Oxidative Stress In Fish Living In Coastal Water Polluted With Sawdust And Wood Waste Along Lagos Lagoon, Nigeria

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Abstract: Increased number of agricultural and industrial wastes enter aquatic environment and being taken up by aquatic organisms induce plural changes. These could result in oxidative stress and later having adverse effect on physiological and biochemical function. The activities of superoxide dismutase (SOD) and lipid peroxidation (MDA) were investigated in liver and gonad of *Pomadys jubelini* from Okobaba along Lagos lagoon. These enzymes play important roles in protecting the cells against potentially toxic effects of environmental pollutants. Fish have been used as indicators for monitoring land-based pollution because they can concentrate pollutants in their tissues, directly from water through respiration and also through their diet. Fish samples were collected from February to June 2012 at Okobaba, a log and sawmill industry that discharge sludge beds of sawdust or wood shavings and domestic waste along the Lagos lagoon and analysed. There was a significant difference ($P<0.05$) in the levels (997.90 Umol/mgprotein) of superoxide dismutase in liver than lipid peroxidation , while in the gonad this enzyme showed insignificant difference ($P >0.05$). Lipid peroxidation level (113.39 Umol/ml) in the gonad showed a significant difference ($P< 0.05$) than superoxide dismutase. However during the sampling periods protein increased relatively in the liver along with the gonads. The results from this study demonstrates the alterations in the levels of superoxide dismutase and lipid peroxidation in liver and gonad which reflects response to stress that could be environmentally induced from the wood waste at the sampling point, seasons or physiological state of the organism. This study provides a rational use of these enzymes as suitable biomarkers with different degrees of specificity and as important tool for environmental monitoring.
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1.Introduction

Organic contamination are continually entering aquatic environments and thence the tissues of resident biota (Pavlović, et al., 2010). Living systems encounter a variety of stress during their continuous interaction with the environment. Environmentally-induced stresses frequently activate the endogenous production of reactive oxygen species (ROS), most of which are generated as side products of tissue respiration (Valavanidis et al., 2009). Increased number of agricultural, industrial and domestic waste enter aquatic environment and being taken up by aquatic organism induce plural changes which enhances the formation of reactive oxygen species (Valavanidis et al., 2009) . Oxidative stress as defined by (Sarkar et al., 2006) is a situation when steady-state reactive oxygen concentration is transiently or chronologically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents. The ecosystems are under the pressure of complex mixtures of contaminants released in the environment due to various human activities exert multiple stress effects; they originate from miscellaneous sources such as chemical and drug manufacture, domestic sewage, polymer and petrochemical-based industries, oil refineries, mining, glass blowing, battery manufacture and many others (Widianarko et al., (2000), Ueno et al., (2004), Huska, et al., (2008). The assessment of alterations in key enzymatic activities of organisms that are exposed to polluted waters have been used as pollution biomarkers, due to the fact that distinct kinds of pollutants may interfere with animal physiology and behavioural processes, which in turn is of ecological importance (Almedia et al., 2009). A number of authors have shown that several biomarkers of oxidative stress can provide satisfactory information on the response of fish to environmental stressors (Achuba and Osakwe (2003), Farombi et al., (2007), Pavlović, et al., (2010). Various enzyme activities have been as indicators or general biochemical markers of stress in fish (Vutukuru et al., (2007), Ozmen et al., (2008). The reason for using biomarker is the generation of information on the biological effect of these contaminant rather than mere quantification of their environmental levels. Recent years have seen the development of biological measurements

(biomarkers) as tools for use in monitoring and environmental impact assessment, such biomarkers being indicative of contaminant exposure and / or impact (Livingstone (2001). Hence, the use of such biomarkers as super oxide dismutase, lipid peroxidation products and other antioxidants which are measurable internal indicators in body fluids, cells or tissues are very important in assessing changes at the cellular level due to the effect of toxicants, since these toxicants are being discharged into the waterways pose a threat to aquatic organism and man (Valavanidis et al., 2009). The present study is aimed at determining oxidative stress in fish (Pomadasys jubelinin) inhabiting coastal water polluted with sawdust and woodwaste, along Lagos lagoon.

