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Metal Content and Oxidative Stress Enzymes in Aquatic Crab, *Goniopsis cruentata* (Latreille, 1802) from Tropical Creeks Adjacent Western Axis of the Lagos Lagoon

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ABSTRACT

Heavy metal analysis and biomarker assessment when combined, can offer more robust and biologically relevant information on the impact of pollutants on organism health. The study evaluated oxidative stress responses in the mangrove crab, *Goniopsis cruentata* inhabiting contaminated locations on nearshore wetland ecosystems adjacent the western axis of the Lagos Lagoon. Heavy metal analysis was done using Atomic Absorption Spectrophotometer while antioxidant enzymes were isolated from the crab tissue by homogenization and centrifugation. Serial dilutions of the crude enzymes were then assayed for residual enzymatic activities using standard enzyme assay protocol. On the average, all the examined heavy metals (nickel, copper, chromium, lead and zinc) exhibited low contamination factor (CF), as their individual values were less than one (1). Relatively, higher CF values were however observed for copper in crabs from Abule-Agege and Abule-Eledu Creeks, suggesting the potential for elevated concentrations in sediment. Nickel was observed to be the most accumulated metal in tissues of *G. cruentata* across creeks. With the exception of superoxide dismutase (SOD) and catalase (CAT), strong positive correlations ($P < 0.05$) were observed between the antioxidant enzyme activities and the heavy metals (nickel, copper, chromium, lead and zinc) across creeks. Presence of heavy metals showed inhibitory effect on SOD and CAT activities, indicating an increasing level of environmental stressors in the area.

Keywords: Biomarkers, Ecological Risk Assessment, Heavy metals, Mangrove Crab.

Introduction

Metal occurring in aquatic ecosystems at varying concentrations may be due to biogeochemical cycling and anthropogenic inputs. The most potentially harmful of these trace elements include; lead, mercury and cadmium.[1] Heavy metal is generally used as a generic term for metals and metalloids associated with environmental pollution, toxicity and adverse effects on living organisms. It is considered the most important constituent of pollution from the aquatic environment due to toxicity and accumulation by aquatic organisms.[2] Aquatic contaminants such as heavy metals are not easily destroyed through the natural process of biological degradation and therefore can accumulate in the environment.[3]

The assessment of biological effects of chemicals, from molecular to tissue levels, has been considered as an effective biological tool in the biomonitoring of marine ecosystem contamination.[4-5] The biological method of detecting aquatic pollution involved the use of fishes, insects and benthos in assessing the quality of aquatic bodies, serving as bio-indicators of environmental pollution.[6] It is believed that sublethal pollutant induced effects in the biological system will first occur at biochemical and subcellular levels before they manifest at higher biological levels.[7] This is more so as aquatic organisms are often exposed to chronic levels of chemicals in their environment, which commonly occur in complex forms.[8,9]

One of the more elusive crab species occurring in brackish water environment in Nigeria is the Purple Mangrove Crab (*Goniopsis cruentata*, Latreille 1802), inhabiting almost every microhabitat in the mangrove ecosystems.[10] Although the mangrove crab does not really constitute a food item for the coastal communities, it however plays a major ecological role in the mangrove ecosystem through processing fallen leaves for feeding. [13] Aquatic crabs as a benthic organism concentrate contaminants from the water column and bottom sediment in which they grow. When heavy metals amass in their tissues, they generate specific reactive oxygen species (ROS), a major precursor of oxidative stress.[11-12] The body acts to counter the effect of these oxidants by activating a series of antioxidant defense systems such as superoxide dismutase (SOD), catalase (CAT), and the glutathione triad: reduced glutathione (GSH), glutathiones-transferase (GST) and glutathione peroxidase (GPx). They all have specific functions in detoxifying the ROS species generated by aquatic pollutants.[6] Chemical biomarkers such as antioxidant enzymes and evidence of oxidative damage to biomolecules are powerful tools for detecting the exposure and biological effects of pollutants, allowing early detection of environmental problems.[5] Hence, biomarker responses are generally considered to be intermediates between pollutant sources and higher-level effects.[13] Many reports have demonstrated the occurrence of heavy metals in the muscle tissue of benthic organisms, [14-19] and crab responses to environmental stressors.[20-21] However, there is paucity of information on the oxidative stress and antioxidant responses in species of grapsid crabs found near shore locations adjacent the Lagos Lagoon. The main thrust of the present study was to use chemical biomarkers to evaluate the health status of crabs inhabiting contaminated locations (via heavy metal assessment) in the mangrove creeks, adjacent the Lagos Lagoon.

