Oxidative stress biomarkers in *Trichiurus lepturus* and *Sardinella maderensis* and the effects of heavy metal pollution in Lagos Lagoon, Nigeria

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Abstract

Contaminants like heavy metals are discharged into aquatic ecosystems from anthropogenic activities. The responses of *Trichiurus lepturus* and *Sardinella maderensis* to heavy metals in three sampling stations from Lagos Lagoon, in the vicinity of wood, textile and power plant industry, as well as control site in Badagry were determined. Heavy metals Pb, Cd, Cu, Cr and Zn were analyzed from fish muscle, water and sediment using atomic absorption spectrometry (AAS) to examine their concentrations and impacts on antioxidant defense enzymes such as superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (LPO) in the fish muscle. The results showed different effects in *T. lepturus* (TL) and *S. maderensis* (SM) in diverse sampling stations with varying degrees of pollution including the control site. Principal component analysis showed that fish samples from Makoko, Ibeshe and Egbin demonstrated a strong correlation between antioxidant defense enzymes and heavy metals concentrations in fish, water and sediment with PCA1 accounting for 56.27% of the loadings and was dominated by heavy metals and antioxidant enzymes in all the media. Variations in activities of antioxidant enzymes were observed in the muscles of *T. lepturus* and *S. maderensis* in relation to metal content. The toxic effects were observed as the activity of antioxidant enzymes increased with increased concentration of metals. Significant differences ($p<0.05$) in SOD (U/mg protein) in TL (96.09±23.08), CAT (U/mg protein) in SM (3.09±1.08) and LPO (µmol/mg protein) in TL (81.28±03) between fish from sampling sites and control site were observed. The fish from the lagoon were found to be under severe stress as compared to the fish from the reference site. Hence, this study reflects the need for integrating antioxidant enzymes to assess the effects of pollutants in aquatic environments under complex interaction of pollutants.

Keywords: antioxidant enzymes;, heavy metals;, *Trichiurus lepturus*;, *Sardinella maderensis*

Introduction

Human and natural factors generate oxidative stress in the aquatic biota (Alkhudhayri *et al*., 2022, Oliva *et al.,* 2012). Pollutants are known to activate the production of reactive oxygen species (ROS) such as O^-_2, H_2O_2, $^\cdot OH$, etc. and elevated amounts often promote antioxidant defense activities (Halliwell and Gutteridge, 1999). Biomarkers of oxidative stress such as antioxidant defense enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) usually functions in a coordinated manner in order to ensure maximum preservation and protection of the cell from oxidative stress (Nwaichi & Anosike, 2016; Foley *et al*., 2022).

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High concentration of heavy metal values have been recorded in Ogun River which flows into the Lagos Lagoon (Ajani *et al*., 2021). Human activities are known to be one of the sources of heavy metal pollution into the lagoon system, urban sewage inputs, wood, textile and power plant along the lagoon and may have suffered neglect (Nwaichi & Osuoha, 2021). Aquatic organisms are mostly exposed to pollutants such as heavy metals from industrial and agricultural activities which discharge their effluents into water ways and are taken up by aquatic biota (Ajani, 2020). As fish are biomarkers of aquatic ecosystem health are being impacted by these environmental stressors, hence the role of oxidative stress biomarkers during bioaccumulation of heavy metals in *Trichiurus lepturus* and *Sardinella maderensis* inhabiting polluted habitats such as Lagos Lagoon are unknown and requires investigation (Foley *et al.,* 2022).

Materials and Methods

The Lagos Lagoon is significant in Nigeria. It is the biggest among the four lagoons within the Gulf of Guinea (Webb, 1958). Osun, Yewa, Ona and Ogun Rivers are the major rivers that drain into the lagoon (Fig. 1.). The major body of the lagoon lies within E3º 23' and 3º 40' and N6º 22' and 6º 38'. The coastline dynamics and distribution of contaminants from the hinterlands as well as immediate shores of the lagoon are incidents that are associated with this brackish water. Three sampling stations were established along the lagoon. These were Makoko, Ibeshe and Egbin and each sampling station has significant human activities operating there.

