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# LEVELS OF OXIDATIVE STRESS MARKERS IN THE MANGROVE OYSTER, *Crassostrea gasar* FROM A COASTAL ECOSYSTEM IN SOUTHWEST NIGERIA

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***ABSTRACT***

***This study investigated biochemical responses in the ecologically important Mangrove Oyster, Crassostrea gasar along a contamination gradient on the Lagos/Badagry lagoon in Lagos, Nigeria. The antioxidant enzymes and oxidative stress biomarkers, catalase enzyme activity (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and Thiobarbituric Acid Reactive Substances (TBARS): Malondialdehyde (MDA) were assessed in gills and muscle tissues of adult Crassostrea gasar using standard biochemical procedures. The muscle tissue of C. gasar exhibited higher oxidative stress enzymes activity than the gills. Relatively higher mean value of MDA was recorded in the gill of C. gasar at Makoko (35.39±1.08 Hmol/mg pro) as compared to the levels in oysters collected from Abule-Agege Creek (26.26±2.78 Hmol/mg pro) and Badagry Creek (25.15±4.54 ± Hmol/mg protein). Similarly, the result of anti-oxidative stress enzyme activities and lipid peroxidation in the muscle tissue of C. gasar from Makoko waters showed significant highest values of SOD (54.24±1.37 Min/mg pro), CAT (6.82 Min/mg pro) and MDA (42.35±0.81 Hmol/mg pro). No significant difference in GPx value was recorded in examined samples of C. gasar across sites. C. gasar collected from Abule-Agege and Badagry Creeks were similar in most values of the measured biochemical enzyme activities. Biological adverse effects were more evident in oysters from Makoko, a recognized contaminated area than those from Abule-Agege Creek and Badagry Creek. The study gave an indication of stress on the health status of the oysters, hence the need for periodic monitoring of the ecosystem.***

**Keywords**: Mangrove Oyster, Antioxidant enzymes, Oxidative stress, Estuary.

## INTRODUCTION

Unregulated discharge of huge amounts of domestic and industrial wastes from increasing human activities has led to unprecedented contamination and subsequent pollution of surrounding coastal waters. The impact of such discharges on aquatic ecosystem functions and services has been a major threat to biodiversity in most region of the world. Of particular concern is the adverse effect on aquatic organisms' biochemical performance. The use of biochemical or physiological and enzymatic biomarker measurements as indicators of toxicity is under constant development and has the advantage of delineating effects prior to the manifestation of diseases (Tejeda *et al*., 2007), thereby leading to better results in environmental risk assessment (Van der Oost *et al*., 2003). Antioxidant enzymes such as superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Malondialdehyde (MDA) and protein assay, have been frequently used as effective biomarkers to assess contaminated areas and native species (Xie *et al*., 2016).

Oysters are among the most valuable socioeconomic group of bivalve species in global fishery landings which provides numerous ecosystem services (Freitas *et al*., 2016). The Mangrove Oyster, *Crassostrea gasar*, a conspicuous inhabitant of the mangrove ecosystem

is an edible bivalve mollusc in the family-Ostreidae. It is found in tropical intertidal zones attached to any hard substrate such as prop roots of mangrove trees, which are exposed during low tides and covered during high tides in the estuarine or coastal environment. It develops well at temperatures between 23 and 31°C and also has a wide salinity tolerance range of between 10-25 parts per thousand (Akinjogunla *et al*., 2017). Generally, oysters filter large volumes of water to extract their food and as such may accumulate contaminants from their immediate surroundings. It is necessary to understand physiological and potential biochemical alterations in the mangrove oyster in the scenario of increasing stress and contamination of valuable ecosystems. There are several reports about the deterioration of the Lagos Lagoon and adjacent water bodies and the adverse impact on fishery resources (Lawal-Are and Babaranti, 2014; Usese *et al*., 2017a; 2017b; 2018; Moruf and Akinjogunla, 2018). The study therefore assessed the antioxidant enzyme activity and oxidative stress biomarker in *Crassostrea gasar* so as to provide information that may allow for better environmental regulation and management in the area.

## MATERIALS AND METHODS

### Site and Sample Collection

Samples of *Crassostrea gasar* (Fig. 1) used in the present study were collected from three sites (Table 1 and figure 2) on the Lagos/Badagry lagoon regions of the Lagos lagoon complex between June and August, 2017 with the help of commercial fishermen. Similar sized oysters (6 - 10cm length; 2 - 5cm width) extracted from the clusters were transported in ice chest box to the Aquatic Toxicology and Ecophysiology Laboratory located at the Department of Marine Sciences, University of Lagos for further processing and analysis.