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Materials and Methods

Study site

Mangrove swamps surround the sampling region, which is located between latitudes 6º 26' – 37' N and longitude 3º 23 – 4º 20'E on the highly populated western axis of the Lagos Lagoon (Figure 1). The selection of sites (Abule-Agege and Abule Eledu Creeks) was based on the increasing anthropogenic effects of household and solid waste disposal.

Sample collection

Random sampling was carried out fortnightly between 17.00 and 19.00 hours on monthly bases from January to March 2021. Six representative samples of water and sediment, each from different sampling station were taken. In total, ninety (90) adult crabs (carapace length 3.72 ± 0.39 cm and total weight 23.90 ± 4.21 g) were collected. Water samples were collected with sampling bottles from three stations each from the two creeks, stored in an ice chest and transported to the laboratory for the determination of the heavy metals. Sediment sampling was performed with Van-veen grab at all stations. Thereafter, the samples were packed in plastic bags and transported to the laboratory located at the Department of Marine Sciences, University of Lagos for processing and analysis. Each sample was preserved by adding 4m nitric acid and stored in a freezer prior to chemical analysis. The test animals, *G. cruentata,* (weighing 23.90 ± 4.21 g) were caught using baited traps and hand-picked with the aid of protective rubber gloves. Crabs were placed in styrofoam boxes without water and immediately transported to the laboratory for further analysis.

Laboratory Analysis

Metal content determination in surface water, sediments and tissues of crab

The concentrations of nickel, copper, chromium, lead and zinc were determined in the samples due to their ecological relevance. Approximately 5 g of sediment samples were air dried, ground into fine powder using pestle and mortar, and sieved using a mesh of 1 mm (pore size). Digestion and analysis of samples for metal content were done according to standard methods. [22,23] In this procedure, 100 mL of water was added into a pre-cleaned beaker and 0.5 mL of 68 % HNO_3 and 5 mL of 35% HCl were added and the mixture heated to 95 ℃ for digestion process. Thereafter, the volume was made up to 10 mL with 0.1 N of 68% HNO_3. The metal concentrations of both the digested water and sediment samples were quantified using GBC (Savant AA Sigma) flame atomic absorption spectrometer (AAS). All chemical reagents used herein were of analytical reagent grade (Merck, United State). The glassware was pre-cleaned with nitric acid and rinsed with double distilled water.

For the determination of metal contents in crab, muscle tissues of the examined thirty (30) samples per site were oven-dried at 70°C for 1 h and ground to powder in ceramic mortars and 0.5 g of each sample were made into a paste by adding double-distilled water. This was followed by digestion using 5 mL of 1 M HNO_3 and mild heat until brown fumes appeared, following previously reported technique.[24] The samples were cooled off, made up to 50 mL in a standard volumetric flask which has been subjected to acid wash to remove any trace of residual metals and then filtered. Thereafter, processed samples were analysed using Atomic Absorption Spectrophotometer (Perkin Elmer series) to determine levels of selected heavy metals.

Ecological risk assessment

In this study, ecological risk of metal distribution in sediments was determined using three ecological risk indices; contamination factor (CF), pollution index (PLI) and bioaccumulation factor (BAF). The Cf and PLI were applied to assess metal distribution and contamination in the sediment using the equations (1) and (2);

$$\textit{Contamination factor: } Cf = \frac{Cmetal}{Cbackground} \qquad (1)$$

C_{metal} is the concentration of metal in sediment, while $C_{background}$ is the background value for the metal. The average composition of shale from Turekian and Wedepohl [25] was used as background values in mg kg^{-1}, for the following metals; nickel (68), copper (45), chromium (90), lead (20) and zinc (95). CF in this study was considered as:

CF < 1 - Low contamination factor

1 < CF < 3 - Moderate contamination factor

3 < CF < 6 - Considerable contamination factor

6 > CF - Very high contamination factor.

