**Faculty of Natural and Applied Sciences Journal of Scientific Innovations**
**Print ISSN: 2814-0877 e-ISSN: 2814-0923**
www.fnasjournals.com
**Volume 4; Issue 2; October 2023; Page No. 42-49.**

# BIOMARKERS OF OXIDATIVE STRESS IN OYSTERS (*Crassostrea gasar*) AND HEAVY METAL LEVELS IN THE MEDIA OF BUGUMA CREEK, NIGER DELTA, NIGERIA

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**Abstract**
The study assessed the activities of biomarkers of oxidative stress: superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), lipid peroxidation product, and malondialdehyde (MDA) levels in oysters (*Crassostrea gasar*) alongside heavy metals: cadmium (Cd), lead (Pb), nickel (Ni), and zinc (Zn) in water, sediments, and oyster (*Crassostrea gasar*) tissues from Buguma Creek, Niger Delta, Nigeria. Sampling was carried out at three stations: Jordan, Amayanabo, and Orubo, respectively. Analysis of biomarkers of oxidative stress revealed that oyster tissues from Orubo had significantly higher GSH (15.98±2.57 μmol/ml) levels than those from Amayanabo (8.34±3.45 μmol/ml) and Jordan (7.69±0.91 μmol/ml), respectively. Orubo also had lower metal content ppm (Cd: 0.66±0.70; Ni: 0.63±0.22; Pb: 179.90±85.32; Zn: 0.00±0.00) compared to Amayanabo (Cd: 1.01±0.61; Ni: 1.45±0.38; Pb: 486.28±94.52; Zn: 36.91±63.94) and Jordan (Cd-0.72±0.14; Ni-1.45±0.38; Pb-353.12±13.31; Zn-5.81±10.06). This observed pattern was, however, not consistent with results from the other stations. Results of heavy metals in water and sediment samples in all sampled stations revealed metal levels to be within the FEPA and DPR permissible limits for water and sediments, respectively. Ni and Pb in oyster (*Crassostrea gasar*) tissues in all the sampled stations were above the limits set by FEPA and FAO for food fish. The levels of the analyzed heavy metals were in the following order: oyster > sediments > water. The findings from this study call for caution in the consumption of oysters (*Crassostrea gasar*), other shellfish, and fish from Buguma Creek.

**Keywords:** Biomarkers, Bioaccumulation, Oyster (*Crassostrea gasar*), Heavy Metals, Pollution, Buguma Creek

## Introduction

Certain aquatic creatures can accumulate and amplify pollutants such as heavy metals, polycyclic aromatic hydrocarbons, and PCBs within their biological systems in aquatic ecosystems. The consumption of these pollutants can have detrimental effects on the productivity and reproductive capacities of these organisms, leading to declines in population numbers. This can result in a reduction in the diversity, stability, and overall health of the ecosystem, ultimately impacting the well-being of the environment. Additionally, the long-term consequences may extend to human health, as humans rely on these organisms as a significant protein source (Davies et al., 2006).

Bivalves like oysters readily accumulate many environmental pollutants, including heavy metals. Due to their sedentary behaviour, relative resistance to chemical contamination, association with sediments and modes of feeding, and respiration, oysters are known to be efficient bioaccumulators (Rittshof & McClellan-Green, 2005). Aquatic pollution is an aspect of global concern due to the large amounts of untreated waste products that find their way into water bodies and their toxicity and persistence in the environment. Dumping of untreated wastes into water bodies leads to an upsurge in the amount of pesticides, fertilizers, heavy metals, and other pollutants in the environment (Hoffman et al., 2003; Vane et al., 2009).