2.Materials and methods

Lagos lagoon is the largest of the four parts lagoon systems of the Gulf of Guinea, located in Lagos state, Nigeria. The lagoon supports tremendous artisanal fishing of capture fisheries sector, it provides necessary ecological habitats such as breeding and nursery grounds for fresh water and marine macrofauna species. It is an open, shallow and tidal lagoon with a surface area of 208km^2 (FAO., 1969) and an average depth of less than two meters. However works have been done on the Lagos lagoon (Edokpayi and Nkwoji (2007), (Onyema et al., (2009), Emmanuel et. al., (2010) that revealed the effect of anthropogenic waste and environmental modifications thus impacting pollution and stress in the water column. The sampling location fig 1 was okobaba with coordinates N06^{0}28'587" E003^{0}23'36.8". Human activities at this location are logging and sawmilling which generate organic waste such as sawdust and particulate woodwaste, as well as domestic sewage. The fishes were caught by local fishermen and then transported immediately in ice to the laboratory and stored in a deep freezer at 4ºC and later dissected to extract organs (liver and gonad).

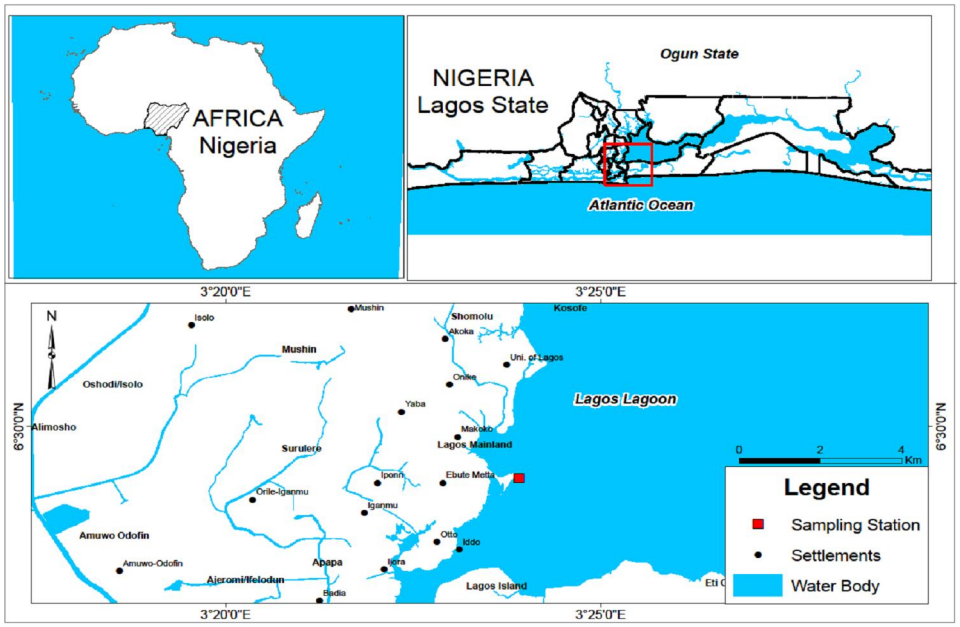

Fig 1. Okobaba sampling station along Lagos lagoon

2a.Method of Homogenization

The fishes were dissected and the liver and gonads were removed. The organs of the fish were washed in ice cold 1.15% KCl solution, blotted and weighed. They were then homogenized with 0.15%M of KCl before putting them into the mortar laboratory sand was added and it was blended in mortar with pestle together. The resulting homogenate was centrifuge at 3500rpm for 15minutes and the supernatant was decanted and stored at -10ºC until analysis (Magwere 1997)

2b.Assay of Superoxide dismutase Activity

Superoxide dismutase activity was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 at 30ºC as described by (Magwere 1997). One unit of superoxide activity is the amount of SOD necessary to cause 50% inhibition of epinephrine auto-oxidation. The assay was performed in 3.0ml of 50M Na_2CO_3 buffer to which 0.02ml of the sample was added. 0.03ml of epinephrine stock solution was added to above before taking the absorbance reading at 480nm for 3-5mins. A blank devoid of the sample (but have all the reagents) was used for background correction.

2c.Assay of Lipid peroxidation (LPO)

LPO was quantified as thiobarbituric acid reactive substances (TBARS) at 535nm estimating the aldehyde (malondialdehide-MDA) formed using a standardard of malonaldehidebis-(dimethylacetal) (Buege and Aust (1978). This means that 1.0ml of sample homogenate was combined with 2.0ml of TCA-TBA-HCl and mixed thoroughly, the solution was heated for 15 minutes in boiling water bath. Allow to cool, the flocculent precipitate was removed by centrifuging at 3500rpm for 10 minutes . The absorbance was determined at 535nm against a blank that contains all the reagent except the sample.

