Internet Journal of Food Safety, Vol.13, 2011, p.134-141

Internet Journal of Food Safety

Lipid Peroxidation Levels and Antioxidant Enzymes Activities as Biomakers in Mangrove Periwinkle, *Tympanotonous fuscatus* in Agboyi Creek and Lekki Lagoon, Lagos State.

Omogoriola, H.O[1]* and L.O, Chukwu[2]

[1]*Marine biology section, Nigeria Institute of Oceanography and Marine Research (NIOMR), P.M.B 12729,*
[2]*Department of Marine Science, Faculty of Science, University of Lagos, Akoka , Lagos,Nigeria.*
3,Wilmot Point Road, Off Ahmedu Bello Way, Victoria Island, Lagos, Nigeria.

Abstract

Levels of lipid peroxidation measured as thiobarbituric acid reactive substance (TBARs) and activities of key antioxidant enzymes including superoxide dismutase, glutathione S-transferase, catalase were determined in Tympanotonus fuscatus collected from two aquatic environments, namely: AAC (Agboyi Creek) and CLL (Lekki Lagoon) in Lagos metropolis where the activities of pollutants and xenobiotics compounds may be high. The mean concentration of SOD, CAT, and GST in Typmpanotonus fuscatus were decreased by 30% (53.4138U/mol/mg protein), 56% (205.7500µmol/min/mg protein) and 5% (264.4838µmol/min/mg/protein) respectively in station AAC (Agboyi creek) compared with station CLL (Lekki Lagoon). LPx mean concentration was decreased by 60%(338.6437Pmol/min/mg protein) in AAC compared with that in CLL. Correlations between enzymes activities and oxidative stress markers were found, for example, an inverse correlation was seen between levels of thiobarbituric acid reactive substance and catalase activities in station CLL. This suggests that catalase plays an important role in the decomposition of hydrogen peroxidation (end products of lipid peroxidation) accumulated during oxidative stress that may have caused by redox cyclic chemicals, transition metals and other compounds. The reduction of their activities as observed in station AAC (Agboyi Creek) would suggest a lower capacity to avoid the activities of pro-oxidant and xenobiotic compounds .This in turn, should decreased the capability to prevent cellular damaged produced by reactive oxygen species (ROS). Also, in station AAC, low antioxidant enzymes response coupled with increases in lipid peroxidation was indicated. This also suggest that catalase and Superoxide dismutase are one of the most responsive biomarkers of oxidative stress.

Keywords: Periwinkle; *Tympanotonus fuscatus*; Antioxidant enzymes; Oxidative stress; Lipid peroxides; Thiobarbituric acid reactive substances.

Introduction

The most notable pollution point source arise from the dumping of untreated or partially treated sewage into the lagoon (Chukwu,2002), deposition of DDT, dyes and heavy metals from industrial effluent (Ajao and Fagade,1990 and Chukwu,2006) and discharge of biodegradable wood wastes from sawmills located along the lagoon (Chukwu and Okeowo,2006). Consequently, aquatic organisms are currently being exposed to multiple chemical contaminants from agricultural run-off, industrial and domestic liquid wastes and oil spillage with different mechanisms of toxicity, each contributing to a final overall adverse effect in aquatic environments. Consequently, in ecological quality monitoring programs, the integration of chemical data with biological

* Corresponding author. mailing address: Nigeria Institute for Oceanography and Marine Research. P.M.P 12729, No 3,Wilmot Point Road, Off Ahmedu Bello Way, Victoria Island, Lagos, Nigeria. Tel: +234-802-949-5588, E-mail: adehannah2002@yahoo.com

responses (biomarkers) is strongly recommended to characterize effects of contaminants to organisms.