The pollution load index (PLI) proposed by Tomlinson *et al.*[26] was also used in this study. The PLI for a single site is the nth root of n number multiplying the contamination factors (CF values) together:

$$\textit{Pollution Load Index: } PLI = (Cf \times Cf \times Cf \times \dots Cfn)^{1/n} \qquad (2)$$

Where Cf is the contamination factor, n is the number of metals.

Bioaccumulation factor (BAF) was calculated to determine the level of heavy metal accumulation in the tissue of the organism using the formula below:

$$\text{BAF} = \frac{\text{Concentration of metals in crab}}{\text{Concentration of metals in sediment/Water}} \qquad (5)$$

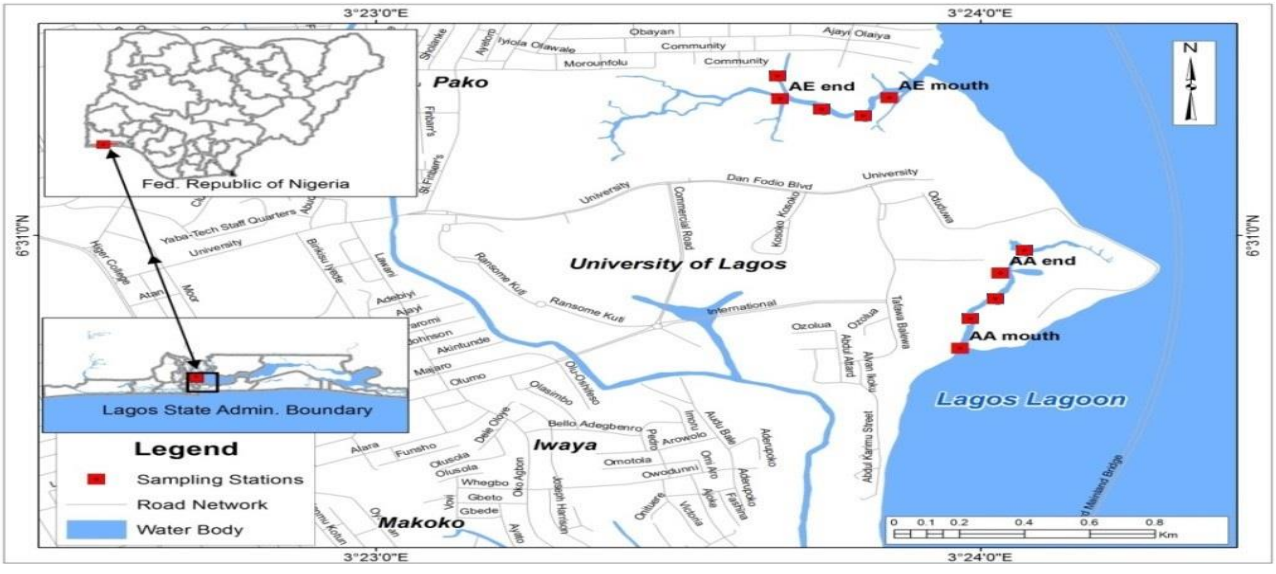

Figure 1: Map showing the selected sampling stations at Abule-Agege and Abule-Eledu Creeks

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Evaluation of oxidative stress biomarkers