2d.Total Protein Estimation

The protein content for each tissue was estimated using the method by (Lowry et. al., (1951). 1g of the sample was weighed and homogenized with 20ml of 0.5M NaOH. The homogenate was poured into a centrifuge tube and centrifuge at 3500rpm for 10 minutes. The supernatant was collected in a tube. To 1ml of the supernatant, 4ml of distilled water was added. A standard protein solution of 0.2mg was prepared (bovine albumin serum) in the same manner. 5cm^3 of alkaline solution was added to each tube and then it was mixed properly and allow to stand at room temperature for 10 minutes. 0.05cm^3 of dilute Folin-ciotteau reagent was added to each tube and mixed immediately to give a blue colour. The absorbance was read at 750nm against a reagent blank.

2e.Statistical analysis

Results of activities of antioxidant enzymes (SOD and LPO) were reported as mean ± SD. Student t test was used to determined the significance level at α=0.05 in activities of each enzyme in the liver and gonad of the fish.

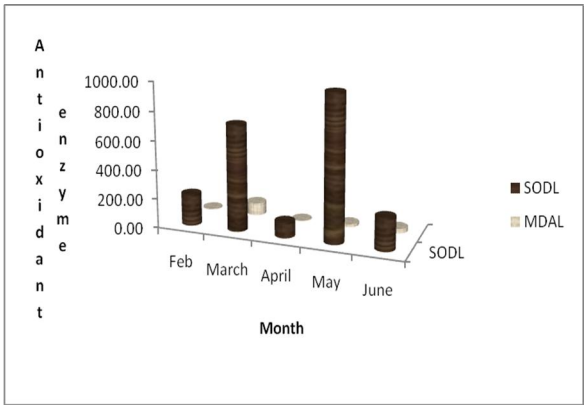

Fig 2. Levels of superoxide dismutase and malonaldehyde (LPO) in the livers of P. jubelini at Okobaba

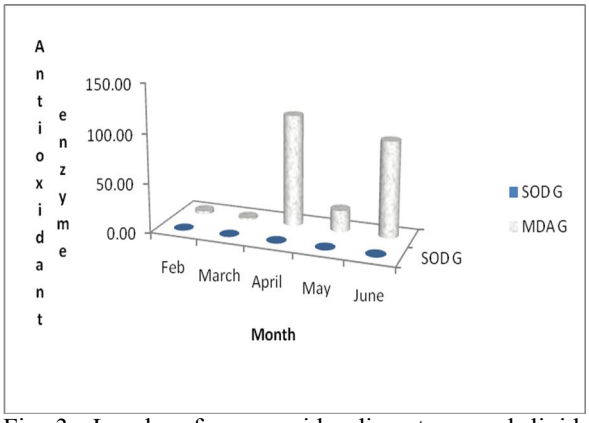

Fig 3. Levels of superoxide dismutase and lipid peroxidation in the gonads of P. jubelinin from Okobaba

3.Result

Fig 2 represents the levels of superoxide dismutase and lipid peroxidation in liver of P. jubelini from Okobaba; fig 3 represents the levels of superoxide dismutase and lipid peroxidation in gonads of P. jubelinin from Okobaba. During the sampling period superoxide dismutase showed a higher levels than lipid peroxidation in the liver. Alternatively, lipid peroxidation showed a higher levels than superoxide dismutase in the gonad of this fish.

4.Discussions

Fish are susceptible to environmental contamination and are widely used as bioindicators for water quality assessment in aquatic environment (Widianarko et. al., (2000), Ueno et al., (2004), Environmental contaminants can have a broad spectrum of sublethal effect on organisms, bioindicators are useful tools for assessing the presence and levels of chemical pollution . Such effect in organism sensitive to contaminant exposures can be used as early warning signs for the degradation of the environment (Huska, et al., (2008), Krizkova et. al., (2008). Among the widely used bioindicators are superoxide dismutase and lipid peroxidation. Malonaldehyde is the product of lipid peroxidation, in other words levels of lipid peroxidation can be assessed by measuring malonaldehyde (MDA) activity. Increased in the activities of superoxide dismutase in the liver suggest induction from organic pollutant which is similar to studies done by (Metwally (2000) on fishes from khomse coast, Libya. Alternatively, elevation in the activities of products of lipid peroxidation, malonaldehyde in the gonads (Fig 3) revealed an increase in antioxidant defense mechanism to prevent formation of excessive free radicals. Bioaccumulation of these contaminants in fish organs