At the biochemical level, biomarkers include studies on enhanced production of reactive oxygen species (ROS) as a general pathway of toxicity induced by many redox cycling chemicals (hydrocarbon quinones, nitro-aromatics, biphenyls, Livingstone,1991); transition metals, Stohs and Bagghi,1995), and many other compounds leading to a condition of oxidative stress. These ROS include superoxide anion radical(O_2^-), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical (OH^-).Under normal conditions, equilibrium exists between the amounts of free radicals generated and antioxidants available to quench or scavenge them, thereby protecting the organism against deleterious effects of pollutants. But cellular metabolism leads to the generation of free radicals causing oxidative damage to macromolecular components (Valavanidis et al; 2006). A suite of biochemical defense mechanisms called the antioxidant defense system is found in aquatic organisms to prevent cellular damage from ROS. Both enzymic and non-enzymic antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione (GSH), ascorbic acid (vitamin C), α-tocopherol (vitamin E) etc., counteract the deleterious action of ROS and protect from cellular and molecular damage (Livingstone,2001). The status of lipid peroxidation and antioxidants in an organism reflects the dynamic balance between the antioxidants defense and pro-oxidant conditions in animals, which serves as a useful index for assessing the risk of oxidative damage (Vijayavel,2006). In molluscs and other crustacean species, oxyradical production as a polluted-mediated mechanism of toxicity and induction of antioxidant enzyme defenses and lipid peroxidation have been observed in individuals exposed to many contaminants (i.e. copper, menadione and paraquat; Livingstone et al., 1990, Livingstone et al., 1992, Livingstone, 2001, Brouwner and Brouwner, 1998 and Correia et al., 2002). For instance, Livingstone measured elevated NADPH–cytochrome c reductase activity (NADPH–cyt c red) in digestive gland microsomes of mussel *Mytilus edulis* and periwinkle *Littorina littorea* exposed to 29 and 123 ppb diesel oil for 4–16 months under natural environmental conditions.

However, little work has been done on Periwinkle, *Tympanotonus fuscatus* in the assessment of environment using antioxidant enzymes and lipid peroxidation. In the view of this, SOD,GST and CAT activities were evaluated from Periwinkle *Tympanotonus fuscatus* on the whole body with reference to oxidative stress biomarker

Materials and Methods

Study Area. Two areas are selected for the purpose of these analyses. They are Agboyi Creek and Lekki Lagoon in Lagos State. Lagos state is situated with low-lying coastal zone which is dominated by creeks, lagoons and rivers. Agboyi creeks is located between Lagos and Ogun States in South-West Nigeria. Lekki Lagoon flows from Epe Lagoon and terminated at Lekki (Eti-osa LGA), in Lagos State (Fig 1).

Reagents. Trichloroacetic acid (TCA), thiobarbituric acid (TBA), ethylenediamine-tetraacetic acid (EDTA), sodium chloride (NACL), 1-chloro-2,4-dinitrobenzen (CDNB),reduced glutathione (GSH), ammonium chloride(NH_4CL), sodium carbonate (Na_2CO_3), ephrinephrine (R)-4-1-hydroxy-2-(methylamino)ethyl benzene-1,2-diol, KH_2PO4, NO_2HPO_4.

Experimental animals. The test organism is benthic macro invertebrate, Periwinkles (*Tympanotonus fuscatus*). Periwinkles were collected from Agoyi creek where anthropogenic and industrial activities were high and Lekki Lagoon in Lagos, Nigeria. The periwinkles were washed with water to remove excess mud and handpicked .They were acclimated to laboratory conditions with temperature $28\pm1^{O}C$ for a 1 week.

Periwinkle dissection and tissue processing. The specimens were weighed and measured and the whole tissue (soft part) of periwinkle were removed from the shell using dissecting knife/pin. They (pooled wet mass 0.29 – 0.809g) were homogenized at $4^{o}C$ in 1:9 wet wt./ buffer volume ratio in 100 mM phosphate buffer, pH 7.4 containing 100mM KCL and 1mM EDTA using glass homogenizer. homogenates were centrifuged at 6,000xg for 15 min and the supernatant were used directly as enzyme sources. Enzymatic measurement were conducted using Optima SP-3000 plus spectrophotometer .Assay were run at least in duplicate.

Lipid peroxidation analysis. Lipid peroxidation measured by the thiobarbituric reactive species (TBARs) assay, which measures the production of malondialdehyde (MDA) that reacts with thiobarbituric acid (Ohkawa et al.,1979). Absorption was measured at 535nm in a spectrophotometer and a molar extinction coefficient of $1.56x10^3M^{-1}CM^{-1}$ was used to determine the concentration of TBARs. 1Mm EDTA was added to a 0.5ml of the supernatant and was mixed with 1.0Ml cold 10%(M/V) trichloroacetic acid (TCA) to precipitate protein. The solution was mixed and centrifuged for 10mins at 5,000xg. The supernatants from the TCA extract were combined with the same volume of TBA and heated in boiling water for 15mins. Control sample contained water instead of supernatant. The