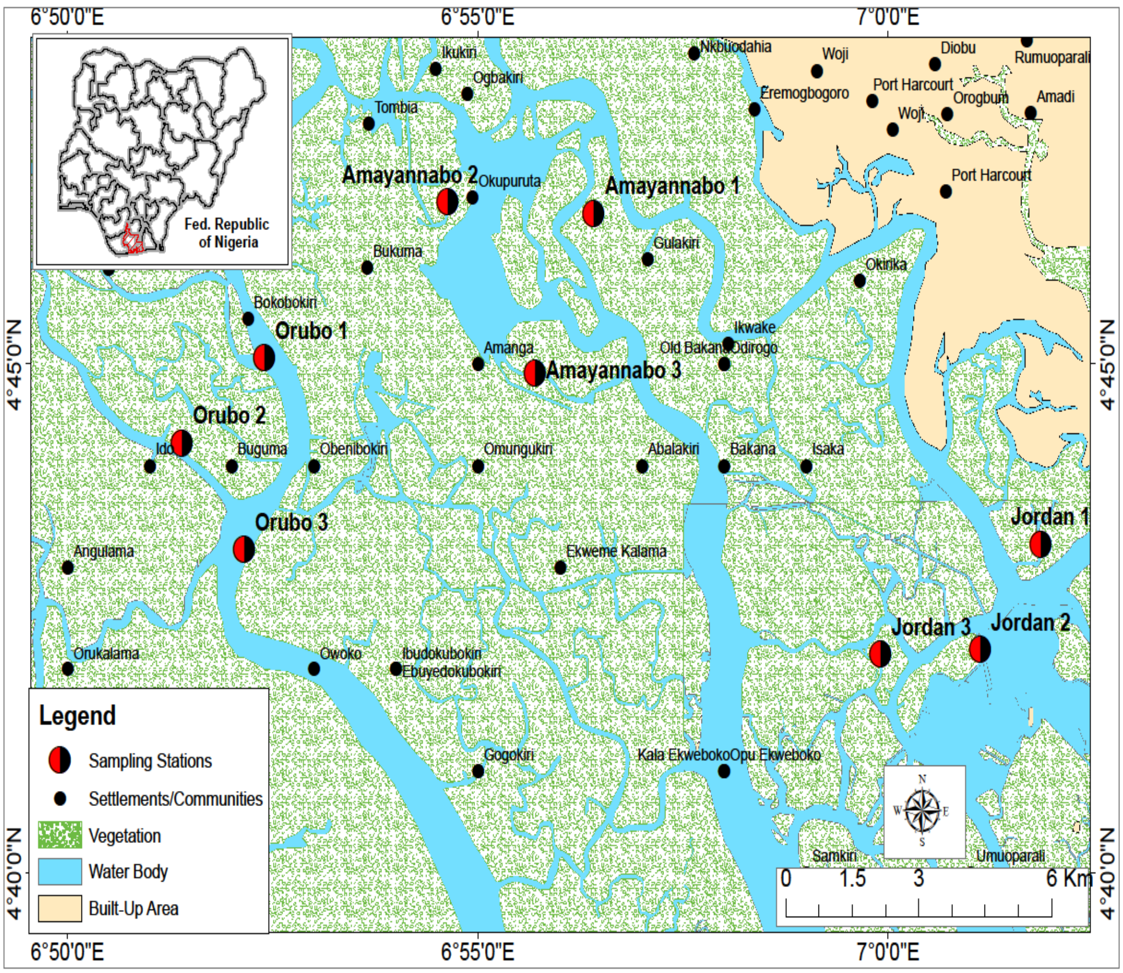
Aquatic ecosystems are susceptible to the presence of heavy metals, which have been identified as pollutants. These heavy metals exhibit toxicity towards aquatic creatures, as evidenced by studies (Gagneten et al., 2007; Farris and Van Hassel, 2007). The issue of heavy metal pollution is a significant challenge to ecological systems since these pollutants can concentrate and undergo biomagnification within water, sediment, and aquatic food chains. Consequently, this process can result in adverse consequences, such as sub-lethal impacts or mortality, on indigenous fish populations (Xu et al., 2004).

