American-Eurasian J. Agric. & Environ. Sci., 7 (3): 359-365, 2010
ISSN 1818-6769
© IDOSI Publications, 2010

Biomarkers of Oxidative Stress and Heavy Metal Levels as Indicators of Environmental Pollution in Some Selected Fishes in Lagos, Nigeria

[1]V.F. Doherty, [1]O.O. Ogunkuade and [1]U.C. Kanife

[1]Department of Biological Sciences, Yaba College of Technology, P.M.B. 2011 Yaba, Lagos, Nigeria

Abstract: Levels of zinc, lead and copper in the flesh of catfish: *Clarias gariepinus, Chrysichthys nigrodigitatus* and Tilapia: Oreochromis *niloticus, Tilapia guinensis,* were determined using Bulk Atomic Absorption Spectrophotometer. *Clarias gariepinus* and *Oreochromis niloticus* were collected from a Fish farm in Ayobo, Lagos Nigeria, which was considered as reference site. *Tilapia guinensis* and *Chrysichthys nigrodigitatus* were collected from the Lagos Lagoon. The activities of superoxide dismutase (SOD), glutathione (GSH) concentration and Malondialdehyde (MDA) formation were also determined. Heavy metals were accumulated in the flesh of the fish to varying extent. The trend of accumulation of the metals is as follows: Catfish-Cu>Pb>Zn: Tilapia-Cu>Pb>Zn. The concentration of heavy metal in the flesh of the fish from the Lagos Lagoon is higher than that from the reference site for all the metals analyzed. SOD concentration in the liver of the *Tilapia guinensis* increased by 12.3% when compared to *Oreochromis niloticus*, the SOD concentration in the gills of *Oreochromis niloticus* increased by 26.22% when compared to Tilapia guinensis. GSH levels increased in the liver and gills of *Tilapia guinensis* and *Chrysichthys nigrodigitatus* by 17.82% and 30.14% respectively and in the gills, GSH increased by 8.6% and 12.64% in *Tilapia guinensis* and *Chrysichthys nigrodigitatus,* respectively. MDA levels were increased in the liver of *Tilapia guinensis* by 15.22% and *Chrysichthys nigrodigitatus* by 12.6%. In the gills, MDA increased by 13.1% in *Tilapia guinensis* and 6.98% in *Chrysichthys nigrodigitatus*. The result demonstrates that alteration in the antioxidant enzymes and induction of lipid peroxidation reflects the presence of heavy metals which may cause oxidative stress in *Tilapia guinensis* and *Chrysichthys nigrodigitatus* from the Lagos Lagoon. The study therefore provides a rational use of biomarkers of oxidative stress in biomonitoring of aquatic pollution.

Key words:

INTRODUCTION

The development of biological monitoring based on fish offers the possibility of checking water pollution with past responses on low concentration of direct acting toxicant [1]. Fish are excellent subjects for the study of various effects of contaminants present in water samples since they can metabolize, concentrate and store water borne pollutants.

Biomarkers for water pollution are early diagnostic tools for biological effect measurement and environmental quality assessment [2].

The presence of metal pollutant both in fresh and marine water has been found to disturb the delicate balance of the aquatic ecosystem [3]. Fish are notorious for their ability to concentrate metals in their body tissues and since they play important role in human nutrition, they need to be screened to ensure that unnecessarily high level of some toxic metals are not being transferred to man through fishes [4].

Many industrial and agricultural processes have contributed to the contamination of fresh water systems therefore causing adverse effects on aquatic biota and human health [5]. The fact that heavy metals cannot be destroyed through biological degradation and have the ability to accumulate in the environment make these toxicants deleterious to the aquatic environment and consequently to humans who depend on aquatic animals and such, tissues concentrations of heavy metals can be of public health concern to both animals and humans [6].