Excised muscle tissues of the crabs stored at −20 °C were later thawed and homogenized for the assays of SOD, CAT, GST, GSH, and GPX following the protocol described by Lushchaks *et al.* [27]. Protein was determined spectrophotometrically using the Bio-Rad DC protein assay kit (Richmond, CA, USA) with bovine serum albumin (BSA) as a standard, based on the method of Lowry *et al.*[28]. The activity of SOD was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 at 30°C. Approximately 3 mL of Na_2CO_3 buffer was added to 0.02 mL tissue homogenate (Tris-HCl buffer, pH 7.5) and treated with 0.03 mL epinephrine reagent and centrifuged at room temperature for 10 min at 3,000 rpm. The clear supernatant was transferred into a 1.5 mL cuvette, and the absorbance was measured against a reference blank at 480 nm using a spectrophotometer. The activity of CAT was determined following the absorbance of hydrogen peroxide at 240 nm, pH 7.0 and 25°C. GSH activity was determined by adding 3 mL of 10% trichloroacetic acid to 3 mL homogenate and centrifuged at 3,000 rpm for 10 min. Then, 1.0 mL of the supernatant was treated with 0.5 mL Ellman's reagent and 3.0 mL phosphate buffer (0.2 M, pH 8.0) before the absorbance was read at 412 nm using a spectrophotometer. GST activity was determined by adding into triplicate wells, 20 L of sample, 150 L of assay buffer (100 mM potassium phosphate, pH 6.5, containing 0.1% Triton X- 100) and 20 L of glutathione. A background or nonenzymatic control was run by adding only 170 L of assay buffer and 20 L of glutathione to three wells. The reaction was initiated by adding 10 L of 1-chloro-2, 4-dinitrobenzene (CDNB) and carefully shaken for several seconds. The absorbance was measured at 340 nm every 30 s for 10 min using a BioTek PowerwaveTM micro-plate reader. The rate of absorbance per min was determined for the background wells and subtracted from the sample wells. GST activity (nmol/min/mL) was normalized to protein, and final GST results were reported as nmol/min/mg protein.

Statistical analysis

Normality of data was tested using the Kolmogorove-Smirnov Test ($\alpha = 0.05$). Homogeneity of variance was tested using Levine's test. Collected data were not normally distributed. Differences between mean concentrations in samples from the two sampling locations were tested using the T-test ($\alpha = 0.05$) while Pearson Correlation Coefficient was performed on the relationship between metal and oxidative stress enzymes. Box plot was created using XLSTAT. Statistical analyses were performed with Microsoft Excel 2010 and Statistical Package for Social Sciences (SPSS) version 12.0.1.

Results and Discussion

Metal concentration in water and sediment

Heavy metals (nickel, copper, chromium, lead and zinc) were recorded in varying and sometimes very low but measureable concentrations in surface water and sediments across sites (Table 1).

Generally, highest mean concentrations were recorded at locations which lie closest to domestic and solid waste dumps. In surface water, significant ($P = 0.03$) variation was observed for chromium between Abule-Agege and Abule-Eledu Creeks. Contrary to this, significant ($P < 0.05$) differences were observed for heavy metal concentrations in sediments, except for lead. The metals with highest concentrations across locations were zinc and copper in water and sediment samples respectively. The recorded mean concentrations for zinc and lead in the water sample were within the permissible guidelines from International regulatory limits (Zn - 5.0 and Pb - 0.015 mg/L),[29] while the concentration of zinc in sediment were lower than what was reported for Ologe (68.73 mg/kg) and Badagry Lagoons (48.12 mg/kg).[30]

Contamination indices in the sediments

The contamination factor (CF) of nickel, copper, chromium, lead and zinc for the sediment samples collected from creeks adjacent the western axis of the Lagos Lagoon are presented in Figure 2. On the average basis, all the examined heavy metals exhibited low contamination, as their individual values were less than 1. However, the highest CF values of copper were 0.0073 and 0.0095 in Abule-Agege and Abule-Eledu Creeks, respectively, suggesting the potential for elevated copper accumulation in the sediment.[19] In comparison with the results of CF in the previous study,[31] the metal contaminations in this study were found to be less than levels reported for Ologe Lagoon.

Computed Pollution Load Index (PLI) values for sediment samples collected from western creeks of the Lagos Lagoon are shown in Table 2.