The persistence, ubiquitous nature, and ability to accumulate and biomagnify in organisms make heavy metals very hazardous to humans and damage the total quality of the environment (Otitoloju et al., 2007). To avoid the pollution of trace metals in aquatic environments, it is necessary to establish the distribution of these metals in such environments and the organisms found there (Vincent-Akpu & Mmom, 2012). The antioxidant defence system provides biological markers that could be used for environmental monitoring. Antioxidant enzymes have a great potential to indicate the toxic effects of heavy metals because of their ability to generate free radicals, which cause lipid peroxidation of cell membranes (Abele et al., 2002).

The Buguma Creek receives salty water from tidal flows from the New Calabar River, which is under the influence of various anthropogenic activities such as agriculture, dredging, and oil exploration. Buguma Creek is one of the finest examples of delta formation in the world. It is brackish with little fresh water discharges that empties from the Port Harcourt end of the New Calabar River (Woke & Babatunde, 2009).

**Materials and Methods**

Buguma Creek is situated in the southeastern region of the Niger Delta, namely between the longitudinal coordinates of 6° 47'E and 6° 59'E and the latitudinal coordinates of 4° 31'N and 4° 59'N, within the country of Nigeria. The creek is comprised of a primary creek channel that is accompanied by a vast network of interconnected creeks (Oribhabor et al., 2009).




Map of Buguma Creek indicating the sampling stations

**Experiment**

Plastic containers with a capacity of 1 litre were used to collect surface water samples from beneath the surface coating of the water body. The water samples were treated with 10 ml of 10N nitric acid for fixation, then placed in chilled containers and sent to the laboratory for further examination.

Sediment samples were obtained via a stainless steel grab device of the Van-Veen type, inside a sampling area measuring approximately 0.5 square metres. The sediment samples were carefully placed into polythene bags that were free from any contamination. These bags were then brought to the laboratory to examine their heavy metal content.

*Crassostrea gasar* was found attached to the sprouting roots of mangrove trees. They were carefully detached from their substrata with the aid of a stainless steel knife. Samples were put in an ice chest to ensure their stability and taken to the laboratory for examination.

The experimental locations' surface water samples were digested according to the procedures described in the APHA (2005) recommendations. Sediment samples were sieved and then transferred to polypropylene test tubes. All protocols for the digestive process were strictly followed. Subsequently, absorbances were compared to those of a standard AAS solution to determine the amounts of heavy metals in the water and sediment samples. An Alpha-4 Cathode, a hollow cathode lamp, a gas cylinder, and an integrated digital computer were used in this investigation utilising an Atomic Absorption Spectrophotometer (Pye Unicamp model 9100).

Agua Agar (wet digesting) approach was used to digest Oyster (*Crassostrea gasar*) tissues, according to methods by Faramobi et al. (2007). Oyster tissue was cut with a stainless steel knife and then dried in an electric oven at 80 degrees Celsius for 36 hours. The samples were then individually pulverised in a dry, clean mortar. After being dried for an extra hour, the powdered samples were put into sterilised polyethylene vials. The Metler analytical balance was used to measure out exactly 5 grammes for each sample. The samples were then weighed again before being placed in a platinum crucible and afterwards ashed at 600 °C for three hours in a muffle furnace.

The ash-covered samples were put into desiccators to cool off. After adding 5 ml of 6NHCl, the samples were kept undisturbed for 30 minutes to allow for optimal digestion. After that, we filtered the solution with Whatman filter paper into a 100-ml conical flask. The flask was brought to a total capacity of 50 ml with distilled water before the heavy metals analysis could begin. An atomic absorption spectrophotometer (type 9100 Pye Unicamp) with a hollow cathode lamp, gas cylinder, and built-in digital computer was used for the analysis of heavy metals.

The purpose of this research was to explore the activities of biomarkers associated with oxidative stress in the tissues of oysters (*Crassostrea gasar*), such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and the lipid peroxidation product malondialdehyde (MDA). The research that was carried out by Idowu et al. (2014) served as the basis for the approaches that were used in the process of determining these biomarkers.

