Research Journal of Environmental and Earth Sciences 4(7): 738-746, 2012
ISSN: 2041-0492

Submitted: May 30, 2012 Accepted: June 29, 2012 Published: July 25, 2012

Long-Term Exposure to Industrial Effluent Induces Oxidative Stress and Affects Growth in *Clarias gariepinus*

[1]Aina O. Adeogun, [1]Ilelabayo M. Ogidan, [2]Oju R. Ibor, [2]Azubuike V. Chukwuka,
[3]Isaac A. Adedara and [3]Ebenezer O. Farombi
[1]Hydrobiology and Fisheries Research Unit,
[2]Ecology and Environmental Biology Unit, Department of Zoology, University of Ibadan, Nigeria
[3]Molecular Toxicology and Drug Metabolism Research Unit, Department of Biochemistry,
College of Medicine, University of Ibadan, Nigeria

Abstract: The study investigated the effects of chronic exposures to binary mixtures of industrial effluents on growth and biomarkers of oxidative stress in *Clarias gariepinus*. Concentrations 7.67, 3.83, 2.56 and 1.92% which were fractions of a preliminary 96 h LC_{50} and control were used in the static/renewal bioassay. Physicochemical parameters (Temperature, pH, DO) of the test media were measured and the activities of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione (GSH) and Malondialdehyde (MDA) formation in the various tissues (kidney, liver and gills) of fish were evaluated. Physicochemical parameters of exposure concentrations ranged from 27.10±1.05-31.00±2.30°C, 5.96±0.09-7.16±0.02, 0.42±0.03-2.11±0.42 mg/L indicating low dissolved oxygen and slightly acidic pH. Exposed fish had poor condition factor (0.74±0.04) compared with control fish (1.25±0.03). Significant increases ($p<0.05$) from control fish were recorded in the activities SOD and CAT in the liver and gills, while the kidney showed no significant increase across all exposure concentrations. Glutathione activity (GSH) increased significantly ($p<0.05$) from control fish in the liver, while the kidney and gills showed no significant increase across all exposure concentrations. Malondialdehyde (MDA) levels were significantly elevated ($p<0.05$) in the kidney of exposed fish, while the gills and liver showed no significant increase across all exposure concentrations. Our results indicated that exposure of fish to industrial effluents induced oxidative stress and were reflected in significant reduction of body weight and poor CF of exposed fish; ultimately these may have significant effects on the sustainability of fish population in polluted aquatic systems.

Keywords: *Clarias gariepinus*, condition factor, industrial effluents, oxidative stress, pollution

INTRODUCTION

The assessment of alterations in key enzymatic activities of organisms following exposure to polluted waters has been one of the major uses of biomarkers in environmental studies (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; Monterio *et al*., 2007; Miller *et al*., 2007; Farombi *et al*., 2007; Pavlović *et al*., 2010). Doherty *et al*. (2010) noted that fish species are suitable candidates for the assessment of biomarkers of oxidative stress induced by pollutants because they play a dual role of being on top of the aquatic chain as vertebrates and respond strongly to stress conditions. Oxidative stress results from an imbalance between the activities of pro-oxidants over antioxidants and might lead to oxidative damage (Kohen and Nyska, 2002). Sensitive and reliable biomarkers are therefore used to determine if there is evidence of significant contaminant exposures that have exceeded detoxification or compensatory mechanisms and are resulting in adverse effects on physiological and biochemical functions (Vijayavel *et al*., 2004). Cellular biomarkers represent early diagnostic tools since they can identify changes at sub-organismal level (i.e., cellular and molecular, etc.) before becoming evident at higher levels of biological organisation (Doherty *et al*., 2010). Stress-induced reduction in growth and fish health (condition) are also well-recognized toxic effects in fish and effects on growth are expected when organisms are exposed to chemical pollutants, if the exposure is sufficient to produce a stress response (Wendelaar, 1997). The unregulated discharge of industrial wastewater into inland waterways in developing countries like Nigeria (Adeogun *et al*., 2011) highlight the importance of evaluating growth response and oxidative stress in commercially important fish species. The African mud catfish (*Clarias gariepinus*) is one of such important fish species and is a widely consumed freshwater fish in Nigeria (Fagade, 1998). This study was therefore aimed at evaluating the effects of chronic exposure to