have been reported to elicit the formation of reactive oxygen species , which may lead to environmental oxidative stress and causes changes in the activities of the enzymes in these organs(Casilas et. al., (1983). The destruction of aquatic ecosystem inform of sublethal pollution by man usually lead to chronic stress of aquatic biota. The transfer of energy from one trophic level to another within the food chain may be disrupted resulting in an ecological imbalance and dwindling fish production . This may eventually affect man who depends on fish as a means of livelihood (Taofik et. al., (2009). Increased lipid peroxidation (LPO) has been demonstrated in response to contaminant exposures in fish and bivalves (Ringwood et. al., 1998). Excessive generation of reactive oxygen species causes irreversible impairment of DNA and damage to membrane lipids leading to the production of malonaldehyde (Gawel et. al., 2004). The biological systems which can defend against oxidative stress includes antioxidant enzymes (Prasanthi et. al., (2005). The data obtained from this study showed that contaminant from this location causes a stress inducing effect on the organs of this fish during exposure, which is reflected by induction and inhibition of the enzymes investigated. In such cases, the fish could be vulnerable or predisposed to any invading pathogens and predators. Growth, development and physiological state of an organism are key factors in determining species sustainability, survival and availability; however these factors are vulnerable to adverse effect of pollutant at all stages of an organisms life cycle (Adeogun and Chukwuka (2012). . Hence it becomes imperative to take cognizance of the adverse effect these contaminants may cause in the nearest future to aquatic biota if not treated effectively before discharge into water body. One of the features of antioxidant enzymes is their induction under condition of oxidative stress and such inductions are important adaptation to pollutant-induced stress in organism generally (Livingstone (2001). Fish responses have been used as biomarker of aquatic pollution, hence the use of a suitable biomarker with different degrees of specificity is an important aspect of environmental monitoring based on biomarkers (Sarkar et. al., (2006).

References

1. Pavlović, SZ, Borković Mitic SS, Radovamovic TB, Perendija BR, Despotovic SG, Gavric JP and Saicic ZS (2010). Seasonal variation of the activity of antioxidant defense enzymes in the Red mullet (Mullus barbatus) from Adriatic sea. Mar. Drugs 8 (3): 413-428.
2. Valavanidis A, Vlachogianni T, Fiotakis K. (2009). *8-Hydroxy-2'-deoxyguanosine (8-OHdG): A Biomarker of Oxidative stress and carcinogenesis*. Journal of Environmental Science and Health, Part C, Environmental Carcinogenesis and Ecotoxicology, 27:1-20, 2009. (abstract).
3. Sarkar A, Ray D, Amulya N. Shrivastavas and Subhodeep Sarker (2006). Molecular Biomarkers: Their significance and application in marine pollution monitoring. Ecotoxicology, 15 (4) : 333-4 340.
4. Widianarko, B, Van Gessel., Verweij CAM, Van Straalen N.M. (2000). Association between trace metals in sediments, water and guppy, Poecilia reticulate (Peters)., from urban streams of Semarang, Indonesia., Ecotox. Environ. Safe. 46, 101-107.
5. Ueno, D, Inove S, Takahashi S, Ikeda, K, Tanaka, H, Subramanian, AN, Fillmann, G, Lam, PKS, Zheng, J, Muchtar, M, Prudente, M, Chung, K, Tanabe, S. (2004). Global pollution monitoring of butyltin compounds using skipjack tuna as a bioindicator. Environ, Pollut. 127, 1-12.
6. Huska, D, Krizkova, S, Beklovah M, Havel, L, Zenalek, J, Diopan, V, Adam, V, Zeman, L, Babula, P, Kizek, R (2008). Influence of Cadmium (11) ions and brewery sludge on the metallothionein level in earthworm (*Esenia fetida*) – Biotransforming of toxic wastes. Sensors. 8., 1039- 1047.
7. Almedia, JA, Barreto RE, Novelli LB, Castro FJ and Moron SE, (2009). Oxidative stress biomarkers and aggressive behavior in fish exposed to aquatic cadmium contamination. Neotrop. Ichthyol., 7(1):103-108
8. Achuba, FI and SA Osakwe (2003). Petroleum induced free radical toxicity in African catfish (*Clarias gariepinus*). Fish Physiol. Biochem.,29(2): 97-103.
9. Farombi, EO, Adelowo OA and Ajimoo YR (2007). Biomaker of oxidative stress and heavy metals as indicators of pollution in African catfish (*C. gariepinus*) from Ogun river, Nigeria. Int. J.Environ. Res. Pub. Health, 4(2): 158-165.
10. Pavlović SZ, Borković Mitic SS, Radovamovic TB, Perendija BR, Despotovic SG, Gavric JP and Saicic ZS (2010). Seasonal variation of the activity of antioxidant defense enzymes in the Red mullet (Mullus barbatus) from Adriatic sea. Mar. Drugs 8 (3): 413-428.
11. Vutukuru, SS, Arun-Prabhath N, Raghavender M and Yerramilli A (2007). Effects of arsenic and chromium on the serum amino-transferases activity in Indian major Carp, Labeo rohita. Int. J. Environ. Res. Public Health, 4: 224-227.