To evaluate the relative metal concentrations in sediment and oysters (*Crassostrea gasar*), bioaccumulation factors (BAFs) were calculated. BAF (bioaccumulation factor) = C (oyster sample) /C (sediment) is the ratio of BAF (bioaccumulation factor) in the oyster sample to BAF (bioaccumulation factor) in the sediment. The heavy metal content in oyster tissues is denoted by the variable C, whereas the sediment's heavy metal concentration is denoted by the variable C (both in ppm).

Descriptive and inferential statistical analyses, such as mean and standard deviation calculations and graphical representations, were performed on the data. A one-way analysis of variance (ANOVA) compared the means of the data obtained from the research of each group for temporal fluctuations and between the organs. IBM SPSS Statistics 20 was used as the statistical analysis tool. The Scheffe multivariate (multiple) comparison approaches were used to identify the underlying factor responsible for a statistically significant difference ($p=0.05$) when an ANOVA was performed.

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## Results

### Heavy metal concentrations of water, sediments and oyster from Buguma Creek

In Table 1, we see the average and standard deviation of the heavy metal concentrations in Buguma Creek water samples. Pd was not discovered at any of the locations, although Ni, Cd, and Zn were. In Table 1 we can see the metal concentrations (Mean±SD) found in Buguma Creek sediment samples. Cd and Zn concentrations were found to vary significantly (p0.05) across the monitoring sites using analysis of variance. Scheffe multivariate tests showed that Cd and Zn concentrations in Jordan were substantially (p0.05) different from those in Amayanabo and Orubo. Sediments from Jordan, Amayanabo, and Orubo had considerably greater concentrations of Cd than water (p0.05), but Ni, Pd, and Zn showed no significant differences (p>0.05).

Oyster (*Crassostrea gasar*) tissue samples from Buguma Creek were analysed for their heavy metal contents (Mean±SD), and the results are shown in Table 1. Ni and Pb concentrations were shown to be statistically different (p0.05) using an analysis of variance. Scheffe multiple variant comparisons showed that the Ni concentration in Amayanabo (1.450.38ppm) was significantly different (p0.05) from the Ni concentration in Orubo (.0.630.22ppm) and the Pb concentration in Orubo (179.9085.32ppm). Furthermore, Pb levels in oyster tissue samples were found to be statistically significant (p0.05) higher in Jordan, Amayanabo, and Orubo than in sediments and water. High levels of heavy metal biomagnification factor (BAF) were found in oyster tissues.

**Table 1:** Heavy metal concentrations (Mean±SD) of the water, sediments and oysters (*Crassostrea gasar*) from Buguma Creek, Niger Delta.

| Stations | Heavy metal | Water | Sediment | Oyster | BAF |
|---|---|---|---|---|---|
| Jordan | Cadmium | 0.33±0.21 | 0.41±0.42 [b] | 0.72±0.14 | 1.76 |
| | Nickel | 0.01±0.01 | 0.12±0.04 | 1.04±0.27 | 8.67 |
| | Lead | ND | 17.39±29.95 | 353.12±13.31 | 20.31 |
| | Zinc | 0.87±0.09 [a] | 0.21±0.02 [b] | 5.81±10.06 | 27.67 |
| Amayanabo | Cadmium | 0.29±0.15 | 1.64±0.12 | 1.01±0.61 | 0.62 |
| | Nickel | 0.02±0.00 | 0.20±0.15 | 1.45±0.38 c | 7.25 |
| | Lead | ND | 33.48±6.11 | 486.28±94.52 [c] | 14.52 |
| | Zinc | 0.37±0.33 | 4.31±2.53 | 36.91±63.94 | 8.56 |
| Orubo | Cadmium | 0.07±0.01 | 1.62±0.13 | 0.66±0.70 | 0.41 |
| | Nickel | 0.02±0.02 | 0.23±0.13 | 0.63±0.22 | 2.74 |
| | Lead | ND | 43.12±3.49 | 179.90±85.32 | 4.17 |
| | Zinc | 0.03±0.01 | 4.25±0.05 | 0.00±0.00 | - |