**Fig. 1:** The Mangrove Oyster, *Crassostrea gasar*
Source: Moruf and Adekoya (2018)

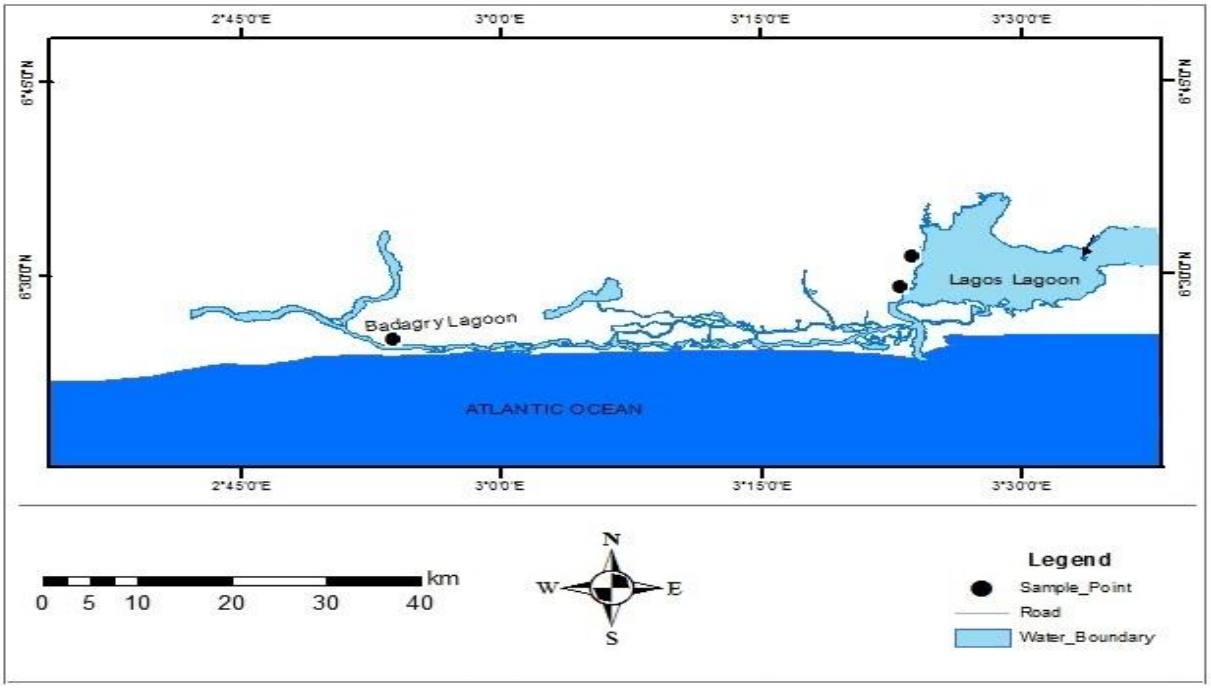


**Fig. 2:** Sampling locations on the coastal water of Lagos

**Table 1: GPS Coordinate Descriptions of Sampling Sites**

| Locations | Latitude | Longitude |
|---|---|---|
| Makoko Waters | 6° 25' 38"N | 3°20'56"E |
| Abule-Agege Creek | 6° 26' 36"N | 3°23'04"E |
| Badagry Creek | 6° 43' 29"N | 3°36'33"E |

**Biochemical Analysis**

In the laboratory, the oyster shells were opened by cutting off the adductor muscles before the gills and muscle tissues were removed. For biochemical analyses, the muscle and gill tissues of twenty individuals were pooled, manually homogenized with a mortar and a pestle under liquid nitrogen and prepared in replicate per site. Homogenates from each specimen were further separated into aliquots to perform individual extractions using specific buffers for each parameter. About 1.3±0.1g of gills and 5.3±1.2g of muscle tissue were homogenized at 4ºC in 0.1M Tris HCl buffer (0.45M NaCl, 26mM CaCl2; 0.5ml of buffer $g^{-1}$ of fresh weight for the gills and 1ml.$g^{-1}$ of fresh weight for the muscle tissue), using an Ultra Turrax (T25 basic, IKA-WERKE) and a Thomas- Potter homogenizer (1KA- Labortechnik RW 20.n, size 0.13- 0.18mm, BB). It was then analyzed for the assay of reduced glutathione, catalase, superoxide dismutase, levels of proteins, and lipid peroxidation following the protocol described by Lushchak *et al*. (2005) and Bertholdo-Vargas *et al*. (2009). Lipid peroxidation estimation was carried out through the determination of thiobarbituric acid reactive substances (TBARS) which are indices of membrane peroxidation.