Water bodies in Nigeria have been reported to be polluted principally due to the discharge of untreated wastes into Rivers by many Industries [7]. Fish are largely being u sed for the assessment of the quality of aquatic

Corresponding Author: V.F. Doherty, Department of Biological Sciences,
Yaba College of Technology, P.M.B. 2011. Yaba, Lagos, Nigeria

environment and as such, can serve as bioindicators of environmental pollution [8]. Heavy metal accumulated in the tissues of fish may catalyze reactions that generate reactive oxygen species (ROS) which may lead to environmental oxidative stress.

African catfish and tilapia are of great commercial importance because they are the most common fish widely consumed in Nigeria [9]. The fishes therefore are good models to study responses to various environmental contaminants. This study reports the levels of Cu, Pb, Zn in the liver and gills of catfish , tilapia and aquatic environmental stress using antioxidant enzyme system and malondialdehyde formation as surrogate biomarkers of aquatic pollution in Nigeria Lagos Lagoon catfish and tilapia.

MATERIALS AND METHODS

Fish: *Tilapia guineensis* and *Chrysichthys nigrodigitatus* were caught from Lagos Lagoon in Lagos and it was transported to the laboratory in ice cold containers on the same day. The fish samples were kept alive for at least 24hrs to minimize stress. *Clarias gariepinus* and *Oreochromis niloticus* were used as control and they were collected from a fish farm in Ayobo, Lagos, Nigeria. The fish farm is devoid of any other facilities that could cause pollution and thus affect the biochemical control responses of the control. Twelve fish samples were collected for the experiment.

Method for Homogenizing Sample: The fishes were dissected and the liver and gills were removed, the flesh of the fish were also cut. The post mitochondria fraction of the organs of the fish was prepared according to Habbu *et al*. [10]. The organs of the fish were washed in an ice cold 1.15% KCL solution, blotted and weighed. They were then homogenized with 0.15%M of KCL before putting the organs each into the mortar laboratory sand was added to it (acid washed sand) and it was blended in the mortar with pestle together. The resulting homogenate was centrifuge at 2500 rpm speed for 15mins then it was removed from the centrifuge after the 15mins and the supernatant was decanted and stored -20°C until analysis [10].

Assay of Glutathione (Gsh) Peroxidase: Glutathione peroxidase (GSH reduced) was determined using 5-5 Dithio-bis 2-Nitrobenzoic acid (DTNB) and Tris-EDTA buffer with the absorbance being read at 412nm. 100µl sample was added to 1ml of 0.2ml of Tris-EDTA buffer at 8.2 pH. 0.9 ml of 20 mM EDTA at pH 4.7 was added, 20 µl of 10 mM DTNB was added and the sample was allowed to incubate at room temperature for 30 mins. The mixture was centrifuged and absorbance of the supernatant was read at 412nm.Blank reagent used was 100µl distilled water + 1ml Tris –EDTA buffer + 0.9 EDTA+ 20µl DTNB [11].

Assay of Malondialdehyde (MDA): 1.0ml of the sample was combined with 2.0ml of TCA-TBA-HCL and was mixed thoroughly. The solution was heated for 15min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000g for 10mins. The absorbance of the sample was determined at 535nm against a blank that contains all the reagents minus the sample. The MDA concentration of the sample was calculated using extinction co-efficient of 1.56×10^{-5} M^{-1} CM^{-1}. Assay of malondialdehyde was determined according to Soon *et al*. [12].

Assay of Superoxide Dismutase Activity (SOD): Superoxide dismutase activity was determined by measuring the inhibition of auto-oxidation of epinephrine at PH 10.2 at 30°C as described Magwere *et al*. [13]. One unit of SOD activity is the amount of SOD necessary to cause 50% inhibition of epinephrine auto-oxidation. The assay was performed in 3.0ml of 50m M Na_2CO_3 buffer to which 0.02mlof the sample was added. 0.03ml of the epinephrine stock solution was then added to the above before taking absorbance readings at 480nm for 3-5mins. A blank devoid of the sample (but having all the reagents) was used for background correction.