Assay of antioxidant enzyme activities. Catalase (CAT) activity was measured by the decrease in absorbance at 240nm due to H_20_2 consumption ($\varepsilon = 40M^{-1}cm^{-1}$) according to Aebi (1974). The reaction volume containing 50mM/0.1ml phosphate buffer at 7 pH , 0.2ml(200µl) of supernatant and 1.8ml of 30mM H_20_2 or 0.9ml of 30Mm H_20_2. Blank were run without supernatant or containing phosphate buffer .The CAT activity was expressed as micromoles of H_20_2 consumed $min^{-1}mg^{-1}$ protein. Superoxides dismutase (SOD) activity was measured by the inhibition of cytochrome c reduction. The reduction of cytochrome c by 0_2^- is monitored y the absorbance increase at 480nm. The reaction volume was 1ml and contained 50Mm Na_2CO_3 (buffer), pH 10.2, $0.005NHCL_3$, $3X10^{-4}$M Epinephrine (0.003) and 0.02ml of supernatant. One unit of SOD is defined as the amount of sample causing 50% inhibition of cytochrome c reduction. The results of this enzymatic assay are given in units of SOD activity per milligram of protein (mol-1min-1mg-1). Glutathione-S-transferase (GST) activity was determined according to the procedure of Habig et al.(1974). The formation of conjugate or adduct between GSH and CDNB was spectrophotometrical measured at 340nm. A reaction solution of 100mM or 1ml of phosphate buffer at pH 6.5 contained 1mM or 0.1ml of 1-chloro-2,4-dinitrobenzen, 1mM or 0.1ml of GSH, 1.7ml of distilled water and 0.1ml of supernatant. Black contained no CDNB. Absorbance was read for 5minutes at 1mm intervals. The GST activity was expressed as µmol of CDNB conjugated/min/mg protein.

Protein determination and statistics. Protein was measured by the method of Lowry et al (1951), using bovine serum albumin as standard. The result were expressed in mg of protein/ml. The statistical significance of differences between the two group was determine using Student's *'t'* test according to the method of Baily N.T.H (1959). Data are presented as means ± SD of eight animals per group and significance was tested at $p < 0.05$. Regression analysis was carried out to determined correlation coefficient (r^2) between concentration of lipid peroxidation and antioxidant enzymes.

Results

The mean concentration of SOD,CAT and GST in tissue of *T. fuscastus* in both stations (Agboyi Creek and Lekki Lagoon) were presented in table 1. The mean concentration of TBARS, SOD,CAT and GST in *T. fuscastus* were low in station AAC (Agboyi Creek) compared to station CLL (Lekki Lagoon) with the following values, 338.64Pmol/min/ mgprotein,53.41U/mgprotein, 205.75µmol/min/ mgprotein and 264.4875µmol/min/mgprotein respectively. Meanwhile, the mean concentration of TBARS was highest in both stations (Agboyi Creek and Lekki Lagoon) compared to antioxidants enzymes. No significance difference ($P > 0.05$) was observed for TBARS and antioxidant enzymes (SOD,CAT and GST) concentration for both stations (Agboyi Creek and Lekki Lagoon). Fig 2 presents the antioxidant enzyme activities of SOD, CAT and GST in tissue of *Tympanotonus fuscastus* in both stations (Agboyi Creek and Lekki Lagoon). The mean concentration of SOD, CAT and GST in the tissue of *Tympanotonus fuscastus* were decreased by 30%.56% and5% respectively in AAC (Agboyi Creek) compared with station CLL (Lekki Lagoon). Correlation analysis showed some relationships between Lipid peroxidation and antioxidant enzymes (Figure 3 & 4). TBARS levels correlated inversely with CAT and SOD activities and directly proportional with GST activities in both stations (Agboyi Creek and Lekki Lagoon).

Discussion

Basic cellular processes oxidizing enzymes and xenobiotic compounds can induce the production of ROS and oxidative tissue damage in aquatic animals (Punchard et al;1996, Fridovich,1974, Halliwell, 1978, Xia et al;1996, Washburn et al; 1988, Thomas and Wofford 1993 and Winston and Di Quilio,1991). Malondialdelyde (MDA) is a marker of membrane lipid peroxidation that results from interaction of ROS and cellular membrane (Aslan et al ; 1997). Membrane damage leads to the loss of cellular homeostatsis (Bradbury et al;1989). The cells of organisms possess a variety of chemical and enzymatic mechanisms to protect the oxidative damage, which include enzymatic oxidant defense system comprising the enzymes like SOD,CAT, GPX,GST and non- enzymatic antioxidant like glutathione, vitamine C and E which capable of neutralizing or scavenging the ROS (Diplock,1994 and Kale et al; 1999).