Water physicochemical parameters temperature, dissolved oxygen (DO), biochemical oxygen demand (BOD), pH, salinity, conductivity were measured *in-situ* using Horiba U-10. One hundred and fifty *Trichiurus lepturus* (Makoko n=52, Ibeshe n=59 and Egbin n=39) with average weight of 230g and two hundred and twenty eight *Sardinella maderensis* (Makoko n=73, Ibeshe n=102 and Egbin n=53), with average weight of 27g caught from three sampling stations and a control site Joforo in Badagry (Fig. 1.) were examined.

Fish was dissected and one gram of muscle was removed. The tissues were placed in a Teflon beaker and digested in an acid solution (HNO_3) in preparation for heavy metal analysis (Kenstar closed vessel microwave digestion) using the microwave digestion procedure (Segbeloyin *et al.,* 2010). The specimens were digested with 5ml of HNO_3 (65%) and filtered with the Whatman Filter Paper no 41 into a 25ml volumetric flask and made up to mark with distilled water (FAO/SIDA, 1983). All digested samples were analyzed in triplicates for the following metals: lead, cadmium, chromium, copper and zinc using atomic absorption spectrophotometer (AAS) (Perkin-Elmer AA 700). The equipment was graded with standard solutions formulated from economically available chemicals purchased from Merck KGaA, Germany (Kingston and Jassie, 1988).

The antioxidant defense enzyme analysis involved the removal of muscle from fish which was washed in ice cold 0.65% sodium chloride before storage. Thereafter, 0.5g of tissue was homogenized with 5ml of 0.4M phosphate buffer using Teflon homogenizer. The tissue homogenates were centrifuged at 3000rpm at 15mins. The resulting supernatant was used for further biochemical analysis (Magwere *et al.*, 1997). Superoxide Dismutase (SOD) activity evaluated using procedures from Magwere *et al.,* (1997). Catalase (CAT) activity followed procedures from Sinha (1972). Malondialdehyde (MDA) constituted part of the final output of lipid peroxidation. MDA levels was analysed according to Buege and Aust (1978) methods. The total protein estimation was measured with Bicinchoninic Acid (BCA) procedure (Smith *et al.,* 1985).

For water analysis, 25ml of each water sample was collected with a conical flask. Aqua regia (concentrated HNO_3) were added to the water samples. The mixture was place on a hot plate in the fume cupboard to evaporate till 5ml at 120^{0}C. The digested samples were allowed to cool and filtered through the Whatman Filter Paper no 41 into a 50ml volumetric flask and made up to mark with distilled water (FAO/SIDA, 1983). All digested samples were analyzed in triplicates for the following metals: lead, cadmium, chromium, copper and zinc using atomic absorption spectrophotometer (AAS) (Perkin-Elmer AA 700). (Perkin-Elmer AA 700). The equipment was graded with standard solutions formulated from economically available chemicals purchased from Merck KGaA, Germany (Kingston and Jassie, 1988).

Van veen grab was used to collect sediment samples from each sampling station, placed in a foil paper and labeled. All sediment samples were air-dried, homogenized and sieved. Sieved samples were digested as follows: 5g of sediment into 100 ml beaker. Freshly prepared mixture of nitric acid and hydrogen peroxide of ratio 1:1 was added to the sediment. Digestion took place on a hot plate in the fume cupboard at 160^{o}C until the volume reduces to 5ml. Allowed to cool and filtered with the Whatman Filter Paper no 41 into a 50ml volumetric flask and made up to mark with distilled water (FAO/SIDA, 1983). All digested sediment samples were analyzed in triplicates for the following metals: lead, cadmium, chromium, copper and zinc using atomic absorption spectrophotometer (Perkin-Elmer AA 700). The equipment was graded with standard solutions formulated from economically available chemicals purchased from Merck KGaA, Germany (Kingston and Jassie, 1988).

All values for the results are expressed as mean±standard error. Unpaired student t-test was used to determine significant differences ($p<0.05$) between fish species. Principal component analysis was used to determine the relationship between antioxidant enzymes and heavy metals.