Determination of Heavy Metals in the Flesh of the Fish: The levels of lead, zinc and copper were determined in the flesh of the fish using, 2000 series Bulk Scientific Atomic Absorption Spectrophotometer as described by Farounbi *et al*. [14]. The flesh of the fish was dried at 80°C in an electric oven for a period of 36hrs, before it was pulverized in a clean dry mortar. The pulverized fish was again dried for another one hour and finally preserved in a clean dry polyethylene bottle. 5g of each sample was accurately weighed with an analytical balance into a platinum crucible and placed in a muffle furnace, it was then ashed at 600°C for 3hrs.It was removed and allowed to cool in a desiccator 5ml of 6N HCL was added and left for 30mins. (For proper digestion to take place). It was then filtered into 100ml conical flask using a filter paper and the filtrate was made up to 50ml with distilled water. The heavy metals were determined using AAS, 2000 Series Bulk Atomic Absorption Spectrophotometer,

with a hollow cathode lamp and was fueled with a flame can acetylene. The samples were aspirated and mean signal responses were recorded with each of the element and their respective wave length. The concentration of each metal was calculated as follow

Total Protein Estimation: The protein content was estimated by the method of Lowry *et al*. [15]. 1gm of the sample was weighed and homogenize with 20ml of 0.5M NaOH. The homogenate was poured into a centrifuge tube and centrifuge at 1000rpm for 10minutes. The supernatant was collected in a test 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 allowed to stand at room temperature for 10minutes. 0.05cm^3 of dilute Folin-ciocalteau reagent was added to each tube and mixed immediately to give a blue colour. The absorbance was read at 750nm against a reagent blank.

RESULTS

Anova method was used to compare the experimental groups, when ($p<0.05$), there is a significant difference but when ($p>0.05$), there is no significance difference. Table 1 above shows the levels of heavy metal in the flesh of *Clarias gariepinus,Oreochromis niloticus, Tilapia guineensis* and *Chrysichthys nigrodigitatus* from lagos. Heavy metals were accumulated in the flesh of the fishes to varying extent. The trend of accumulation of the metals is as follows: Catfish-Cu>Pb>Zn: Tilapia-Cu>Pb>Zn. The order of concentration in the flesh of the fish is as follows: Zinc-Tilapia>Catfish; Lead-Tilapia>Catfish; Copper-Catfish>Tilapia.

The concentration of Copper, Lead and Zinc in flesh of *Clarias gariepinus* and *Chrysichthys nigrodigitatus* is shown in Table 1, the level at which Zinc accumulates in the two fishes is low compare to the other two metals. Copper was more accumulated in the flesh of the wild fishes compare to the reared one.

Catfish shows the highest level of accumulation for Copper while Tilapia showed the highest level for Lead. In adition, the level of metal in the flesh of the fishes From the wild is higher compare to the reared ones. The percentage at which Zinc increase in *Chrysichtys nigrodigitatus* flesh compare to *Clarias gariepinus* is

Table 1: Result of Percentage Change in the Level of Heavy Metals in the Flesh of the Fishes

Heavy Metal		Catfish Flesh	Tilapia Flesh
Zn (ppm)	Test sample	0.335±0.0071	0.435±0.0354
	Control	0.26±0.071	0.205±0.0212
	% Change	12.6	35.94
	P-value	0.2741	0.0157
Pb (ppm)	Test sample	0.395±0.0636	0.55±0.0707
	Control	0.145±0.0636	0.205±0.0071
	% Change	35.94	45.7
	P-value	0.0591	0.0206
Cu (ppm)	Test sample	3.91±0.0141	1.8±0.5657
	Control	0.405±0.0636	0.2±0.0424
	% Change	81.22	80
	P-value	0.0002	0.0575

Table 2: Result of Antioxidant and Enzymatic Activities

Sample I.D Catfish	SOD	GSH	MDA
(*Clarias gariepinus*)			
L1	20.87	4.45	18.65
L2	24.83	3.84	17.12
G1	27.94	6.26	25.58
G2	27.14	6.11	24.42
(*Chrysichtys nigrodigitatus)*			
L1	30.17	8.29	24.03
L2	34.06	8.82	27.12
G1	20.00	8.07	29.42
G2	21.84	7.16	32.31
Tilapia			
(*Oreochromis niloticus)*			
L1	15.15	2.79	10.19
L2	15.94	3.09	11.73
G1	13.00	3.24	15.96
G2	13.55	2.94	13.65
(*Tilapia guineensis*)			
L1	19.86	4.00	13.85
L2	20.06	3.69	14.81
G1	7.60	3.85	20.77
G2	7.69	3.09	18.46