The observed increased in the lipid peroxidation activity in tissue of *Tympanotonus fuscatus* at the both stations (Agboyi Creek and Lekki Lagoon) (Table 1) might be due to the effect of ROS generated during microsomal metabolism of xenobiotic compounds present. Also, the increased level of detoxifying and antioxidant enzymes are described as response to environmental pollution. Laboratory exposures to copper have shown increased lipid peroxidation levels in digestive gland

tissue from Crassoostrea virginica (Ringwood et al.,1998 and Conner and Ringwood,2002). Also , there was significant increased in lipid peroxidation measured as TBARS for menadione endosulfan and Cu in the tissue of *Daphina magna* (Carlos et al;2005).

he mean concentration of SOD,CAT and GST in periwinkle (*Tympanotonus fuscatus*) were low (Table 1)and decreased by 30%.56% and 5% respectively (Fig. 2) in station AAC (Agboyi Creek) compared to station CLL (Lekki Lagoon). Among the enzymatic antioxidant defense mechanisms the first line of defense is initiated by SOD (Fridovich,1996). Since SOD is directly related to dismutation of superoxide (O_2^-) into hydrogen peroxidation (H_2O_2) and oxygen (O_2) while catalase (CAT) catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen within the cells. Reduction of their activities as observed in station AAC (Agoyi Creek) would suggest a lower capacity to avoid the activities of pro-oxidants and xenobiotic compounds. This in turn , should decrease the capability to prevent cellular damage produced by reactive oxygen species (ROS). GST is an important enzyme that catalyzes the conjugation of xenobiotics with the help of glutathione, thereby facilitating their elimination from cell organelles. The role of conjugation process in understanding the mechanism of toxicity is of key importance since it aids the removal of reactive electrophiles, protecting vital nucleophilic groups in macromolecules such as proteins and nucleic acids (Carro et al; 2004). Since glutathione plays a protective role, its depletion will enhance the toxicity of chemical which are normally detoxified by glutathione conjugation. For example, in toxicity of *Daphina* magna, Dier,CKX (1986) and LeBlanc and Cochrane (1985) reported that the prior exposure *Daphia magna* to CDNB will increase the susceptibility to Pentachlorophenate of CDNB. An algicide used in cooling water, is enhanced by previous depletion of GSH. The rise of CAT activity in both stations (Agboyi Creek and Lekki Lagoon) was probably responsible for the subsequent decrease in TBARS level. This is supported by a negative correlation (R^2 = 0.014 and 0.001 respectively) between TBARS and CAT activity in both stations (Agboyi Creek and Lekki Lagoon) (Fig 3 & 4) and probably reflects the importance of CAT in decomposition of aldehydic products of lipid peroxidation, such as the fatty acid, hydroperoxides, 4-hydroxy-2-nonenal and malondialdehyde as reported by (Hermes-Lima,2004).

Conclusion

Application of various complimentary biomarkers is a promising approach for assessing the health of aquatic animals. The biomarker approach coupled with ecotoxicity bioassays applied to species allows detection of early biological changes which may result in physiological disturbances in the long term. Thus, it is worthwhile studying biomarkers that predict and enhance the understanding of chemical toxicity.

References

Aebi,H . (1974). Catalase. In: H.U. Bergmeyer, Editor, Methods of enzymatic analysis, Academic Press, London. pp. 671–684.

Ajao,E.A and Fagade,S.O. (1990). The ecology of *Neritina glabrata* in Lagos Lagoon. Nigeria .*Arch hydrobio*.**119** (3): 339-350

Aslan, R., Sekeroglu,M.R., Gultekin .F and Bayiroglu, F. (1997).Blood lipid peroxidation and antioxidant enzymes in healthy individuals: relation to age sex habits, life style and environment, *J. Environ. Sci. Health* **32** (8) (1997), pp. 2102–2109.

Baily, N.T.J (1959).Statistical Methods in Biology, The English University Press, London, p. 200.

Bradbury, S., Steven.P and Coats,R.J. (1989) Comparative toxicology of pyrethroid insecticides, *Rev.Environ. Contam. Toxicol.* **108** pp. 133–177.