Corresponding Author: Aina O. Adeogun, Hydrobiology and Fisheries Research Unit, Department of Zoology, University of Ibadan, Nigeria, Tel.: +2348055261105

composite mixtures of food and beverage industry effluents on the activity of antioxidant defense enzymes: Superoxide Dimustase (SOD), Catalase (CAT), Gluthathione (GSH) and Malondialdehyde (MDA) formation in the liver, kidney and gills and growth (condition) of *Clarias gariepinus*.

MATERIALS AND METHODS

Sources and acclimatization of fish: Fingerlings of *C. gariepinus* with an average weight of 4.20±0.06 g were collected from a private fish farm in Ibadan Nigeria, transported to the laboratory in oxygenated waterproof bags and kept in outdoor holding tanks in the Department of Zoology, University of Ibadan, Ibadan, Nigeria. These tanks were filled with dechlorinated tap water and fish were kept for 7 days to allow them acclimatize to environmental conditions. Holding medium was changed every three days. Fish were fed daily with 40% crude protein diet at 3% body weight and uneaten food was siphoned out regularly to prevent accumulation of metabolites.

Collection of effluents: Whole unfiltered effluents collected from the points of discharge of a food and beverage industry were taken as stock solutions. Required volumes were measured into graduated plastic containers and made up to the required concentration according to the methods described by Reish and Oshida (1987) for toxicity evaluation.

Chronic exposures: Nominal fractions of a 96 hr LC_{50} value from exposures to predetermined ratios 3:1 (v/v) of a food industry and beverage industry effluent gave concentrations of 7.67, 3.83, 2.56 and 1.92%, respectively. Exposure concentrations and control exposures (0.00%) devoid of effluent mixtures were used in a six month static/renewal bioassay from February - July, 2011. Ten outdoor tanks measuring 1.14×0.80×0.3 m (LBH) each was used for the chronic exposure and each tank was stocked with 100 fingerlings. Exposure media was renewed every 72 h to maintain the requisite concentrations (OECD, 2002).

Physico-chemical analysis: Physico-chemical parameters such as pH, temperature and Dissolved Oxygen (DO) were measured bi-weekly with a digital CS-C933T Electrochemistry multimeter (Topac Instrument, Inc., USA).

Growth analysis: Subsamples of 12 fish were randomly selected and total length and body weight of fish was measured weekly for the duration of the study. The total length and body weight were measured with the Absolute digital caliper (Trensa Instruments, Inc.) and an Ohaun Compact digital weighing balance (Mettler Instruments) respectively.

Condition factor: The condition factor (k) of fish was calculated using the formula: $k = 100\ W/L^3$ where W = Weight (g); L = Total Length (cm) (Fulton, 1902)

Biochemical assay: At the end of the exposure period, 5 fish samples were randomly selected from all exposure concentrations including the control to investigate the effects of industrial effluents on biomarkers of oxidative stress in fish. Fish were sacrificed by medullar transection (Lucky, 1977) and dissected within 3 min on ice. The liver, kidney and gills were quickly removed and the post-mitochondria fraction was prepared as follows: The kidney, liver and gills were washed in ice-cold 1.15% KCl solution, blotted and weighed. They were then homogenized in 4 volumes of homogenizing buffer (50 nM Tris – HCl mixed with 1.15% KCl and the pH adjusted to 7.4), using Teflon homogenizer. The resulting homogenate was centrifuged at 10,000 g for 20 min in a Beckman L5-50B centrifuge at 0-4°C. The resulting supernatant was decanted and stored -20°C until further analysis.