L1- Liver for fish (1)
L2- Liver for fish (2)
G1- Gill for fish (1)
G2- Gill for fish (2)

Table 3: Result of Percentage Difference in Enzymatic Activities of Catfish; (*Clarias gariepinus* and *Chrysychthys nigrodigitatus)*

Catfish: Farm (reared) and Lagoon (wild) Sample I.D	%SOD	%GSH	%MDA
REARED L1	40.89	34.93	43.7
WILD L1	59.11	65.07	56.3
%Change	18.22	30.14	12.6
REARED L2	42.13	30.33	38.7
WILD L2	57.87	69.67	61.3
%Change	15.74	39.34	22.6
REARED G1	58.28	43.68	46.51
WILD G1	41.72	56.32	53.49
%Change	16.56	12.64	6.98
REARED G2	55.41	46.04	43.05
WILD G2	44.59	53.96	56.95
%Change	10.82	7.92	13.9

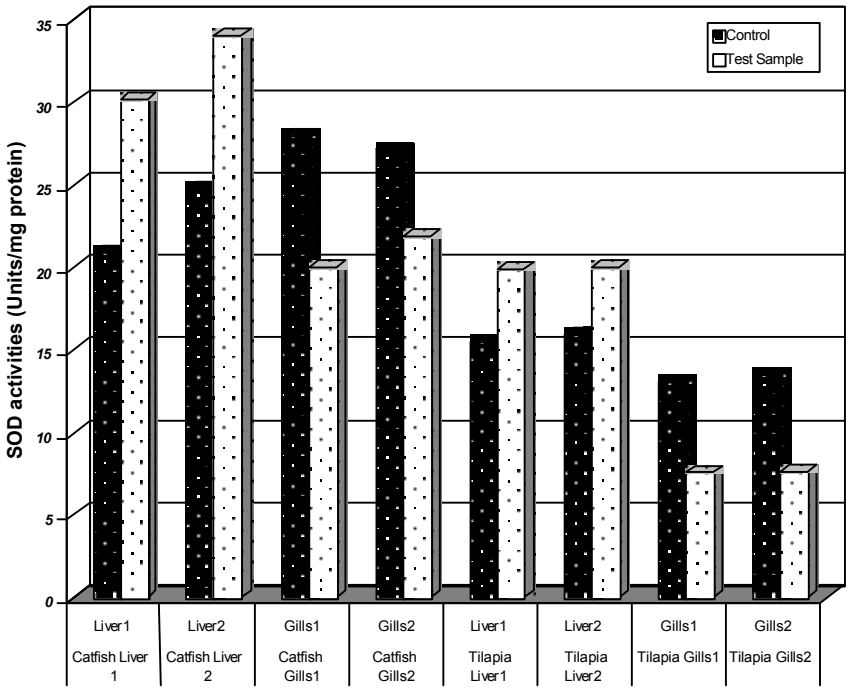

Fig. 1: Sod Concentration

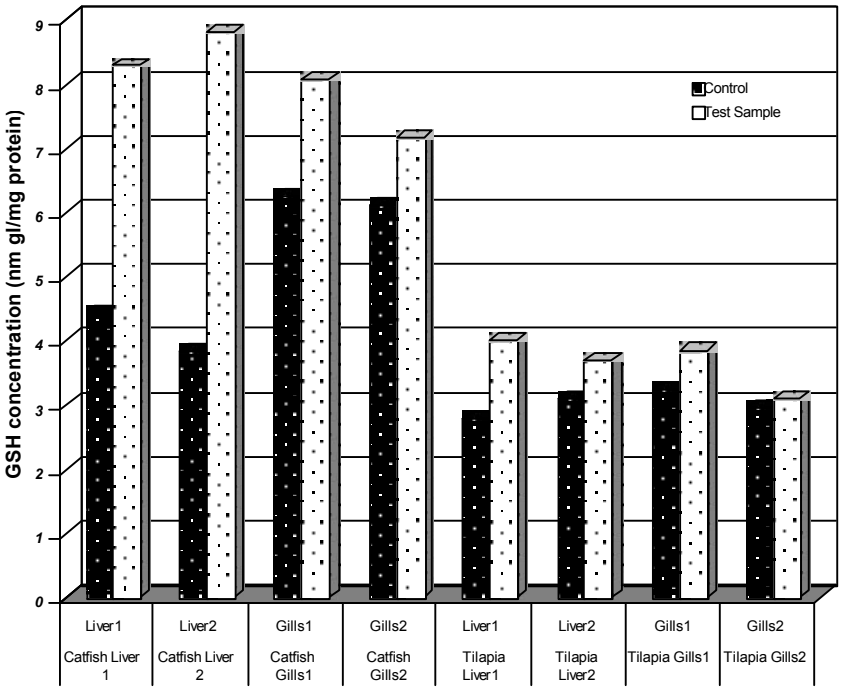

Fig. 2: Gsh Concentration