Brouwner .M and Brouwner T.H. (1998) Biochemical defense mechanisms against copper-induced oxidative damage in the blue crab, *Callinectes sapidus*, *Arch. Biochem. Biophys.* **351** pp. 257–264.

Cairrao,E., Couderchet,M., Soares,A.M.V.M and Guilhermino, L.(2004)Glutathione-*S*-transferase activity of *Fucus* spp. as a biomarker of environmental contamination, *Aquat. Toxicol.* **70** pp. 277–286.

Chukwu.L.O (2002). Histophysciological indices of osmotic stress in the *Euryhaline teleost.Elops lacerta* (Val) adapted to different salinity regimes . *Journal of*

Science Technology and Environment Vol.**2**(2): 1-

Chukwu.L.O. (2002). Physicology-hemical characterization of pollutant load of treated industrial effluents in Lagos metropolis. *Journal of Industrial Pollution Control* Vol.**22**(1):147-153.

Chukwu.L.O and Okeowo,O.H. (2006).Piscicidal properties of aqueous and ethanolic bark extracts of floating timber logs in a tropical open lagoon. Ecology, *Environment and Conservation* Vol. **12**(1):37-41.

Correia,A.D., Livingstone ,D.R and Costa,M.H. (2002).Effects of water-born copper on metallothionein and lipid peroxidation in the marine amphipod *Gammarus lacusta*, *Mar.Environ. Res.* **54** pp. 357–360.

Fridovich,I. (1997). Superoxide anion radical (O_2) superoxide dismutase and related matters, *J. Biol. Chem.* **272** pp. 515–517.

Habig,W., Pabst.M.J and Jacob, W.B.(1974)Glutathione-*S*-transferase. The first enzymatic step in mercaptoric acid formation, *J. Biol. Chem.* **249** pp. 7130–7139.

Hermes-Lima, M. (2004). Oxygen in biology and biochemistry: Role of free radicals. In: K.B. Storey, Editor, *Functional metabolism: Regulation and adaptation*, Wiley-Liss, Hoboken, pp. 319–368.

Kale,M., Rathore,N., John .S and Bhatnagar,D. (1999). Lipidperoxidative damage on pyrethroid exposure and alteration in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species, *Toxicol. Lett.* **105** (3) pp. 850–852.

Livingstone, D.R. (1991). Organic xenobiotic metabolism in marine invertebrates. In: R. Gilles, Editor, *Advances in comparative and environmental physiology*, Springer-Verlag, Berlin, pp. 45–185.

Livingstone, D.R. (2001). Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms, *Mar. Pollut. Bull.* **42** (2001), pp. 656–666.

Lowry,O.H ,Rosebrough, N.J ,Farr.N.J and Randall, R.J(1951).Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* **93** pp. 265–275.

Stohs S.J and Bagghi D. (1995).Oxidative mechanisms in the toxicity of metal ions, *Free Radic. Biol. Med.* **18** , pp. 321–336.

Swann, J.D., Smith, M.W., Phelps,P.C., Maki,A., Berezesky I.K and Trump,B.F. (1991) Oxidative injury induced influx dependent changes in intracellular calcium homeostasis, *Toxicol. Pathol.* **19** pp. 128–137.

Thomas,P. and Wofford,H.W. (1993). Effects of cadmium and Aroclor 1254 on lipid peroxidation,glutathione peroxidase activity, and selected antioxdiants in Atlantic croacker tissues. *Aquat. Toxicol.**27:159 – 178.***

Valavanidis, A., Vahogianni,T., Dassenakis , M and Scoullos,M. (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants, *Ecotoxicol. Environ. Safety* **64**, pp. 178–189.

Vijayavel, K., Gopalakrishnan, S., Thilagam .H and Balasubramanian,M.P (2006). Dietary ascorbic acid and -tocopherol mitigates oxidative stress induced by copper in the thorn fish *Terapon jarbua*, *Sci. Tot. Environ.* **372** pp. 157–163.