Total protein estimation: The total protein content of the various fractions was estimated by the method of Lowry *et al*. (1951), using bovine serum albumin as standard.

Assay for antioxidant enzymes: Superoxide Dismutase (SOD) activity was determined by measuring the inhibition of autoxidation of epinephrine at pH 10.2 (30ºC) as described by Magwere *et al*. (1997). One unit of SOD activity is the amount of SOD necessary to cause 50% inhibition of epinephrine autooxidation. Activity of Catalase (CAT) was determined according to the procedure of Clairborne (1995) following the absorbance of hydrogen peroxide at 240 nm, pH 7.0 and 25ºC.

Assay of reduced Glutathione (GSH): Reduced Glutathione (GSH) was determined in the 10,000 g supernatant fraction of the liver, kidney and gill homogenates of *C. gariepinus* according to methods described by Jollow *et al*. (1974), using 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) and Tris-EDTA buffer with the absorbance being read at 412 nm.

Determination of Malondialdehyde (MDA): Lipid peroxidation was determined by measuring the Thiobarbituric Acid Reacting Substances (TBARS) as

described by Farombi *et al*. (2000). Malondialdehyde (MDA) was quantitated by using extinction co-efficient of $\sum = 1.56 \times 10^5 M^{-1} cm^{-1}$ (Buege and Aust, 1978).

Statistical analysis: All data were presented as mean±S.D (standard deviation). One way ANOVA was used to test for significant difference between means (SPSS 17) and differences in means were considered significant when $p<0.05$.

RESULTS

Physico-chemical parameters: The mean values obtained for physico-chemical parameters (temperature, DO and pH) of exposure concentrations and composite mixtures of effluents compared with National Environmental Standards Regulations and Enforcement Agency (NESREA) are presented in Table 1. Temperature values were 27.10±1.05ºC in exposure concentrations and was higher (31.00±2.30ºC) in effluent samples. DO values were very low (2.11±0.42 mg/L) in exposure concentrations compared to NESREA standards but much lower values were recorded (0.42±0.03 mg/L) in effluent samples. The effluent was characterized by slightly acidic pH (5.96±0.09) while higher values (7.16±0.02) were recorded in exposure concentrations.

Growth evaluation: Exposed fish in the 7.67, 3.83, 2.56 and 1.92% concentrations were 13.15, 9.98, 6.19 and 1.94%, respectively shorter in length compared with the control (0.00%) fish. Significant decrease ($p<0.05$) in length from control fish was recorded compared to fish in other exposure concentrations (Table 2). Exposed fish were also 36.57, 27.15, 16.89 and 10.39% lighter in weight than the control fish (0.00%) and a significant increase ($p<0.05$) was recorded in the body weight of control fish compared to other exposure concentrations (Table 2).

Condition factor: The initial and final Condition Factor (CF) of *C. gariepinus* exposed to composite mixtures of food and beverage industry effluents are presented in Table 2. Evident in this result is a relatively better condition factor (1.25±0.03) in the control fish compared to exposed fish across all concentrations and a significant decrease ($p<0.05$) in fish condition (poor CF) in all exposure concentrations (0.74±0.04–0.81±0.04) compared to the control (1.25±0.03).

Biochemical assays:

Superoxide Dismutase activity (SOD): The activity of SOD was significantly higher ($p<0.05$) in the gills and liver of exposed fish, while the kidney showed no increase across exposure concentrations compared to the control (Fig. 1).

Catalase activity (CAT): There was a significant increase ($p<0.05$) in CAT activity in the liver and gills of exposed fish, while the kidney showed no significant increase across all exposure concentrations compared to the control (Fig. 2).

Glutathione activity (GSH): The activity of GSH was significantly higher ($p<0.05$) in the liver, while the gills and kidney showed no significant increase across other exposure concentrations compared to the control (Fig. 3).

Lipid peroxidation (MDA): MDA activity was significantly higher ($p<0.05$) in the kidney of exposed fish while the liver and gills showed no significant increase across all exposure concentrations compared to the control (Fig. 4).