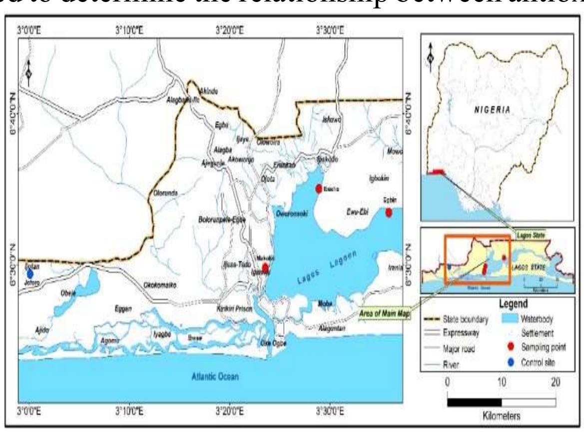

Fig. 1.: Map of Lagos Lagoon showing the sampling stations

Results and Discussion

There was a marked variation in physicochemical parameters of water sample with DO, pH, salinity and conductivity showing the highest values in Makoko (Figs. 2;, 3). This can be attributed to proximity to the sea.

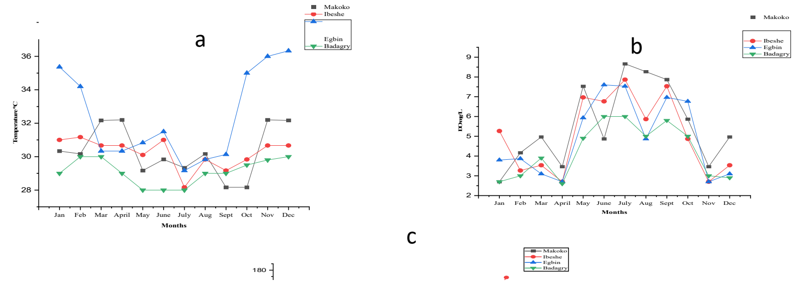

Fig. 2.: Physicochemical parameters of surface water recorded from sampling stations a= temperature, b= dissolved oxygen, c= biochemical oxygen demand

Egbin sampling station showed the highest temperature values, owing to the power plant at the station, while Ibeshe recorded the highest BOD values implicating point source pollution at the station. Our results further showed enriched heavy metals (Pb, Cr, Cu and Zn) in *T. lepturus* than *S. maderensis* (Figs. 4;, 5), while Cd showed a reverse concentration in the fish species. These results may not be unconnected with the feeding habits and trophic status of the two fish species. Notably, the factors responsible for variation in metal accumulation in aquatic ecosystems are: water temperature, salinity, geographical location, season, food availability, age, size of the fish, and maturation status (Majouobi *et al*., 2021, Tramice *et al*., 2021).

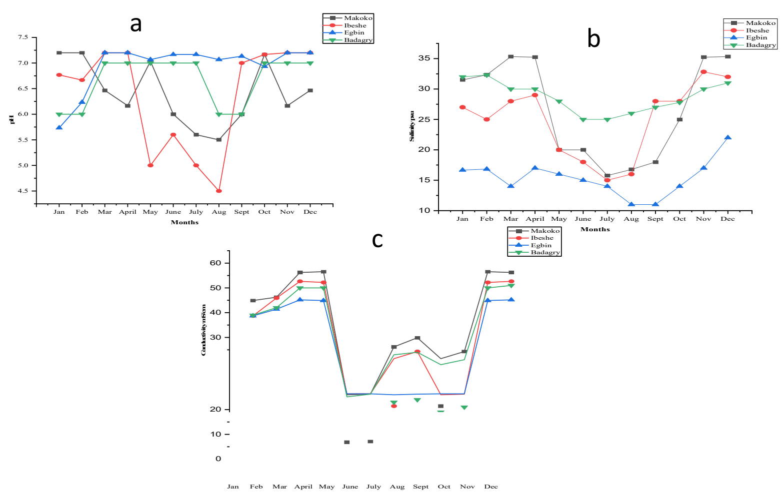

Fig. 3.: Physicochemical parameters of surface water recorded from sampling stations a= pH, b= salinity, c = conductivity