12.6%, for Lead, it is by 35.94% while for copper which has the highest percentage is 81.22%. The percentage at which Zinc increase in the flesh of *Tilapia guineensis* compare to *Oreochromis niloticus* is 35.94%, for Lead, it is 45.7% while for copper it is 80%.

The activities of antioxidant enzymes in the Liver and Gills of *Chrysichthys nigrodigitatus* (1and 2) and *Tilapia guineensis* (1 and 2) from the Lagoon (Lagos) are presented in Tables 2,3 and 4. In Figure 1.the SOD concentration in the liver of *Tilapia guineensis* (1)

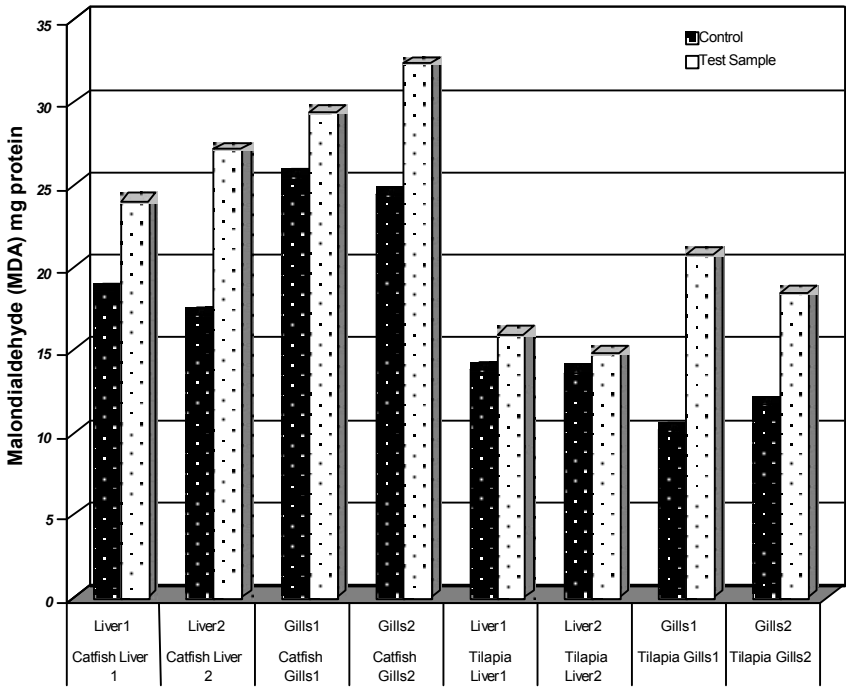

Fig. 3: Mda Concentration

Table 4: Result of Percentage Difference in Enzymatic Activities of Reared and Wild Tilapia (*Oreochrois niloticus* and *Tilapia guineensis)*