Table 1: Physicochemical parameters of industrial effluents and exposure concentrations compared with NESREA standards

Parameters	Exposure concentrations	Levels in composite mixtures of effluents	NESREA permissible limit
Temperature (ºC)	27.10±1.05	31.00±2.30	40.00
pH	7.16±0.02	5.96±0.09	6.00-9.00
Dissolved Oxygen (mg/L)	2.11±0.42	0.42±0.03	4.00

NESREA: National Environmental Standards and Regulations Enforcement Agency

Table 2: Body weight, Total length and Condition Factor (CF) of *C. gariepinus* exposed to industrial effluents

Concentrations (%)	Initial total length(cm)	Final total length(cm)	Initial body weight (g)	Final body weight (g)	Initial condition factor	Final condition factor
0.00	9.54±0.92^{a}	35.06±2.34^{a}	5.64±1.69^{a}	539.01±52.27^{a}	0.64±0.06^{a}	1.25±0.03^{a}
1.92	9.58±0.69^{a}	33.40±3.31^{b}	5.57±1.98^{a}	302.59±82.50^{b}	0.63±0.07^{a}	0.81±0.04^{b}
2.56	9.59±0.95^{a}	31.95±2.23^{bc}	5.67±1.53^{a}	251.32±57.11^{bc}	0.64±0.07^{a}	0.77±0.03^{b}
3.83	9.57±0.78^{a}	30.66±2.74^{cd}	5.66±1.63^{a}	220.83±61.15^{cd}	0.64±0.08^{a}	0.76±0.04^{b}
7.67	9.66±0.92^{a}	29.58±1.72^{d}	5.73±1.37^{a}	191.56±41.68^{d}	0.63±0.06^{a}	0.74±0.04^{b}

Values with the same superscript along the same column are not significantly different ($p>0.05$)

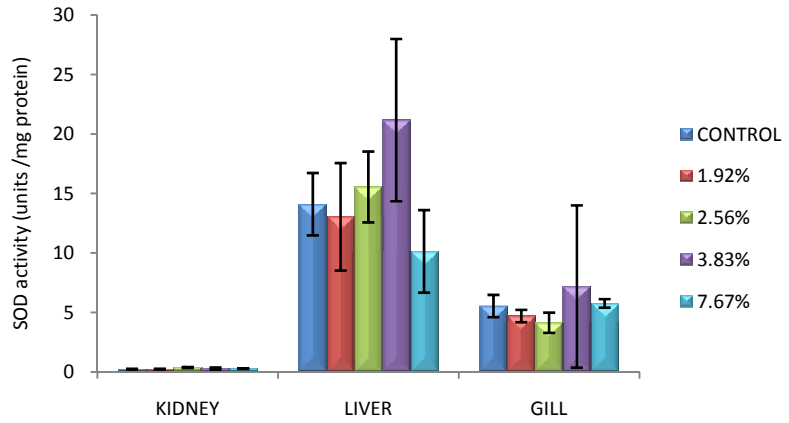

Fig. 1: The activity of SOD in the kidney, liver and gills of *Clarias gariepinus* exposed to industrial effluents