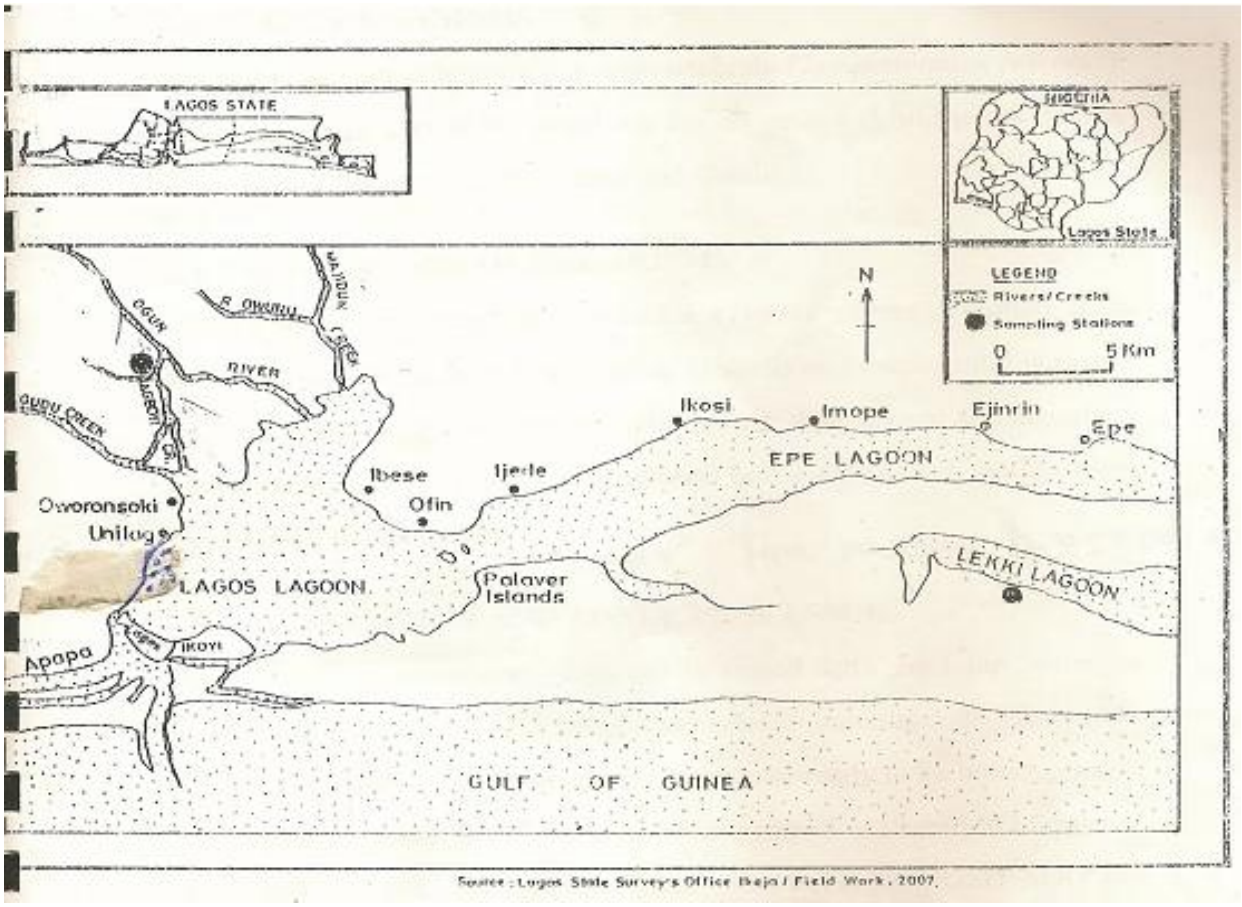

Fig 1: Map of Lagos State showing the Lekki Lagoon and Agboyi Creek as sampling stations (•)

Table 1. Mean concentration of TBARS, SOD, CAT and GST in tissue of *Tympanotonus fuscatus* for station AAC and CLL.

Parameters	Station AAC	Station CLL
Lipid peroxidation (P mol of MDA released/mg protein)	338.59±216.69	856.94± 758.22
Superoxides dismutase (U/mg protein)	53.41 ± 39.22	76.87 ± 67.92
Catalase (µmol of H_2O_2 consumed/min/mg protein	205.75 ± 166.17	467.45 ± 187.94
Glutathione-S-transferase (µmol of CDNB conjugated/min/mg protein)	264.48 ± 115.61	278.89 ± 156.05

Data are means ± SD; n = 8. *P < 0.05*.

Percentage decreased of station AAC on CLL: SOD(30%), CAT(56%), GST(5%).

Fig. 2: Antioxidant enzyme activities: Superoxides dismutase(SOD), Catalase(CAT) and glutathione-S-transerase(GST) in station AAC and CLL.