12. Ozmen, M, Ayas Z, Gungordu A, Ekmeci GF and Yerli S (2008). Ecological assessment of water pollution in Sariyar Dam lake, Turkey, Ecotoxicology. Environ. Saf., 70:163-173.
13. Livingstone, DR (2001). Contaminant-stimulated Reactive Oxygen Species Production and Oxidative Damage in Aquatic Organisms. *Marine Pollution Bulletin,* Vol. 42, pp.
656–666, ISSN 0025-326X
14. FAO., 1969. Fisheries survey in the western and mid-western regions of nigeria. FAO, Rome, SF74/NIR6.
15. Edokpayi, CA and Nkwoji, JA (2007). Annual changes in the physico-chemical and macrobenthic invertebrate characteristics of the Lagos lagoon sewage dump site at Iddo, Southern Nigeria. *Ecol. Env. and Cons*. 13(1): 13-18.
16. Onyema IC, Nkwoji JA and Eruteya OJ. Journal of American Science 2009; 5(8): 13-24
17. Emmanuel, BE, Chukwu, LO and Bakare, SO (2010). Hydro-Chemistry, Macro-Invertebrate Fauna And Fish Production Of Acdja Fishing Sites In A Tropical Lagoonal Ecosystem Journal of American Science 2010;6(1):42-48.
18. Magwere, T, Naik YS and Hasler YS (1997). Effects of chloroquine treatment on antioxidant enzymes in rat liver and kidney. Free Rad. Biol. Med., 22(1-2):321-327.
19. Buege, JA and SD Aust, (1978). Microsomal lipid peroxidation. Methods Enzymol., 52: 302-310
20. Lowry, O. H., Rosebrough NJ, Farr AL and RJ Randall (1951). Protein measurement with the folin phenol reagent. The Biological Chemistry, 193(1): 265-75.
21. Widianarko B, Van Gessel CAM, Verweiji RA, Van Straalen NM (2000). Association between trace metals in sediments, water and guppy, Poecilia reticulate (Peters)., from urban streams of Semarang, Indonesia, Ecotox. Environ. Safe. 46, 101-107.
22. Ueno D, Inove S, Takahashi S, Ikeda K, Tanaka H, Subramanian AN, Fillmann G, Lam PKS, Zheng J, Muchtar M, Prudente M, Chung K, Tanabe S (2004) Global pollution monitoring of butyltin compounds using skipjack tuna as a bioindicator. Environ, Pollut. 127, 1-12.
23. Krizkova, S., Ryant, P., Krystofova, O., Adam, V., Galiova, M., Beklova, M., Babula, P., Kaiser, J., Novotry, K., Novotny, J., Hubalek, J., Havel, L., Kizek, R. (2008). Multi instrumental analysis of tissues of sunflower plants treated with silver (I) ions. Plants as bioindicators of environmental pollution. Sensors 8., 445-463.
24. Metwally AAM (2000). Biochemical changes induced by heavy metal pollution in marine fishes at Khomse coast, Libya. Global Veterinaria 2 (6) : 308-311.
25. Casilas E, Meyers M and Ames W (1983). Relationship of serum chemistry values to liver and kidney histopathology in English sole (Parophrys vetulus) exposed to environmental contaminants. Arch. Environ. Contam. Toxicol., 20: 462-473.
26. Taofik OS, Olufemi DO, and Oyelola BO (2009). Anthracene-Induced Enzymatic changes as stress indicators in African catfish, Heterobranchus bidorsali Geoffroy saint hilaire (1809). Research Journal of Environmental Sciences., 3 :677-686.
27. Ringwood AH, Conners DE and Di Novo A (1998). The effects of copper exposures on cellular responses in Oysters. Marine Environmental Research 46: 591-595.
28. Gawel S, Wardes M, Niedworok E, Wardes P,Waid lek (2004). 57 9-10: 453-455
29. Prasanthi K and Rajini PS Toxicol in vitro (2005). 19: 449-456.
30. Adeogun AO and Chukwuka AV (2012). Altered reproduction in Clarias gariepinus exposed to industrial effluents. Am J. Agric. Biol. Sci. , 7 (1): 61-70.
31. Sarkar A, Ray D, Amulya N, Shrivastava W and Subhodeep S (2006). Molecular Biomarkers: Their significance and application in marine pollution monitoring. Ecotoxicology, 15 (4): 333-340

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