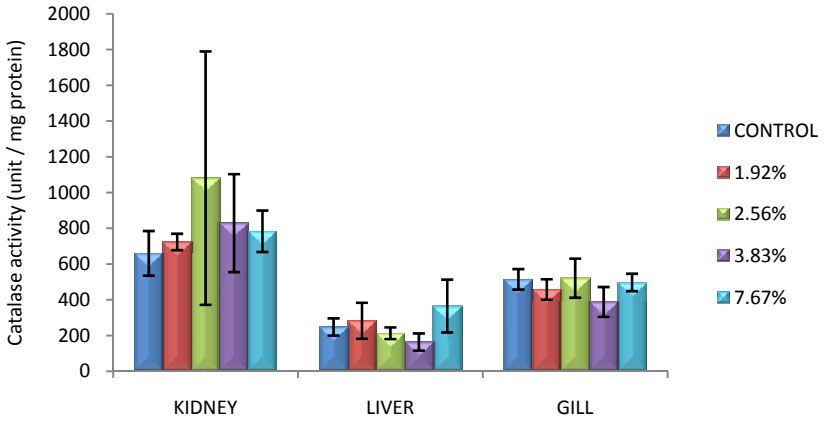

Fig. 2: The activity of catalase (CAT) in the kidney, liver and gills of *Clarias gariepinus* exposed to industrial effluents

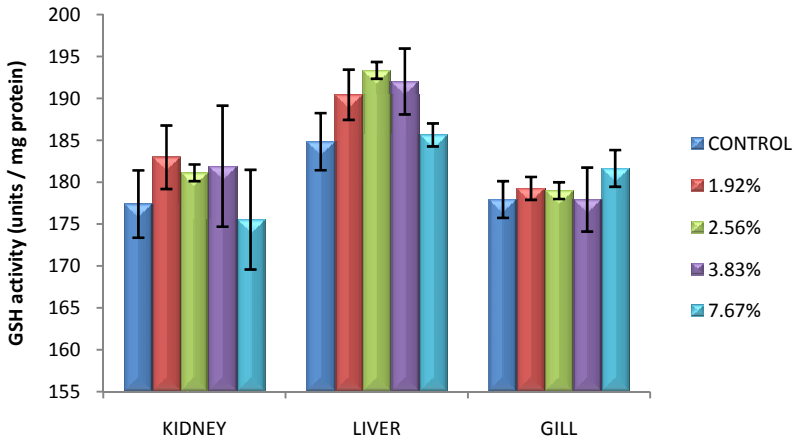

Fig. 3: The activity of reduced glutathione (GSH) in the kidney, liver and gills of *Clarias gariepinus* exposed to industrial effluents

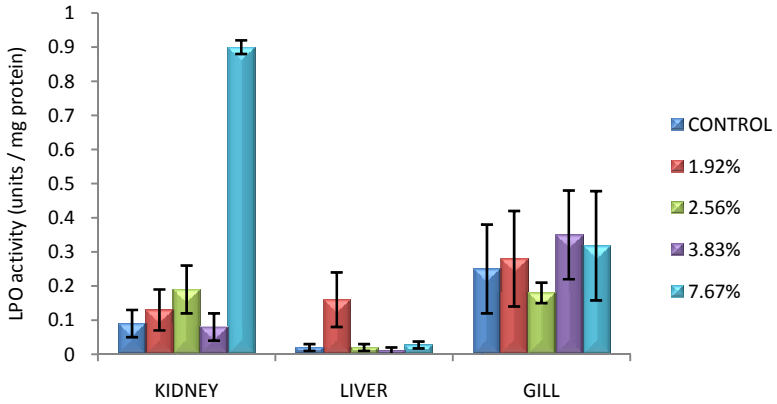

Fig. 4: The levels of lipid peroxidation (LPO) in the kidney, liver and gills of *Clarias gariepinus* exposed to industrial effluents

DISCUSSION

Growth, development and physiological state of an organism are key factors in determining species sustainability, survival and availability. Consequently, these factors are all susceptible to the effects of xenobiotic compounds (pollutants) at all stages of an organism life cycle (Adeogun and Chukwuka, 2012). Effects of pollutants at higher levels such as population, community and ecological systems are usually preceded by changes in earlier biological process viz; molecular and cellular events, which includes lipid peroxidation, antioxidant status, mixed function oxidase system and a more general physiological index such as growth (Vijayavel *et al*., 2004; Zhu *et al*., 2008).