Standards for water(mg/kg) FEPA (2003): Cadmium: 1.00; Nickel: 1.00; Lead: 1.00; Zinc: 20.00

Standards for sediment (mg/kg) DPR (2002): Cadmium: 12.00; Nickel: 210.00; Lead: 510.00; Zinc: 720.00

Standards for food fish(mg/kg) FEPA (2003): Cadmium: 2.0; Nickel: 0.50; Lead: 2.00; Zinc: 75.00

ND = Not detected; a, b, c = Different letters indicate a statistically significant difference (P<0.05).

### Biochemical Studies: Activities of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and lipid peroxidation product, malondialdehyde (MDA) in oyster (*Crassostrea gasar*) from Buguma Creek.

In Table 1, we see the average and standard deviation of the heavy metal concentrations in Buguma Creek water samples. Pd was not discovered at any of the locations, although Ni, Cd, and Zn were. In Table 1 we can see the metal concentrations (Mean± SD) found in Buguma Creek sediment samples. Cd and Zn concentrations were found to vary significantly (p0.05) across the monitoring sites using analysis of variance. Scheffe multivariate tests showed that Cd and Zn concentrations in Jordan were substantially (p0.05) different from those in Amayanabo and Orubo.


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**Table 2:** Concentrations of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and lipid peroxidation product, malondialdehyde (MDA) (Mean±SD) in oyster tissues (*Crassostrea gasar*) from Buguma Creek, Niger Delta.

| | **Stations** | | |
|---|---|---|---|
| | **Jordan** | **Amayanabo** | **Orubo** |
| GSH(µmol/ml) | 7.69±0.91 | 8.34±3.45 | *15.98 ±2.57 |
| SOD(Um/mg) | 84.60±4.93 | 90.28±8.32 | 95.47±1.91 |
| CAT(ml mol/g) | 589.91±89.49 | 526.84±19.56 | 613.22±78.37 |
| MDA(Umol/ml) | 3.74±0.71 | 2.76±1.14 | 4.60±1.24 |

*Indicates statistically significant differences (P<0.05) across rows

**Discussion**

Antioxidant defence changes are useful indicators for oxidative stress in aquatic species produced by a variety of pollutants (Gorinstein et al., 2003). Increased activity of oxidative defence enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) is a common response to the oxidative stress caused by exposure to pollutants (Doherty et al., 2010). However, MDA, SOD, CAT, and GSH levels may all drop due to the pervasive influence of contaminants (Saliu & Bawa-Allah, 2012).

Oyster (*Crassostrea gasar*) tissues collected from various locations within Buguma Creek showed low levels of antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), as well as lipid peroxidation (MDA). This study's results are consistent with those of Sole et al. (1996), who found that the bivalve species M. edulis had a reduction in antioxidant levels after being exposed to the oil spill in the Aegean Sea. The enzyme superoxide dismutase (SOD) is essential for protecting cells against oxyradical damage. It does this by hastening the transformation of superoxide (O2) into hydrogen peroxide (H2O2), which is toxic to cells and may destroy biological structures. Therefore, increased intracellular reactive oxygen species (ROS) levels due to exposure to heavy metals or other contaminants may account for the decreased superoxide dismutase (SOD) concentration found in this research.

Reduced glutathione (GSH) is a powerful antioxidant that protects cell membranes from lipid peroxidation. It does this by binding to and neutralising oxygen radicals. The results given in Table 2 suggest that *Crassostrea gasar*, more popularly known as oysters, may have been exposed to dangerous compounds from polluted areas due to the high levels of glutathione (GSH) activity seen in their tissues. Oysters from Buguma Creek were discovered to have consistently low amounts of lipid peroxidation product (malondialdehyde, MDA) in their tissues. Species living in polluted settings have been shown to have lower levels of lipid peroxidation (MDA) activity, as demonstrated by Idowu et al. (2014).