Tilapia Reared and Wild	SOD	GSH	MDA
REARED L1	43.85	41.09	42.39
WILD L1	56.15	58.91	57.61
%Change	12.30	17.82	15.22
REARED L2	44.28	45.58	44.20
WILD L2	55.72	54.42	55.80
%Change	11.44	8.84	11.60
REARED G1	63.11	45.70	43.45
WILD G1	36.89	54.30	56.55
%Change	26.22	8.60	13.10
REARED G2	63.79	48.76	42.51
WILD G2	36.21	51.24	57.49
%Change	27.58	2.48	14.98

increased by 12.3% compare to the *Oreochromis niloticus* (1) from Ayobo (control), while the SOD concentration in the liver of the *Chrysichthys nigrodigitatus* (1) from the lagoon increase by 18.22% compare to the *Clarias gariepinus* (1) from Ayobo (control). The SOD concentration in the liver of *Tilapia guineensis* (2) increased by 11.44% compare to the *Oreochromis niloticus* (2) from Ayobo (control), while the SOD concentration in the liver of the *Chrysichthys nigrodigitatus* (2) from the lagoon increase by 15.74% compare to the *Clarias gariepinus* (2) from Ayobo (control). For the gills, the SOD concentration in *Oreochromis niloticus* (1) increase by 26.22% compare to the *Tilapia guineensis* (1) from the Lagoon, while for *Oreochromis niloticus* (2) the SOD concentration increase by 27.58%. The SOD concentration in the gills of *Clarias gariepinus* (1) increase by 16.56 compare to the *Chrysichthys nigrodigitatus* (1) while *Clarias gariepinus* (2) increase by 10.82% compare to the *Chrysichthys nigrodigitatus* (2).

Glutathione Level: Figure 2 shows the levels of GSH in the liver and gills of *Chrysichthys nigrodigitatus* (1and 2) and *Tilapia guineensis* (1 and 2).GSH levels in the liver of *Tilapia guinensis* (1) increased by 17.82% compare to the *Oreochromis niloticus* (1) from Ayobo (control), while the GSH concentration in the liver of the *Chrysichthys nigrodigitatus* (1) from the lagoon increase by 30.14% compare to the *Clarias gariepinus* (1) from Ayobo (control). The GSH concentration in the liver of *Tilapia guineensis* (2) increased by 8.84% compare to the *Oreochromis niloticus* (2) from Ayobo (control), while the GSH concentration in the liver of the *Chrysichthys nigrodigitatus* (2) from the lagoon increase by 39.54% compare to the *Clarias gariepinus* (2) from Ayobo (control). For the gills, the GSH concentration in *Tilapia guineensis* (1) increase by 8.6% compare to the *Oreochromis niloticus* (1) from the Lagoon, while for *Tilapia guineensis* (2) the GSH concentration increase

by 2.48%. The GSH concentration in the gills of *Chrysichthys nigrodigitatus* (1) increase by 12.64 compare to the *Clarias gariepinus* (1) while *Chrysichthys nigrodigitatus (2)* increase by 7.92% compare to the *Clarias gariepinus (2)*.

MDA Level: The level of MDA formation in the liver and gills of the fishes are shown in Figure 3. MDA levels in the liver of *Tilapia guineensis* (1) increased by 15.22% compare to the *Oreochromis niloticus (1)* from Ayobo (control), while the MDA concentration in the liver of the *Chrysychthys nigrodigitatus(1)* from the lagoon increase by 12.6% compare to the *Clarias gariepinus(1)* from Ayobo (control). The MDA concentration in the liver of *Tilapia guineensis* (2) increased by 11.6% compare to the *Oreochromis niloticus(2)* from Ayobo (control), while the MDA concentration in the liver of the *Chrysichthys nigrodigitatus(2)* from the lagoon increase by 22.6% compare to the *Clarias gariepinus(2)* from Ayobo (control). For the gills, the MDA concentration in *Tilapia guineensis* (1) increase by 13.1% compare to the *Oreochromis niloticus (1)* while for *Tilapia guineensis* (2) the MDA concentration increase by 14.98%. The MDA concentration in the gills of *Chrysichthys nigrodigitatus(1)* increase by 6.98% compare to the *Clarias gariepinus(1)* while for *Chrysichthys nigrodigitatus(2)* MDA concentration increase by 13.9% compare to the *clarias gariepinus(2)*.