The low levels of DO in effluent mixture and exposure concentrations compared to NESREA (2011) standards could be due to the high levels of nutrients, organic loads and total solids content of effluents from these industries and may have grave implications for the survival of aquatic organisms. Adeogun and Chukwuka (2012) reported high levels of lead, chromium and iron in effluent samples of the industries used in this study and concluded that the high concentration of iron in effluent sample resulted in a decrease in DO concentrations of effluent samples and exposure concentration. The slightly acidic pH in effluent mixture may be attributed to mineral acids such as hydrochloric, nitric, phosphorus and sulphuric acid which are essential reagents or raw materials in many industries. Aquatic organisms are affected by pH because most of their activities are pH dependent (Wang *et al*., 2002) and acidic pH have been reported in Nigeria for some industrial effluents (Fakayode, 2005; Adeogun *et al*., 2011).

It is evident that continuous exposure of *C. gariepinus* to composite mixtures of these effluents inhibited fish growth (body weight and length) in a time-and-dose-dependent manner, with an overall significant effect being exerted by as little as the lowest concentration i.e., 1.92%. The significant reduction in growth observed in exposed fish compared to the control in this study may be attributed to the presence of pollutants for example heavy metals in the industrial effluents (Zhu *et al*., 2008). Pavlović *et al*. (2010) reported that the response of fish to a variety of metal and organic pollutants are transient and are dependent on the species, enzymes and single or mixed contaminants. This may be due to the fact that under oxidative stress, the toxic effects of pollutants may overwhelm the antioxidant defenses of fish; hence the energy budget for growth, reproduction, development and other biological activities is used to overcome stressful environmental conditions. Some authors have reported that the induction of oxidative stress have an inhibitory effect on fish growth as animals inhabiting chronically polluted environments can develop some adaptations or compensatory mechanisms (Wendelaar, 1997; Zhu *et al*., 2008; Almedia *et al*., 2009; Pavlović *et al*., 2010).

In fish, Condition Factor (CF) is a reflection of the physiological state in relation to its wellbeing. Fish in the untreated (control) exposure were in better condition than those in exposure concentrations. The poor Condition Factor (CF) recorded in exposed fish may be attributed to physiological stress due to physical and chemical conditions of the test media. Nwabueze and Ekelemu (2011) reported a similar condition factor of 0.9 in exposed and 1.3 in untreated control *C. gariepinus* fingerlings exposed to different concentrations of domestic leachates and concluded that exposure of fish to leachates may reduce the growth and survival in a time and dose dependent manner.

One of the features of antioxidant enzymes is their induction under conditions of oxidative stress and such

inductions are important adaptation to pollutant-induced stress in organisms generally (Livingstone, 2001; Li *et al*., 2007). SOD is a group of metalloenzymes that play crucial antioxidant role and constitute a defense system against natural and chemical pollutants by catalyzing the dismutation of the highly reactive superoxide anion radical (O_2^-) which is an important agent of oxygen toxicity to the less reactive species H_2O_2 (Ozmen, 2005). CAT is a peroxisomal haemoprotein that catalyzes the removal of H_20_2 formed during the reaction catalyzed by SOD. The significant increase ($p<0.05$) in the activity of SOD and CAT observed in the gills and liver was not surprising considering the low level of dissolved oxygen, acidic pH and high temperature recorded in the industrial effluent. These increases may be attributed to the presence of heavy metals (lead, chromium and iron) reported earlier in the effluent used (Adeogun and Chukwuka, 2012). It is well known that heavy metals induce the formation of free radicals within an organism leading to oxidative stress and if prolonged may result in oxidative damage (Farombi *et al*., 2007; Doherty *et al*., 2010). Increase in the activity of CAT and SOD is usually observed in the presence of environmental pollutants (McCord, 1996; Dautremepuits *et al*., 2004) since SOD-CAT system represents the first line of defense against oxidative stress. Elevated levels of antioxidant defense enzyme systems (SOD and CAT) may be due to the fact that under oxidative stress, the toxic effects of pollutants may trigger the production of antioxidant defenses to overcome stressful conditions generated by such pollutants (Bebianno *et al*., 2004). Yildirin *et al*. (2011) reported that stressful conditions lead to the formation of excessive free radicals which are major internal threat to cellular homeostasis of aerobic organisms. Also the significantly ($p<0.05$) elevated levels in the activities of SOD and CAT in the gills may be attributed to the fact that the gills are in direct contact with the contaminated medium (water) and have the thinnest epithelium of all the organs, as such, metal ions can penetrate through their thin epithelial cells inducing more effect on this organ compared to other organs (Nwadozie, 1998; Farombi *et al*., 2007). Also, the gills are the main site of gas exchange and other important functions such as ionic and osmotic regulation takes place in this organ (Bebianno *et al*., 2004). The significant increase ($p<0.05$) in SOD and CAT activities in the liver probably reflects the fact that the liver is the most important target organ and center for detoxification of metabolites. Our findings also indicate a positive relationship in SOD and CAT activities in the liver and gills, which may reflect a reinforced response to oxidative stress in these two organs.