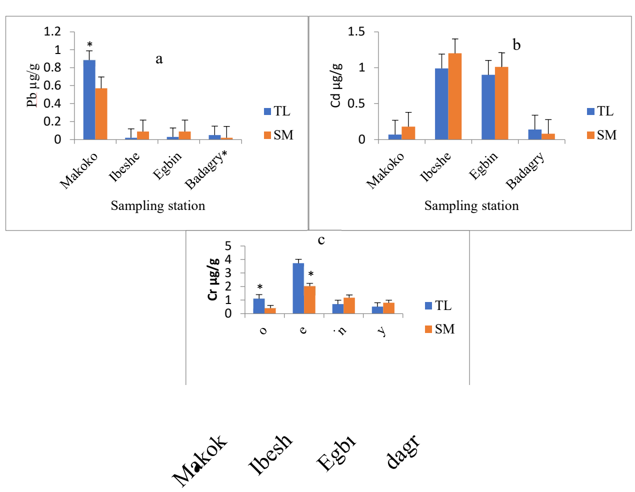

Fig. 4.: Heavy metal concentrations in *T. lepturus* (TL) and *S. maderensis* (SM) from Lagos Lagoon a= Pb, b= Cd, c= Cr

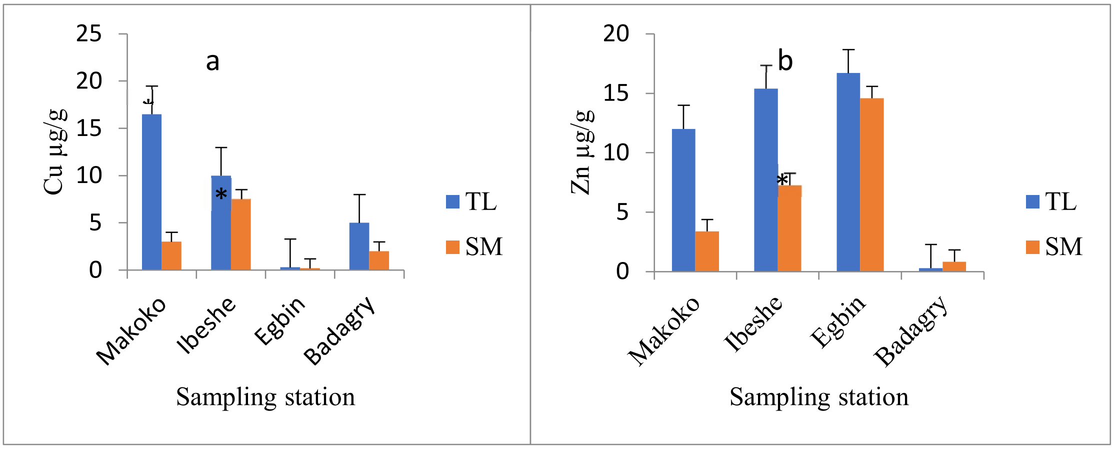

Fig. 5.: Heavy metal concentrations in *T. lepturus* (TL) and *S. maderensis* (SM) from Lagos Lagoon a= Cu, b= Zn

Zinc and Cadmium concentrations were higher in the sediment at Makoko than in the water (Table 1). Cr and Cu concentrations were higher in water than sediment at Ibeshe, and this may be attributed to the textile effluent and sand mining activities (Ajani, 2021). The metal values at the control from Joforo in Badagry did not show any significant difference based on the relationship between antioxidant defense enzymes and heavy metal concentration in fish samples using principal component analysis. Fish species from Makoko, Ibeshe and Egbin showed strong correlation with heavy metals and antioxidant enzymes (Fig. 6). This study shows the essential role played by antioxidant defense enzymes against oxidative stress in aquatic ecosystem (Brix *et al.,* 2022). Studies that link stress antioxidant enzymes activities and heavy metal load in fish are very few however, the integration of oxidative stress biomarkers in toxicological studies is necessary to understand the effect of complex pollutants in aquatic environment.