DISCUSSION

The levels of heavy metals and certain biomarkers of oxidative stress were evaluated in *Clarias gariepinus, Chrysichthys nigrodigitatus, Tilapia guineensis* and *Oreochromis niloticus* from Lagos. From this result, copper and lead were highly accumulated in the flesh of *Chrysichthys nigrodigitatus* and *Tilapia guineensis* from the Lagoon, the high level of accumulation for copper in *Chrysichthys nigrodigitatus* suggests that either the Lagoon has high concentration of these metals or the fish species have poor digestive mechanism for digesting and eliminating this metal and may provide good monitoring levels of copper pollution [16].

The *Chrysichthys nigrodigitatus* value for copper (3.92ppm) and lead (0.44ppm) are above the statutory limit set by WHO and FAO (0.5ppm and 0.29ppm respectively). This suggest that the water is not safe for consumption. The concentration of zinc analyzed showed that *Chrysichthys nigrodigitatus* (0.33ppm) and *Tilapia guineensis* (0.41ppm) are within the confine value set by WHO/FAO (5.0ppm), which makes it safe for consumption.

This result indicates a significant elevation of lipid peroxidation in all the organs, this is in agreement with the findings of Olaifa *et al*. [9] in which the level of metals and certain biomarker of oxidative stress were elevated in *Clarias gariepinus* from the Ogun River. The apparent increase in lipid peroxidation may be attributed to the accumulation of the heavy metals in the flesh of the fish, as the data indicates significant concentration of heavy metals in the flesh of the fishes. Metal catalyzed formation of ROS capable of damaging tissues such as DNA, protein and lipids is well documented [17].

Furthermore, the activities of SOD and the redox sensitive thiol compound GSH were elevated in the liver and for SOD, it was reduced in the gills. The accumulation of heavy metals might have led to the production of superoxide radical to H_2O_2. SOD catalytically scavenges superoxide radical which appears to be an important agent of toxicity of oxygen and this provides a defense against this aspect of oxygen toxicity [18]. GSH is known to be a substrate for the activity of GST. The apparent increase in GSH levels in the organs suggests an adaptive and protective role of this biomolecule against oxidative stress induced by the heavy metals. This result is in agreement with the findings of Pandey *et al*. [19] on *wallago attu* fish from the Panipal River in India. The decreased levels of antioxidant enzymes in the gills could account for the marked lipid peroxidation observed. The gills are more exposed to contaminated water and as such, metal can penetrate through their thin epithelial cells [20].

Under acute oxidative stress, the toxic effect of the pollutants may overwhelm the antioxidant defense [21]. Furthermore, the apparent decrease in detoxification system in the gills, the first point of contact with environmental xenobiotics indicates that this system is a sensitive biochemical indicator of environmental pollution in the fishes [22].

The high level in MDA of the fishes from the Lagoon compared with the relatively unpolluted farm suggests that the Lagoon is polluted.

REFERENCES

1. Poele, C.L. and J.J.T. Strik, 1975. Sublethal effects of toxic chemicals on aquatic animals, In: J.T. Koeman and J.J.T.W.A. Strick, (eds), Elsevier, Amsterdam, pp: 81-91.
2. Cajaraville, M.P., M.J. Bebianno, J. Blasco, C. Porte, C. Sarasquete and A. Viarengo, 2000. The use of biomarkers to assess the impact of Pollution in coastal environment of the *Iberian Peninsula* A practical approach. Sci. Total. Environ., 247: 295-311.

