Antioxidant Defense System Alternations in Four Crab Species as a Bio-Indicator of Environmental Contamination

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Abstract

The ecological health status of aquatic environment is a determinant for the survival and growth of organisms within such niche. An investigative study was carried out on four crab species – *Cardiosoma armatum*, *Goniopsis pelli*, *Callinectes amnicola*, *Portunus validus* inhabiting contaminated sites in Lagos Lagoon- exploring their antioxidant defense mechanism in the light of heavy metal concentration in the crab tissues. Amongst the measured heavy metals, cadmium level proved to be significantly highest ($P < 0.05$) with range concentration of 0.42 ± 0.12 mg/kg (*G. pelli*) - 0.79 ± 0.06 mg/kg (*C. armatum*). Contrastingly, lead was marginally low with concentration below 0.01 mg/kg in all the crab species. Organismal responses to environmental pollution showed a high level of biomarkers. *C. armatum* was observed to have elevated level of superoxide dismutase (123.04 ± 0.01 min/mg/pro), catalase (7.74 ± 0.05 min/mg/pro), glutathion transferase (18.21 ± 0.02 Hmol/mg pro), reduced glutathione (2.92 ± 0.04 Hmol/mg pro) and glutathione peroxidase (61.85 ± 0.06 Hmol/mg pro) above other species with *C. amnicola* recording the lowest concentration of the biomarkers. With the low level of heavy metals and corresponding high concentration of these biomarkers, the pollution indices within the study habitat are quite modest.

Keywords: brachyuran, ecosystem, heavy metal, antioxidant, Lagos Lagoon.

Introduction

Crabs have flourished predominantly in the invertebrate fauna because of its ubiquitous existence in almost all part of the world oceans, fresh water, and even on land (Akin-Oriola *et al.*, 2005). Many species actively forage on land and several species have become semi-terrestrial (Cumberlidge, 1999; Moruf and Ojetayo, 2017). The marine littoral ecosystem and estuaries are threatened by increasing levels of various pollutants originating from human activities, urban, agricultural and industrial discharges. Heavy metals are potent toxicants

which have a wide spectrum of adverse effects on different levels of organismic integration. They accumulate in the bodies of organisms and increase the formation of reactive oxygen species (ROS) which can lead to protein, DNA, and lipid damage (Frenzilli *et al.*, 2001; Regoli *et al.*, 2004), enzyme inhibition, cell signalling impairment, disruption of calcium homeostasis, and changes in gene regulation (Elbekai and El-Kadi, 2005).

The assessment of biological effects of chemicals, from molecular to tissue levels, has been considered as an effective biological tool

in the bio monitoring of marine ecosystem contamination (Jebali, *et al.*, 2012). Biological monitor or pollution biomarker is early diagnostic tool for biological effect measurement and environmental quality assessment and algae, macro-invertebrates and fish are considered as important indicators (Mandaville, 2002, Gadzała-Kopciuch *et al.*, 2004). Biomarkers are measurements in body fluids, cells or tissues indicating biochemical or cellular modifications due to the presence and magnitude of toxicants, or of host response (Maharajan and Shanmugavel, 2015). Examples include superoxide dismutase (SOD), catalase (CAT), glutathione transferase (GT), glutathione peroxidase (GPx), reduced glutathione (GSH) and lipid peroxidation (LPx). They are used after exposure to environmental or anthropogenic sources of pollution, to elucidate cause-effect and dose-effect relationships in health risk assessment, in clinical diagnoses and

for monitoring purposes. Generally, biomarker responses are considered to be intermediates between pollutant sources and higher-level effects (Suter, 1990). When these compensatory responses are activated, the survival potential of the organism may already have begun to decline because the ability of the organism to mount compensatory responses to new environmental challenges may have been compromised (Depledge, 1993). The most compelling reason for using biomarkers is that they can give information on the biological effects of pollutants rather than a mere quantification of their environmental levels.