Table 1: Metal Concentration in Water and Sediment of Tropical Creeks adjacent Western Axis of the Lagos Lagoon

Sample	Parameters	Abule-Agege	Abule-Eledu	*P-value*
Water	Nickel	0.0003 ± 0.00	0.0002 ± 0.00	0.29
	Copper	0.0045 ± 0.00	0.0241 ± 0.02	0.29
	Chromium	0.0001 ± 0.00	0.0003 ± 0.00	0.03*
	Lead	0.0003 ± 0.00	0.0001 ± 0.00	0.07
	Zinc	0.1136 ± 0.00	0.1442 ± 0.03	0.32
Sediment	Nickel	0.0032 ± 0.00	0.0108 ± 0.00	0.01*
	Copper	0.3272 ± 0.00	0.4268 ± 0.00	0.00*
	Chromium	0.0343 ± 0.01	0.0006 ± 0.00	0.01*
	Lead	0.0003 ± 0.00	0.1883 ± 0.02	0.00*
	Zinc	0.2809 ± 0.06	0.1639 ± 0.02	0.14

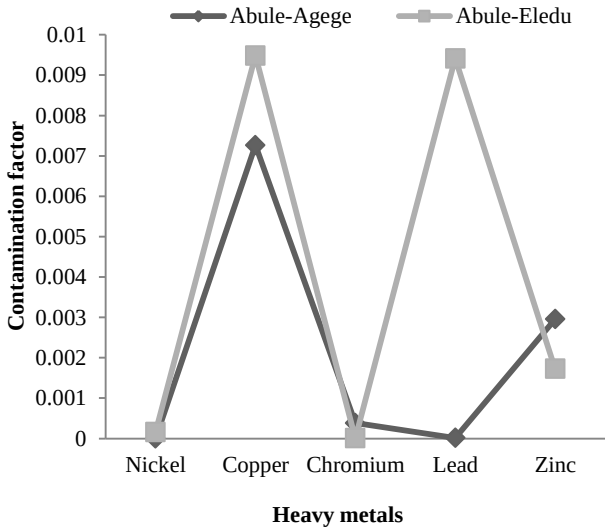

Figure 2: Contamination factor for sediments from tropical creeks adjacent western axis of the Lagos Lagoon

Table 2: Pollution Load Index for Sediments from Tropical Creeks adjacent Western Axis of the Lagos Lagoon

Creeks	Stations	Pollution Load Index
Abule-Agege	1	0.0881
	2	0.0891
	3	0.0892
	Mean	0.0888
Abule-Eledu	1	0.2677
	2	0.2147
	3	0.2247
	Mean	0.2357

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PLI values of sediments of the Abule-Agege Creek ranged from 0.088 to 0.089 with an average of 0.088, while that of Abule-Eledu Creek vary between 0.215 and 0.268 with an average of 0.236. This result indicates that sediments from both creeks are unpolluted; therefore similar to the report on creeks of the Great Kwa River.[32]. According to Tomlinson *et al.*[26], the PLI provides a simple, comparative means for assessing a site or estuarine quality: a value of zero (0.0) indicates perfection, a value of one (1.0) indicate only baseline levels of pollutants present and values above one (> 1.0) indicate progressive deterioration of the site and estuarine quality.

Metal levels and accumulation in crab

Certain forms of metals have been shown to readily accumulate within crustacean tissues at much higher levels. Crab species mostly absorbed heavy metals from its feeding diets, sediments and surrounding waters resulting to their accumulation in reasonable amounts.[13] Generally, our results showed measurable but low concentrations of heavy metals amongst examined samples and across creeks (Figure 3). All the examined metals (with the exception of copper and zinc) showed a narrow range of accumulation in *G. cruentata* across stations in examined creeks. Zinc had the highest concentration (0.1211 - 0.2312 mg kg^{-1}) while lead recorded the lowest concentration (0.0001 – 0.0003 mg kg^{-1}) in the crab tissues for both creeks. Relatively higher metal contents were recorded in *G. cruentata* when compared to water, which can be attributed to biological buildup. Similarly, Lawal-Are *et al.*[33] found elevated levels of zinc (0.74 ± 0.13 mg kg^{-1}), copper (1.21 ± 0.03 mg kg^{-1}), chromium (0.28 ± 0.10 mg kg^{-1}), lead (0.26 ± 0.07 mg kg^{-1}) and nickel (0.28 ± 0.03 mg kg^{-1}) in *Callinects amnicola* from Igbese River. According to Ezemonye *et al.*[34], elevated heavy metal concentrations in aquatic organisms imply cumulative exposure through water and/or food.

