* 2010).

CONCLUSION

The use of a suite of antioxidant enzymes, Lipid perioxidation and histopathology biomarkers responses as biological end points in *C. amnicola* has established PCBs levels of some selected sites of the Lagos lagoon thus useful in assessment of environmental pollution. It has also provided discrimination between Abule-eledu and the Atlantic Ocean sampled sites of the Lagos lagoon with different contamination levels though these responses are site dependent. The sensitivity of these biomarkers has been utilized in expressing oxidative stress as a complementary biological tool for biomonitoring of marine ecosystems. Intensive regulatory controls for monitoring and mitigating wastewater emissions into Nigerian waters should be implemented and emphasized by the relevant government organs.

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