**Statistical Analysis**

The results were reported as mean and its standard error. Data were tested using One way Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) at 5% level of significance ($\alpha$=0.05) to determine if the means are statistically significant from each other for the three different locations.

**RESULTS**

The result of the biomarkers of oxidative stress in the gill and muscle tissue of *Crassostrea gasar* from Makoko sampling site is presented in Table 2. Relatively, higher range values of protein (13.2-19.49 mg), CAT (5.36-7.68 Min/mg pro) and MDA (28.06-63.54 Hmol/mg pro) were recorded in the muscle tissue than the gills. However, relatively higher range values for SOD (38.46-85.1 Min/mg pro) and GPx (35.5-59.94 Hmol/mg pro) were obtained in the gills of *C. gasar* from Makoko. On the other hand, the muscle tissue of *C. gasar* from Abule-Agege Creek exhibited higher PRO (18.35-23.81 mg), SOD (36.56-58.89 Min/mg pro) and CAT (2.03-3.48 Min/mg pro) activities but lower in GPx (22.87-82.11 Hmol/mg pro) and MDA (22.45-23.16 Hmol/mg pro) when compared to its gill (Table 3). With the exception of GPx (23.12-53.9 Hmol/mg pro), the muscle tissue of *C. gasar* from Badagry also exhibited higher oxidative stress enzymes activity than in the gills (Table 4).

**Table 2: Biochemical Enzyme Activity in *Crassostrea gasar* from Makoko Waters**

| Biomarkers | Gill | | Muscle tissue | |
|---|---|---|---|---|
| | Minimum | Maximum | Minimum | Maximum |
| Protein (mg) | 12.84 | 15.99 | 13.2 | 19.49 |
| SOD (Min/mg pro) | 38.46 | 85.1 | 27.77 | 86.97 |
| CAT (Min/mg pro) | 2.96 | 6.56 | 5.36 | 7.68 |
| GPx (Hmol/mg pro) | 35.5 | 59.94 | 25.8 | 56.58 |
| MDA (Hmol/mg pro) | 22.91 | 57.49 | 28.06 | 63.54 |

**Keys**: SOD: Superoxide dismutase , CAT: Catalase, MDA: Malondialdehyde, GPx: Glutathione Peroxidase

**Table 3: Biochemical Enzyme Activity in *Crassostrea gasar* from Abule-Agege Creek**

| Biomarkers | Gill | | Muscle tissue | |
|---|---|---|---|---|
| | Minimum | Maximum | Minimum | Maximum |
| Protein (mg) | 13.86 | 18.65 | 18.35 | 21.81 |
| SOD (Min/mg pro) | 37.15 | 57.44 | 36.56 | 58.89 |
| CAT (Min/mg pro) | 0.73 | 3.75 | 2.03 | 3.48 |
| GPx (Hmol/mg pro) | 50.28 | 77.05 | 22.87 | 82.11 |
| MDA (Hmol/mg pro) | 20.73 | 29.58 | 22.45 | 23.16 |

**Keys**: SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde, GPx: Glutathione Peroxidase

Table 4: Biochemical Enzyme Activity in *Crassostrea gasar* from Badagry Creek

| Biomarkers | Gill | | Muscle tissue | |
|---|---|---|---|---|
| | Minimum | Maximum | Minimum | Maximum |
| Protein (mg) | 13.21 | 19.51 | 12.49 | 20.82 |
| SOD (Min/mg pro) | 21.05 | 64.02 | 23.15 | 79.49 |
| CAT (Min/mg pro) | 1.32 | 6.77 | 3.5 | 6.95 |
| GPx (Hmol/mg pro) | 23.12 | 53.9 | 16.05 | 58.6 |
| MDA (Hmol/mg pro) | 19.45 | 34.13 | 14.66 | 53.04 |

**Keys**: SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde, GPx: Glutathione Peroxidase

The biochemical enzymes activity in the gill and muscle tissue of *C. gasar* from the three sampling stations (Table 5 and 6), shows significant differences ($P<0.05$) in anti-oxidative stress enzyme activities and lipid peroxidation across sites. In the gills (Table 5), the lowest (38.5 ± 3.04 Min/mg pro) and highest values (55.57 ± 1.83 Min/mg pro) of SOD activity were obtained in samples of *C. gasar* from Badagry Creek and Makoko water respectively.

Values for anti-oxidative stress enzyme activities and lipid peroxidation in the muscle tissue of *C. gasar* (Table 6) from Makoko were higher than the other sampling sites. There was no significant difference ($P>0.05$) in GPx values recorded in examined samples of *C. gasar* across sites. *C. gasar* collected from Abule-Agege and Badagry Creeks were similar in values of the measured biochemical enzyme activities.