The usefulness of glutathione in fish for evaluating the detoxification of xenobiotics has been reported by Rodriguez-Ariza *et al*. (1993), Hasspielar *et al*. (1994), Shams and Ahmad (2011), Sahan *et al*. (2010) and Pavlović *et al*. (2010). Glutathione (GSH) is a metabolizing phase 11 enzyme involved in the biotransformation of xenobiotics (Ferrari *et al*., 2007) and as a redox sensitive thiol compound, GSH has a protective role against noxious chemicals and is known to be a substrate for the activity of GST (Kohen and Nyska, 2002). Glutathione is responsible for the regulation of intracellular levels of lipid peroxidation and also act as a reactant in conjugation with electrophilic substances, therefore a change in GSH level may be a very important indicator of the detoxification ability of an organism (Vijayavel *et al*., 2004). In addition to being a necessary cofactor for GST activity, GSH is an effective protectant capable of detoxifying oxyradicals (Ross, 1988). Evidence from various pathological and toxicological conditions such as chemical induced oxidative injury, aging and degenerative disease indicate that GSH is a primary component of the protection system of cells against oxidative and free radical damage (Kohen and Nyska, 2002). The apparent significant increase ($p<0.05$) in GSH levels in the liver suggests an adaptive and protective role of this biomolecule against oxidative stress induced by pollutants. Some authors have reported similar findings in fish from polluted waters. For example, Di Giulio *et al*. (1993) reported high levels of GSH in catfish exposed to polluted waters compared with control and Pandey *et al*. (2003) observed an increase in GSH activity in *Wallago attu* fish from the Panipal River in India. Sahan *et al*. (2010) also reported higher hepatic glutathione concentration (GSH) in *Cyprinus caprio* inhabiting the polluted Ceyhan river in Turkey.

Lipid peroxidation is one of the major mechanisms involved in oxidative cell injury and an increase in Malondialdehyde (MDA) level is frequently observed during oxidative stress and has generally been used as a marker of oxidative damage (Yildirin *et al*., 2011). It is also a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content can be related to degradation of an environment due to poor water quality (Charissou *et al*., 2004). The significant increase in lipid oxidation (MDA) may indicate the susceptibility of lipid molecules to reactive oxygen species and the extent of oxidative damage imposed on these molecules. The observed significant increase in MDA activity in the kidney could be attributed to high antioxidant (CAT, SOD and GSH) activity recorded in this study. This is in line with reports (Farombi *et al*., 2007; Pavlović *et al*, 2010,

Doherty *et al*., 2010) that when antioxidants defenses are impaired or overcome, oxidative stress may exert effects on biomolecules like proteins, lipids and DNA. This may apparently explain the significant increase in the MDA activity recorded in this study.