Table 1.: Heavy metal concentration in water and sediment from Lagos lagoon

Heavy metal	Sampling stations	Water µg/L	Sediment µg/g
Pb	Makoko	0.043±0.01	1.13± 0.85
	Ibeshe	0.02 ±0.04	0.99±0.9
	Egbin	0.040±0.02	0.62±0.43
	Badagry	0.11±0.01	0.72±0.02
Zn	Makoko	0.130±0.04	21.27±9.63
	Ibeshe	15.38±3.10	11.27±9.8
	Egbin	0.041±0.02	17.53±6.84

	Badagry	0.6±0.011	2.18±0.03
Cu	Makoko	0.173±0.09	0.35±0.26
	Ibeshe	7.79±6.23	0.50±0.23
	Egbin	0.08±0.09	0.21±0.09
	Badagry	0.043±0.00	1.16±0.21
Cr	Makoko	0.06±0.05	0.34±0.20
	Ibeshe	1.72 ±0.52	0.50±0.44
	Egbin	0.04±0.01	0.27±0.30
	Badagry	0.211±0.00	1.03±0.02
Cd	Makoko	0.117±0.13	3.83±3.05
	Ibeshe	0±0.00	4.83±3.05
	Egbin	0.07±0.05	3.72±1.96
	Badagry	0.09±0.01	0.21±0.02

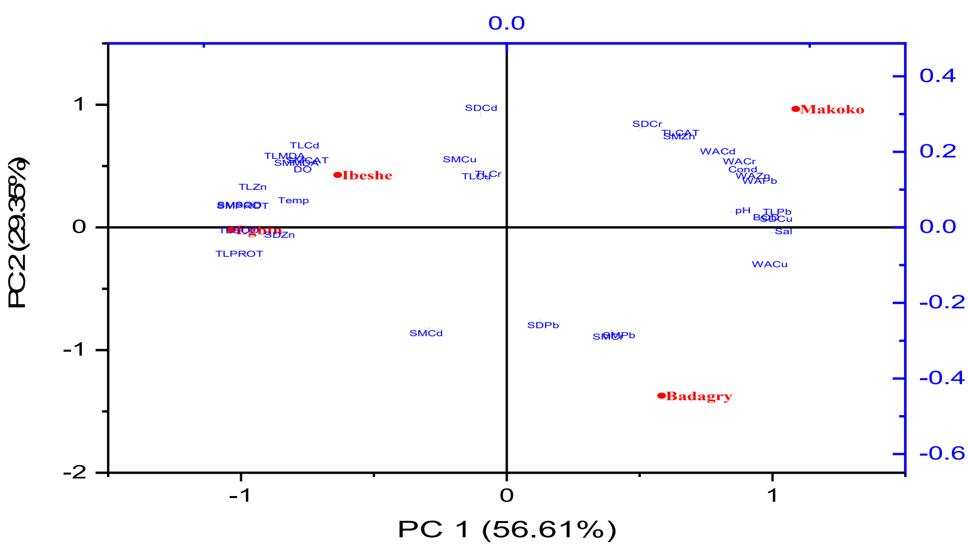

Fig. 6.: Principal component analysis (PCA) showing the relationship between antioxidant enzymes and heavy metal concentrations in *T. lepturus* and *S. maderensis* from Lagos Lagoon.

Conclusion

Research on the serious problem of heavy metals in fish and environments is increasing to understand their distribution and fate. The continuous ingestion of heavy metals may result in hazardous consequences for populations and communities. Extended monitoring studies focusing on biomarker activities with heavy metal concentrations are required especially since the heavy metals are persistent in nature.

References

Ajani, G.E. 2021. Comparative study on heavy metal concentrations and biochemical responses in *Chrysichthys nigrodigitatus* and *Sardinella maderensis* from Lagos Lagoon. *Proceedings of the 36th annual conference of Fisheries Society of Nigeria* (FISON), 24th -29th October, 2021, page 72-76.

Ajani, G.E. 2020. Biomarker profile of silver catfish *Chrysichthys nigrodigitatus* in heavy metal polluted areas of the Lagos Lagoon. *Proceedings of the Nigeria Academy of Science*. Themed Edition on Sustainable Development Goal 14 Volume 13, 2: 15-35.

Ajani, G.E., Popoola, S.O. and Oyatola, O.O. 2021. Evaluation of the pollution status of Lagos coastal waters and sediments using physicochemical characteristics, contamination factor, Nemerow pollution index, Ecological Risk and potential Ecological Risks index. *International Journal of Environment and Climate Change*, 11:1-16.