The antioxidants enzymes such as catalase, SOD and GPx are the primary enzymes of the antioxidant defense against oxidative damages generated by multiple environmental stressors and have been studied in fish and shellfish species (Vijayavel *et al.*, 2004; Martin Diaz *et*

Figure 1. Map of the Lagos Lagoon showing sampling sites

al., 2005; Usese *et al.*, 2018). Their induction reflects a specific response to variable pollutants including hydrocarbons and metals (El-Khayat *et al.*, 2015). In addition, malonedialdehyde (MDA) is a biomarker of oxidative damage that reflects the state of lipid peroxidation of the membranes of many living organisms (Lykkesfeldt, 2007). The main aim of the present study was to use chemical biomarkers to evaluate the health status of *Cardiosoma armatum*, *Goniopsis pelli*, *Callinectes amnicola* and *Portunus validus* inhabiting contaminated locations in the Lagos Lagoon ecosystem.

Materials and Methods

Study site

The Lagos Lagoon (3°10'E and 3°45' E and 6°15'N and 6°36'N) is a part of the continuous system of lagoons found along the coast of Nigeria from the border of the Republic of Benin to Niger-Delta (Lawal-Are and Owolabi, 2012; Akinjogunla and Moruf, 2018). The influx of seawater gives rise to brackish conditions in the lagoon and thus encourages the growth of mangroves (Emmanuel *et al.*, 2010). The lagoon serves as a sink receiving large quantities of domestic and industrial wastes with implication for environmental degradation. The highly populated western axis of the Lagos Lagoon is fringed by mangrove swamps. Selection of study sites (Figure 1) was based on increasing anthropogenic effects from heaps of domestic and solid waste dumps.

Sample Collection

The mangrove crab species (*Goniopsis pelli* and *Cardiosoma armatum*) - were collected at the mudflats area of the Lagos Lagoon along Abule-Eledu refuse site using the removal method by hand picking. The fishing of the swimming crab species (*Callinectes amnicola* and *Portunus validus*) was done using circular lift nets at depth of 1 – 2.5m. The description and design characteristics of the circular lift nets were documented by Solarin *et al.* (2003). Approximately 30 adult crabs each of the crab species were collected over a twelve-week period (March-May, 2018). Crabs were immediately placed in styrofoam boxes and were transported to the Aquatic Toxicology and Ecophysiology laboratory at the Department of Marine Sciences, University of Lagos for proper identification, processing and analysis.

Laboratory Analysis

Heavy metal levels in tissues of the crabs

Muscle tissues of the examined samples were oven dried at 70°C for 1 hr and ground to powder in ceramic mortars and 0.5 g of each sample were made into paste by adding double distilled water. This was followed by digestion using 5 ml of 1 M HNO₃ and mild heat until brown fumes appeared, following an established technique (Don-Pedro *et al.*, 2004). The samples were cooled off, made up to 50 ml in a standard volumetric flask which have been subjected to acid wash to remove any trace of residual metals and then filtered. Thereafter, processed samples were analysed using Atomic Absorption Spectrophotometer (Perkin Elmer series) to determine levels of selected heavy metals (zinc, copper, cobalt, lead and cadmium).

Evaluation of defense responses

Excised muscle tissues of the crabs stored at -20 °C were later thawed and homogenized for the assays of superoxide dismutase, catalase, glutathione transferase, reduced glutathione and glutathione peroxidase, following the protocol described by Lushchaks *et al.* (2005) and Bertholdo-Vargas *et al.* (2009). 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.* (1951). The activity of SOD was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 at 30°C. Three milliliters of Na₂CO₃ 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 three milliliters 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. Glutathione-S-transferase (GST) activity was determined by adding into triplicate wells, 20 IL of sample, 150 IL of assay buffer (100 mM potassium

Table 1. Mean heavy metal concentrations (mg/kg) in crab tissues

Metal (mg/Kg)	<i>Cardiosoma armatum</i>	<i>Goniopsis pelli</i>	<i>Callinectes amnicola</i>	<i>Portunus validus</i>
Zinc	(0.42-0.63) 0.53±0.06 ^a	(0.19-0.54) 0.31±0.12 ^b	(0.44-0.51) 0.48±0.02 ^c	(0.40-0.43) 0.41±0.01 ^c
Copper	(0.20-0.41) 0.31±0.06 ^a	(0.11-0.46) 0.23±0.12 ^b	(0.11-0.32) 0.22±0.06 ^b	(0.42-0.63) 0.52±0.06 ^c
Cobalt	BDL	BDL	BDL	BDL
Lead	(0.005-0.014) 0.01±0.00 ^a	(0.01-0.02) 0.01±0.00 ^a	BDL	(0.008-0.013) 0.01±0.00 ^a
Cadmium	(0.6-0.8) 0.79±0.06 ^a	(0.3-0.65) 0.42±0.12 ^b	(0.57-0.59) 0.58±0.00 ^c	(0.64-0.67) 0.65±0.01 ^a