In tissues of the crabs, nickel was observed to be the most bioaccumulated metal from Abule-Agege Creek while chromium was the most bioaccumulated in Abule-Eledu Creek with respective values of 0.77 and 0.83 (Figure 4). The heavy metal bio-accumulation patterns found in *G. cruentata* from Abule-Agege Creek followed decreasing order as nickel > lead > zinc > copper > chromium. On the other hand, the heavy metal bioaccumulation patterns found in the crab from Abule-Eledu Creek followed the decreasing order: chromium > zinc > nickel > copper > lead. Bioaccumulation of heavy metals in crustaceans critically influences the growth rate, physiological and biochemical status[13]. Furthermore, a number of deleterious effects including oxidative stress from heavy metal bioaccumulation in biological systems have been reported.[5, 6, 13, 21]

Oxidative stress and relations to metal concentration

The results of the activity of the biochemical enzymes are presented in Table 3. Examined samples of *G. cruentata* showed significant difference ($P<0.05$) only in GPX between Abule-Agege and Abule-Eledu Creeks. With relatively higher mean values of 16.82±2.00 g/L protein recorded in samples obtained from Abule-Agege Creek. The highest values (109.88±2.77 Min/mg/pro) and lowest (105.90±2.63 Min/mg/pro) of SOD activity were obtained in samples of *G. cruentata* from Abule-Agege and Abule-Eledu Creeks respectively. Relatively higher mean values of CAT, GST, GSH, GPX were recorded at Abule-Eledu Creek as compared to the levels in crabs obtained from Abule-Agege Creek.

Oxidative stress in aquatic organisms is induced by many chemical pollutants which may stimulate the production of reactive oxygen species and other oxygen free radicals that can lead to alteration in antioxidant systems.[35] Moreover, increase in antioxidant enzymes activities have been attributed to adaptive responses of the organism to counteract the oxidative effect of generated ROS or resistance to water pollutants toxicity against the damage caused by the excessive amount of oxygen free radicals and oxidative stress.[36] SOD-CAT system is the primary defense mechanism against such oxidative stress. The recorded high and relatively low levels of SOD activity observed in crabs from Abule-Agege and Abule Eledu Creek respectively suggest potential stress from those locations. In a related study, Usese *et al.*[13] reported a relatively low level of SOD activities in crabs from Abule-Agege and Okobaba, suggesting potential stress from the locations.

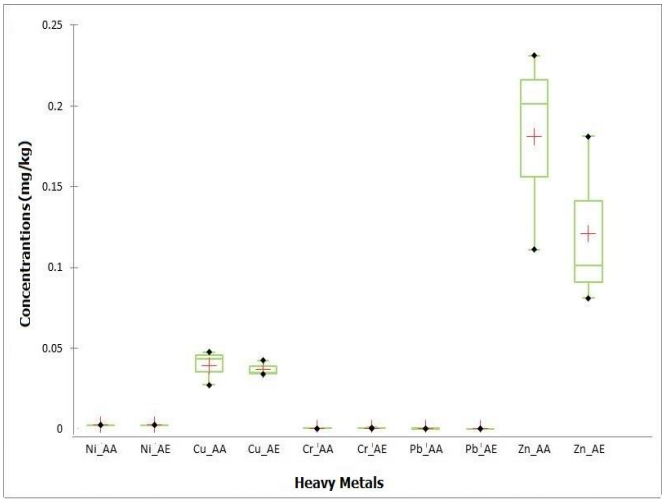

Figure 3: Box plot for metal concentration in the tissues of *Goniopsis cruentata* from western creeks of the Lagos Lagoon (Lower and upper box boundaries 25th and 75th percentiles, respectively, line inside box median, lower and upper error lines 10th and 90th percentiles, respectively, filled circles data falling outside 10th and 90th percentiles). AA: Abule-Agege Creek. AE: Abule-Eledu Creek.