**Table 5: Biochemical Enzyme Activity in *C. gasar* (Gill) from Lagos Coastal Ecosystem**

| Biomarkers | Makoko Waters | Abule-Agege Creek | Badagry Creek |
|---|---|---|---|
| Protein (mg) | $14.58\pm0.92^{a}$ | $16.68\pm1.45^{a}$ | $15.32\pm2.09^{a}$ |
| SOD (Min/mg pro) | $55.57\pm1.83^{a}$ | $44.27\pm6.59^{b}$ | $38.5\pm3.04^{b}$ |
| CAT (Min/mg pro) | $5.29\pm1.17^{a}$ | $1.77\pm0.99^{b}$ | $3.56\pm1.65^{b}$ |
| GPx (Hmol/mg pro) | $57.61\pm1.83^{a}$ | $57.56\pm0.75^{a}$ | $42.37\pm9.69^{b}$ |
| MDA (Hmol/mg pro) | $35.39\pm1.08^{a}$ | $26.26\pm2.78^{b}$ | $25.15\pm4.54^{b}$ |

**Keys**: SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde, GPx: Glutathione Peroxidase

**Table 6: Biochemical Enzyme Activity in *C. gasar* (Muscle) from Lagos Coastal Ecosystem**

| Biomarkers | Makoko Waters | Abule-Agege | Badagry Creek |
|---|---|---|---|
| Protein (mg) | $16.53\pm1.83^{a}$ | $19.95\pm1.80^{a}$ | $17.53\pm2.56^{a}$ |
| SOD (Min/mg pro) | $54.24\pm1.37^{a}$ | $44.32\pm1.29^{b}$ | $45.61\pm1.24^{b}$ |
| CAT (Min/mg pro) | $6.82\pm0.74^{a}$ | $2.78\pm0.42^{b}$ | $4.1\pm1.58^{b}$ |
| GPx (Hmol/mg pro) | $42.57\pm2.18^{a}$ | $41.04\pm19.54^{a}$ | $39.02\pm12.4^{a}$ |
| MDA(Hmol/mg pro) | $42.35\pm0.81^{a}$ | $22.8\pm0.21^{b}$ | $31.42\pm1.34^{b}$ |

**Keys**: SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde, GPx: Glutathione Peroxidase

## DISCUSSION

Over the years, several studies have emphasized the enormous threats posed to ecological receptors within Lagos Lagoon and other interconnected ecosystems (Chukwu, 2006; Lawal-Are *et al*., 2018) and biomarkers including antioxidant enzymes allow early detection of environmental problems (Bouraoui *et al*., 2010). In the present study, relatively higher range values of biochemical enzymes activity were recorded in the muscle tissue than in gills of *C. gasar*. This is similar to the report of Usese *et al*. (2018) on the biochemical enzymes activity in the tissues of *Sesarma huzardii* from polluted location along Lagos Lagoon.

The recorded high level of SOD activity observed in *C. gasar* from Makoko suggests potential stress from this location. In a related study, Achuba *et al*., (2014) reported increased SOD and CAT activity in Hetero-clarias exposed to environmental pollutants while decreasing levels were however obtained in the different tissues of some other fish species. The observed increase in antioxidant enzymes activities indicates adaptive responses of the organism to counteract the oxidative effect of generated reactive oxygen species ROS or resistance to water pollutants toxicity against the tissue damage caused by excessive amount of oxygen free radicals and oxidative stress (Carvalho *et al*., 2012). Increase in the activity of CAT and SOD is usually observed in the face of environmental pollutants since SOD-CAT system represents the first line of defense against oxidative stress (McCord, 1996).

Lipid peroxidation in muscle tissues of *C. gasar* expressed by the malondialdehyde levels (MDA) was inconsistent among examined samples across all sites. A high level of MDA, SOD and CAT was recorded at Makoko. Physiological stress response, although initiated as an adaptive response to destabilizing factors, could have damaging effects if prolonged by increasing susceptibility to infections, through immune depression, that leads to mortality (Ugwu and Soyinka, 2018; Moruf and Lawal-Are, 2018). The oysters collected from Abule-Agege and Badagry Creeks were similar in values of the measured biochemical enzyme activities. Oxidative stress is caused by several factors including inactivity (Laufs *et al* 2005; de Lemos 2012), radiation and inflammation (Yoshikawa and Naito 2002) as well as pollution.

## CONCLUSION

Since a sedentary lifestyle is implicated in the spike of oxidative stress markers and oysters are sedentary, it becomes inappropriate to allude the high level of MDA, SOD and CAT in Makoko to pollution alone. A good look at the tide structure may show that oyster movement via tide is common in Abule-Agege and Badagry hence the low levels.

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