Keys: Range (in brackets); Mean±Standard Error; BDL =below detection limit; Values with different superscript across row are significantly different at (P < 0.05).

phosphate, pH 6.5, containing 0.1% Triton X- 100) and 20 IL of glutathione. A background or non-enzymatic control was run by adding only 170 IL of assay buffer and 20 IL of glutathione to three wells. The reaction was initiated by adding 10 IL 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 Powerwave™ 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 reported as nmol/min/mg protein.

Statistical Analysis

Statistical analyses were carried out using SPSS version 17. Data were tested using Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) at a level of significance of alpha of 5% ($\alpha=0.05$) to determine if the means are statistically significant among the four different crab species.

Results and Discussions

Heavy metal concentrations in crab tissues

With the importance of a healthy environment to the survival of aquatic organisms; their inherent capacity to reproduce, develop and growth could be massively affected in environments strained by

external pressures (Ochang *et al.*, 2007; Lawal-Are *et al.* 2018; Moruf and Lawal-Are, 2018). The presence of pollutant; measured as the relative concentration of heavy metals found in the tissue of the crabs showed slight variations (Table 1). Amongst all the measured heavy metal, cadmium concentration was the highest and significant (P<0.05) across species, ranging from 0.42±0.12mg/kg (*G.pelli*) to 0.79±0.06 mg/kg (*C.armatum*), followed by zinc and copper with concentrations of 0.41±0.01mg/kg (*P.validus*) to 0.53±0.06mg/kg (*C.armatum*) and 0.22±0.06mg/kg (*C.amnicola*) to 0.52±0.06mg/kg (*P. validus*) respectively. However, lead concentration was relatively low, averaging 0.01±0.00 mg/kg across samples, while cobalt was largely below detection level. In respect to the organisms, heavy metals were more abundance in the *C. armatum* and recorded the lowest in *G.pelli*.

From the heavy metal result, the levels of metal pollution is mild, and in some cases negligible. However, metals such as zinc and copper were modest, showing minimal episode of heavy metal infusion to the adjoining water channel where these organisms dwell. There are many earlier surveys on effects of heavy metals on aquatic animals. Pan *et al.* (2011) have reported that heavy metal ions' (Cu²⁺, Pb²⁺, and Cd²⁺) exposure caused DNA damage in haemocytes of marine crab *Charybdis*

japonica. In freshwater crab *Sinopotamon yangtsekiense*, the mobilization of carbohydrate and protein was increased by cadmium stress (Xuan *et al.*, 2011). Uncharacteristically, cadmium was recorded to be highest relative to the other heavy metals. This comes as a surprise given the almost traced nature of the cadmium in several literature studies in the particular environment where the crab species were caught (Edokpayi and Nkwoji, 2007). Contrary to the report of Lawal-Are and Babaranti (2014), where cadmium was the least abundant metal recorded in the smooth swim crab (*P. validus*) with the value of 0.42 mg/kg. The highest copper concentration observed in *P. validus* than all other species could be attributed to biological bioaccumulation process and the fact that benthic organisms are highly sensitive to environmental stress such as trace metal pollution (Ajao and Fagade, 1990; Moruf and Akinjogunla, 2018). According to Elghobashy *et al.*, (2001), bioaccumulation of heavy metals in crustaceans critically influences the growth rate, physiological and biochemical status. Furthermore, a number of deleterious effects including oxidative stress from heavy metals bioaccumulation in biological systems have been reported by some researchers (Farombi *et al.*, 2007; Soundararajan *et al.*, 2009; Usese *et al.*, 2018).