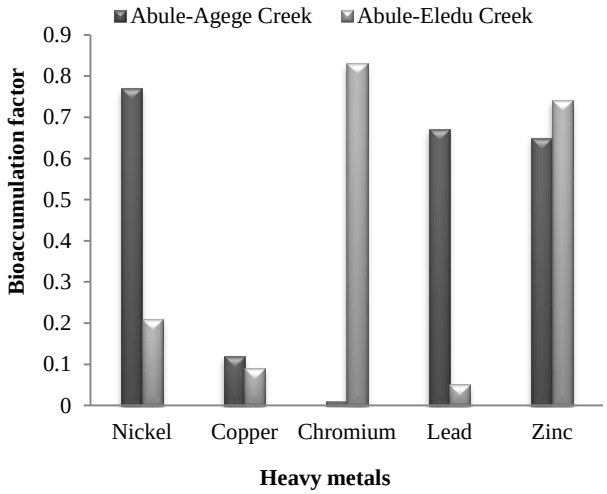

Figure 4: Metal bioaccumulation of *Goniopsis cruentata* from tropical creeks adjacent western axis of the Lagos Lagoon

Table 3: Oxidative Stress Enzyme Activities in Tissues of *Goniopsis cruentata* from Tropical Creeks adjacent Western Axis of the Lagos Lagoon

Parameters	Abule Agege Creek	Abule Eledu Creek	*P*-value
PRO (g/L)	16.82 ± 2.00	13.27 ± 2.44	0.12
SOD (Min/mg/pro)	109.88 ± 2.77	105.90 ± 2.63	0.15
CAT(Min/mg pro)	5.35 ± 2.73	6.64 ± 2.21	0.56
GST(Hmol/mg pro)	12.45 ± 2.43	15.42 ± 2.89	0.24
GSH(Hmol/mg pro)	1.87 ± 0.11	2.02 ± 0.20	0.31
GPX(Hmol/mg pro)	48.56 ± 4.10	60.07 ± 2.55	0.01*

Keys: PRO- Protein, SOD- Superoxide dismutase, CAT- Catalase, GST- Glutathione transferase, GSH- Glutathione, GPx: Glutathione Peroxidase, *- Significant difference ($P < 0.05$)

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Table 4: Relations between Tissue Metals and Antioxidant Enzyme Activities in *Goniopsis cruentata* from Abule-Agege Creek

Heavy metal	PRO	SOD	CAT	GST	GSH	GPX
Nickel	0.98	-0.90	-0.92	0.94	0.98	0.88
Copper	0.98	-1.00	-1.00	1.00	0.80	1.00
Chromium	1.00	-0.98	-0.99	1.00	0.90	0.97
Lead	0.88	-0.75	-0.77	0.81	1.00	0.71
Zinc	0.98	-0.90	-0.92	0.94	0.98	0.88

Table 5: Relations between Tissue Metals and Antioxidant Enzyme Activities in *Goniopsis cruentata* from Abule-Eledu Creek

Heavy metal	PRO	SOD	CAT	GST	GSH	GPX
Nickel	0.95	-0.94	-0.92	0.94	0.98	0.93
Copper	1.00	-1.00	-0.99	1.00	1.00	0.99
Chromium	1.00	-1.00	-1.00	1.00	0.99	1.00
Lead	0.99	-0.99	-0.98	0.99	1.00	0.98
Zinc	0.95	-0.94	-0.92	0.94	0.98	0.93

Some major correlations between the antioxidant enzyme activities and analysed heavy metals were shown by the relationship matrix for Abule Agege Creek (Table 4) and Abule-Eledu Creek (Table 5). With the exception of SOD and CAT, strong positive correlations (P> 0.05) were observed between the antioxidant enzyme activities and the heavy metals across creeks. Over the years, studies have shown that chemical analysis and biomarker assessment when combined, can offer more complete and biologically relevant information on the impact of pollutants on organism health.[5] Presence of heavy metals showed inhibitory effect on CAT activity. Antioxidant enzymes, as SOD increase their activity in low metal levels and time exposures, but their activities are inhibited with higher metal concentrations.[21]

Conclusion

All examined metals (nickel, copper, chromium, lead and zinc) were observed to accumulate in varying and sometimes very low but measurable concentrations in water and sediment samples across creeks. With the exception of SOD and CAT, strong positive correlations were observed between the antioxidant enzyme activities and the heavy metals (nickel, copper, chromium, lead and zinc) across creeks. Obtained levels of antioxidant enzymes in *G. cruentata* further indicate increasing level of environmental stressors in the area.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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