Alkhudhayri, A., Felwa, A., Thagfan, A., Al-Quraishy, S., Abdel-Gaber, R., Mohamed, A.D. 2022. Assessment of the oxidative status and goblet cell response during eimeriosis and after treatment of mice with magnesium oxide nanoparticles. *Saudi Journal of Biological Sciences* 29, 1234–1238.

Brix, K. V., De Boeck, G., Baken, S., and Forte, D. 2022. Adverse Outcome Pathways for Chronic Copper Toxicity to Fish and Amphibians *Environmental Toxicology and Chemistry*, 1, 1–17.

Buege, J. and Aust, S. 1978. Microsomal lipid peroxidati on. Methods in Enzymology 52: 302-310

Celina Obeka and Aroloye O. Numbere 2020. Heavy metal concentration and public health risk in consuming *Sardinella maderensis* (Sardine), *Sarotherodon melanotheron* (Tilapia), and *Liza falicipinis* (Mullet) harvested from Bonny River, Nigeria. *Journal of Oceanography and Marine Science,* 11, pp. 1-10.

Foley, M., Askin, N. and Wittnich, M.B. 2022. Anadromous fish as biomarkers for the combined impact of marine and freshwater heavy metal pollution. *Ecotoxicology and Environmental Safety* 230, 113153.

Food and Agricultural Organization (FAO)/Swedish International Development Cooperation Agency

(FAO/SIDA), 1983. Manual of methods in aquatic environmental research, part 9. Analyses of metals and organochlorines in fish. *FAO Fisheries. Technical paper* 212pp.

Halliwell, B. and Gutteridge, J.M.C., 1999. Free Radicals in Biology and Medicine. Oxford University Press, Oxford.

Kingston, H. and Jassie, L. 1988. Introduction to microwave sample preparation. *American Chemical Society* 54.

Magwere, T., Naik, Y. and Hasler, J. 1997. Effect of chloroquine treatment on antioxidant enzymes in rat liver and kidney. *Free Radical and Biological Medicine* 22:321-327.

Majdoubi, F.Z., Ouizgane, A., Farid, S., Mossetti, L., Droussi, M., Guerriero, G., Hasnaoui, M. 2021. Fry Survival Rate as a Predictive Marker of Optimal Production of Silver Carp (Hypophthalmichthys molitrix, Valenciennes 1844): A Biostatistical Study in Deroua Fish Farm, Morocco. *Proc. Zool. Soc*. 1: 12-19

Nwaichi, E. O. and Anosike, E. O. (2016). Plant response on exposure to Ag Nanoparticles: a study with Vigna subterranea. Biochemistry & Analytical Biochemistry, 5(4), 299.

Nwaichi, E.O. and Osuoha, J. O. (2021). Has the National policy on environmental pollution control in Nigeria been neglected in the Niger Delta region? An update. Environment, Development and Sustainability. 24, 12494–12517. https://doi.org/10.1007/s10668-021-01973-1

Oliva, M., Vicente, J., Gravato, C., Guilhermino, L. and Galindo-Rian, M. 2012. Oxidative stress biomarkers in Senegal sole, *Solea senegalensis*, to assess the impact of heavy metal pollution in a Huelva estuary (SW Spain): Seasonal and spatial variation. *Ecotoxicology and Environmental Safety* 75, 151–162.

Segbeloyin, J.N., Onyimonyi, A.E., Ujam, O.T., Ukwueze, N.N. and Ukoha, P.O. 2010. Assessment of toxic trace metals in selected fish species and parts of domestic animals. *Pakistan Journal of Nutrition* 9:213-215.

Sinha, A.K. 1972. Colorimetric assay of catalase. Analytical Biochemistry 47:389-394.

Tramice, A., Trifuoggi, M., Ahmad, M.F., Lam, S.S., Iodice, C., Velotto, G., Giarra, A., Inglese, S., Cupo, A., Guerriero, G. 2021. Comparative fatty acid profiling of edible fishes in Kuala Terengganu, Malaysia. *Foods* 10, 2456.