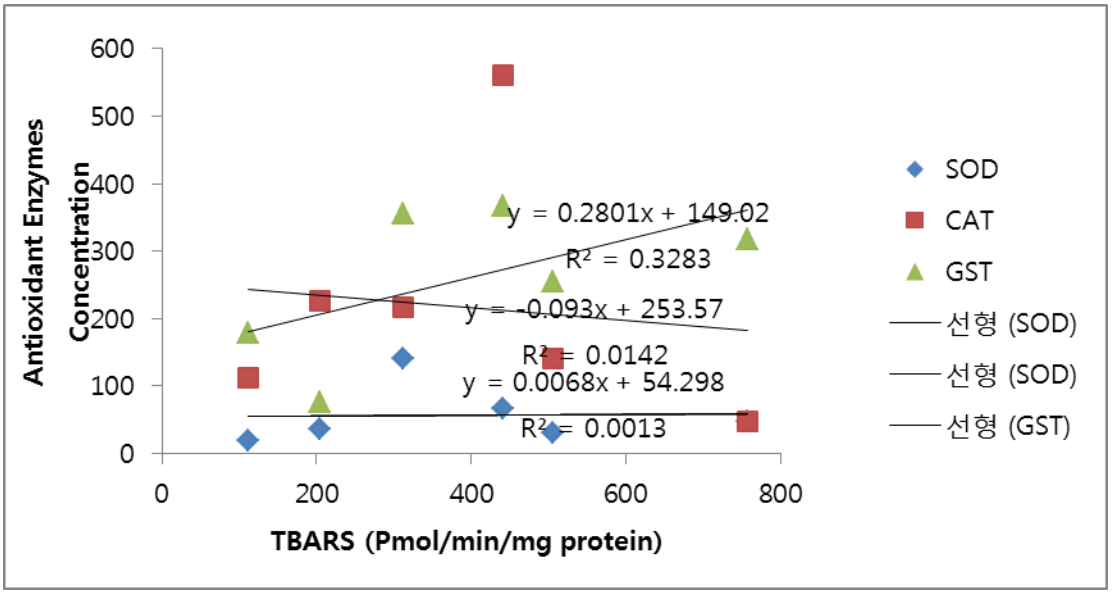

Fig.3: Regression curve between TBARS and Antioxidant enzymes at station AAC.

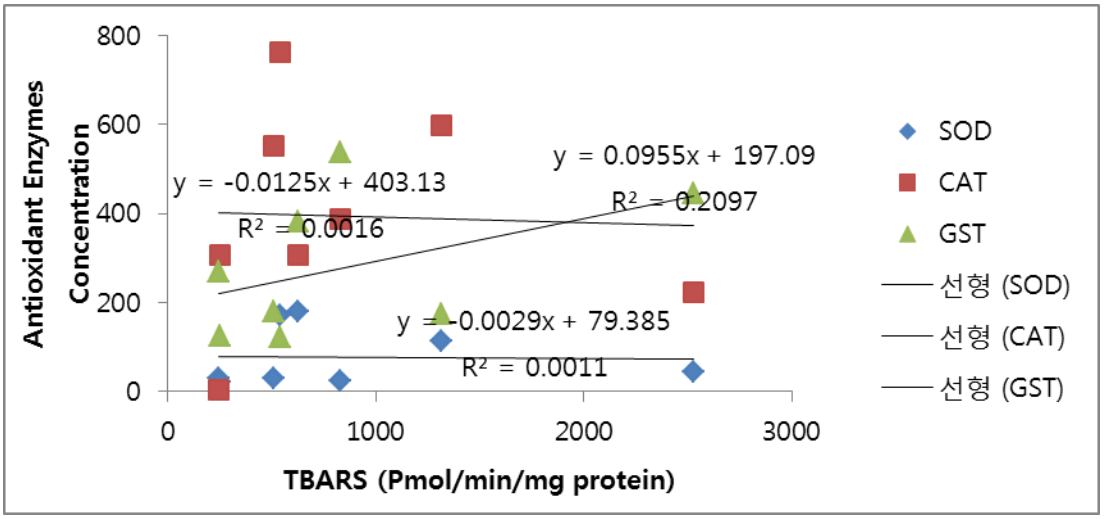

Fig.4: Regression curve between TBARS and Antioxidant enzymes at station CLL.