Assessment of oxidative stress in crab tissues

Biochemical enzymes activities in tissues of the crabs (Table 2) under study showed slight organismal variation with PRO ranging from 12.35±0.02g/l in *C. armatum* to 19.23±1.80g/l

in *C. amnicola*. *P. validus* recorded a mean concentration as low as 14.81±1.23 g/l while *G. pelli* was 15.05±0.03 g/l. The lowest SOD activity was recorded in *C. amnicola* (81.04±2.01 min/mg/pro) and the highest in *C. armatum* (123.04±0.01 min/mg/pro). Usese *et al.* (2018) also noted variations in the antioxidant response to oxidative stress in the various tissues of the Hairy Mangrove Crab, *Sesarma huzardii* from a tropical lagoon mudflat in southwest Nigeria. According to Viarengo *et al.* (1999), high SOD concentration suggests a correspondingly less polluted nature of the environment. As such, ROS activities should be low in the crab specimen because of the induced SOD that cleansed generated ROS spiked by heavy metal pollution.

Mean CAT activity in *P. validus* was 5.46±2.02 min/mg/pro while *C. armatum* exhibited significant highest value of CAT (P<0.05) with mean activity of 7.74±0.05 min/mg/pro closely followed by *G. pelli* (5.99±1.01 min/mg/pro) with *C. amnicola* recording the lowest (4.61±3.02 min/mg/pro). The low range of CAT activity might lead to less scavenging of excess hydrogen peroxide, if any exist in the cell membrane of the crab species. According to Pellerin-Massicotte (1997), catalase activity is considered as the primary defense against oxidative damage and has been well studied in marine invertebrates due to its ability to convert hydrogen peroxide into water.

The maximum significant concentration of GST was found in *C. armicola* with value of 18.21±0.02Hmol/mgpro and the minimum was recorded in *P. validus* with value of 9.28±1.03Hmol/

Table 2. Antioxidant enzyme activity in tissues of crab species

Biomarkers	<i>Goniopsis pelli</i>	<i>Cardiosoma armatum</i>	<i>Callinectes amnicola</i>	<i>Portonus validus</i>
PRO (g/l)	15.05±0.03 ^a	12.35±0.02 ^a	19.23±1.80 ^a	14.81±1.23 ^a
SOD(min/mg/pro)	107.89±0.02 ^a	123.04±0.01 ^a	81.04±2.01 ^b	88.63±1.41 ^b
CAT(min/mg/pro)	5.99±1.01 ^a	7.74±0.05 ^b	4.61±3.02 ^a	5.46±2.02 ^a
GST(Hmol/mg pro)	13.93±0.21 ^a	18.21±0.02 ^b	12.45±2.04 ^a	9.28±1.03 ^c
GSH(Hmol/mg pro)	1.94±1.01 ^a	2.92±0.04 ^a	1.32±1.12 ^a	2.53±3.01 ^a
GPx(Hmol/mg pro)	54.31±0.05 ^a	61.85±0.06 ^b	43.72±2.01 ^a	55.24±1.05 ^a

Keys: PRO: Protein, SOD: Superoxide Dismutase, CAT: Catalase, GT: glutathione transferase, GSH: reduced glutathione, GPX: glutathione peroxidase (GPX)

mg pro, significantly different from values obtained in other crab species. According to Fitzpatrick *et al.* (1997) and Hoarau *et al.* (2004), GST is regulated by a structurally diverse range of xenobiotics and at least 100 chemicals, including organochlorine compounds that have been identified to induce GST activity in marine invertebrate. In the present study, GSH was fairly low in concentration with value ranging from 1.32 ± 1.12 Hmol/mg pro in *C. amnicolato* 2.92 ± 0.04 Hmol/mg pro in *C. armatum*. GSH depletion suggested that the crabs might be suffering from oxidative damage (Chelomin *et al.*, 2005; Ivanina *et al.*, 2008). Average GPX activity was 55.74 Hmol/mg pro with slight species variation. According to Liu *et al.* (2014), with low concentration of heavy metal, GPx could protect membrane lipids from oxidative damage detoxifying the organic peroxides and act on organic hydroperoxides.

Conclusion

The presence of pollutant; measured as the relative concentration of heavy metals and the evaluated oxidative stress responses found in the tissue of the crabs suggests a correspondingly less polluted nature of the environment. With the positive conclusion of the ecological status of the crab, there should be a continue effort by environmental actors and stakeholder involved in coastal management in Nigeria in ensuring that the trend stay the same while further studies should proceed naturally from this back-end investigation.

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