3. Asaolu, S.S. and O. Olaife, 2005. Biomagnification's of some heavy and essential metals in sediment, fishes and crayfish from Ondo state coastal region, Nig. Pak. J. Sei. Ind. Res., 48: 96-102.
4. Kakulu, S.E. and O. Osibanjo, 1988. Trace heavy metal pollutional status in sediments of Niger Delta area. Nig. J. Chem. Soc., 13: 9-11.
5. Wang, W.X., 2002. Interaction of trace metals and different marine food chain. Mar. Ecol. Prog. Ser., 243: 295-309.
6. Kalay, M. and A.Y.P. Canil, 1999. Heavy metal concentration in fish tissues from the northeast Meditereansea. Bull Environ. Contam. Toxicl., 63: 673-671.
7. Allinor, I.J., 2005. Assessment of elemental contaminants in water and fish Samples from Aba River. Environmental Monitoring Assesment, 102: 15-25.
8. Lopes, P.A, T. Pinheiro, M.C. Santos, Da Luz M. Mathias, M.J. Collares-Pereira and A.M. Viegas-Crespo, 2001. Response of antioxidant enzymes in freshwater fish populations (*Leuciscus alburnoides* complex) to inorganic pollutants exposure. Sci. Total. Environ., 280: 63-1.
9. Olaifa, F.G., A.K. Olaifa and T.E. Onwude, 2004. Lethal and sublethal effects of copper to the African cat fish (*clarias gariepinus*) African J. Biomed. Res., 7: 65-70.
10. Habbu, P.V., R.A. Shastry, K.M. Mahadevan, Hanumanthachar Joshi and S.K. Das, 2008. Hepatoprotective and antioxidant effects of *Argyreia speciosa* in rats. African J. Traditional Complementary and Alternative Medicines, 5(2): 158-164.
11. Jollow, D.J., J.R. Michelle, N. Zampaglione and J.R. Gillete, 1974. Bromobenzene induced liver necrosis protective role of GSH and evidence for 3,4- Bromobenzene oxideas the Hepatotoxic metabolite. Pharmacol., 11: 151-169.
12. Soon, Y.Y. and B.K.H. Tan, 2002. Evaluation of the hypoglycaemic and antioxidant activity of *Morinda officinalis* in streptozotocin induced diabetic rats. Singapore Med. J., 43(2): 077-085.
13. Magwere, T., Y.S. Naiks and J.A. Hasler, 1997. Effect of chloroquine treatment on antioxidant enzymes in rat liver and kidney Free. Rad. Biol. Med., 22: 321-327.
14. Farounbi, E.O., O.A. Adelowo and Y.R. Ajimoko, 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias garienpinus)* from Nigeria ogun river.
15. Lowry, O.H., N.M. Rosenbrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin phenol reagent. J. Biol. Chem., pp: 193-265.
16. Vinikour, W.S., R.M. Goldstein and R.V. Anderson, 1980. Bioconcentration patterns of zn, cu, cd and pb in selected fish species from the toxic river, Illinois. Bull Environ. Contam. Toxicol., 25: 727-734.
17. McCord, J.M., 1996. Effects of positive iron status at a cellular level. Nutr. Rev., 19(54): 8-85.
18. Kadar, E., V. Costa and R.S. Santos, 2005. Distribution of microessential (Fe,Cu ,Zn) and toxic (Hg) metals in tissues of two nutritionally distinct hydrothermal shrimps. Sci. Total Environ., pp: 65-70.
19. Pandey, S., S. Parvez, I. Sayeed, R. Haque and B. Bin-Hafeez Raisuddin, 2003. Biomarkers of oxidative stress: A comparative study of river Yamuna fish Wallago attu (BI. and Schn.). Sci. Total. Environ., 309: 15-105.
20. Nwaedozie, J.M., 1998. The determination of heavy metal pollutants in fish samples from River Kaduna. J. Chem. Soc. Nigeria, 23: 21-23.
21. Bebianno, M.J., F. Geret, P. Hoarau,' M.A. Serafim, M.R. Coelho, M. Gnassia-Barelli and M. Romeo, 2004. Biomarkers in *Ruditapes decusstus*: a potential bioindicator species. Biomarkers, 9: 30-305.
22. Kono Y. and L. Fridovich, 1982. Superoxide radical inhibits catalase. J. Biol., 257: 4-5751.