A previous study by Oribhabor and Ogbeibu (2009) confirms the presence of heavy metals, including cadmium (Cd), nickel (Ni), lead (Pb), and zinc (Zn), in both the media of Buguma Creek and oysters (*Crassostrea gasar*). These scientists uncovered evidence of zinc, lead, and nickel in Buguma Creek's sediments and water. Heavy metal contamination of the New Calabar River has also been demonstrated by the works of Chinda et al. (2004) and Davies et al. (2006). Heavy metal studies conducted on Buguma Creek sand samples showed concentrations distinct from those found in the matching water samples. Cd concentrations in Jordan, Amayanabo, and Orubo sediments were found to be statistically (p0.05) greater than those in water. However, no statistically significant increases in Ni, Pd, or Zn concentrations ($p>0.05$) were seen across any of the monitoring sites. These results corroborate those of a 2006 research by Howard et al., which found that heavy metals have accumulated in Buguma Creek sediments. Sediments often have greater amounts of heavy metals compared to the water column, which is consistent with the results of prior research by Chinda et al. (2004), Davies et al. (2006), and Vincent-Akpu and Mmom (2012). Researchers found higher concentrations of metals in sediment samples than in water samples from many sites along the New Calabar River.

Heavy metal concentrations were determined in oyster tissue samples taken from Buguma Creek. Cd concentrations were found to be far below the 2003 Federal Environmental Protection Agency (FEPA) guidelines in all sampling sites. However, it was discovered that these concentrations exceeded those set by the FAO in the same year. Lead (Pb) levels in oyster tissue samples from all sampling sites were found to exceed the permitted limits established for food fish by both the Federal Environmental Protection Agency (FEPA, 2003) and the Food and Agriculture Organisation (FAO), while zinc (Zn) levels were found to be within the permissible limits established by the Food and Environmental Protection Agency (FEPA, 2003) for food fish. The population of Buguma and the surrounding towns are at risk due to the presence of high quantities of certain metals over the permitted criteria and their neurotoxic effects, especially in children. This is especially worrisome since oysters are a vital food source for many people.

Oysters (*Crassostrea gasar*) have a high bioaccumulation factor (BAF), suggesting they can store a lot of heavy metals in their bodies. Therefore, oysters may be used as reliable bioindicators of the health of the aquatic ecosystems they inhabit. Consistent with prior research (Davies et al., 2006; Obasohan, 2008; Vincent-Akpu and Mmom, 2012), it has been discovered that oyster (Crassostrea gasar) tissues accumulate more heavy metals than their surrounding environment. Heavy metal concentrations in fish and shellfish have been observed to be higher than those found in their soil and water habitats.

**Conclusion**

Oyster (*Crassostrea gasar*) samples collected from Buguma Creek were found to be contaminated with nickel and lead in the current study. FEPA (2003) and FAO (2003) established maximum values for pollutants in fish used for human consumption, and these levels were deemed to be excessive. The oyster species, *Crassostrea gasar* has been shown to bioaccumulate heavy metals to an extent that much exceeds the quantities in the water and sediments it inhabits, according to studies. Those who live in Buguma and the surrounding communities who eat oysters (*Crassostrea gasar*) and other fish species from Buguma Creek may be putting their health at risk due to the high levels of heavy metals in these waters. Superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and the lipid peroxidation product malondialdehyde (MDA) activities, as well as their relationship to heavy metal contamination, did not show a consistent pattern across the stations studied.

**Recommendations**

1. The study showed that oyster (*Crassostrea gasar*) has a good bioaccumulation factor (BAF). With its potential for accumulating metals, oysters should be considered as a good test organism for ecotoxicological studies.
2. Caution should be taken in the consumption of oysters (*Crassostrea gasar*) and other fishes from Buguma Creek due to the high levels of Ni and Pb found in oyster tissues from the Creek.

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*Cite this article as*:
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