CONCLUSION

Our findings indicate that exposure of *Clarias gariepinus* to industrial effluents induced oxidative stress and was reflected in a direct physiological effect resulting in significant reduction of exposed fish growth and poor fish condition. The use of antioxidant defense systems (SOD, CAT and GSH), biomarker (MDA) and a physiological index (growth) for detecting pollution stress in fish can be suggested as a valuable biological indicator not only because of its sensitivity to environmental contamination but also due to its ability to further shed more light on understanding why small sized fish and decline in fish population structure and dynamics has characterized landings by artisanal fisheries in recent times. Further investigations encompassing multispecies population and wider bio-geographical ranges are currently on-going as this is important for meeting the larger objectives of improved yield in fish culture and harvesting techniques.

REFERENCES

Achuba, F.I. and S.A. Osakwe, 2003. Petroleum induced free radical toxicity in African catfish (*Clarias gariepinus*). Fish Physiol. Biochem., 29(2): 97-103.

Adeogun, A.O. and A.V. Chukwuka, 2012. Altered reproduction in *Clarias gariepinus* exposed to industrial effluents. Am. J. Agric. Biol. Sci., 7(1): 61-70.

Adeogun, A.O., A.V. Chukwuka and O.R. Ibor, 2011. Impact of abattoir and saw-mill effluents on water quality of upper Ogun River (Abeokuta). Am. J. Environ. Sci., 7(6): 525-530.

Almedia, J.A., R.E. Barreto, L.B. Novelli, F.J. Castro and S.E. Moron, 2009. Oxidative stress biomarkers and aggressive behavior in fish exposed to aquatic cadmium contamination. Neotrop. Ichthyol., 7(1): 103-108.

Bebianno, M.J., F. Geret, P. Hoarau, M.A. Serafim, M.R. Coelho, M. Gnassia-Barelli and M. Romeo, 2004. Biomarkers in ruditapes decussatus: A potential bioindicator species. Biomarkers, 9(4-5): 300-305.

Buege, J.A. and S.D. Aust, 1978. Microsomal lipid peroxidation. Methods Enzymol., 52: 302-310.

Charissou, A.M., C. Cossu-Leguille and P. Vasseur, 2004. Relationship between two oxidative stress biomarkers, malondialdehyde and 8-oxo-7, 8-dihydro-2'-deoxyguanosine, in the freshwater bivalve Unio tumidus. Sci. Total Environ., 322(1-3): 109-122.

Clairborne, A., 1995. CRC Handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, pp: 447, ISBN: 0849329361.

Dautremepuits, C.S., S. Paris-palacios, S. Betoulle and G. Vernet, 2004. Modulation in hepatic and head kidney parameters of carp (*Cyprinus carpio* L.) induced by copper and chitosan. Toxicol. Pharmacol., 137(4): 325-333.

Di Giulio, R.T., C. Habig and E.P. Gallagher, 1993. Effects of black river harbor sediments on indices of biotransformation, oxidative stress and DNA integrity in channel catfish. Aqua. Toxicol., 26: 1-22.

Doherty, V.F., O.O. Ogunkuade and U.C. Kanife, 2010. Biomarkers of oxidative stress as indicators of Environmental pollution in some selected fishes in Lagos, Nigeria. American-Eurasian J. Agric. Environ. Sci., 7(3): 359-365.

Fagade, S.O., 1998. Credit in fisheries development. Symposium Fisheries Society of Nigeria, 5: 10.

Fakayode, S.O., 2005. Impact Assessment of Industrial Effluent on Water Quality of the Receiving Alaro River in Ibadan, Nigeria. Department of Chemistry and Biochemistry, Baylor University, Ajeam-Range, 10: 1-13, Retrieved from: http://www.ajeam-ragee.org/vol10p1t13.htm.

Farombi, E.O., O.A. Adelowo and Y.R. Ajimoo, 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.

Farombi, E.O., J.G. Tahnteng, O.A. Agboola, J.O. Nwankwo, J.O. and G.O. Emerole, 2000. Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron--a Garcinia kola seed extract. Food Chem. Toxicol., 38(6): 535-541.

Ferrari, A., A. Venturino and A.M Pechen de Angelo, 2007. Effects of carbaryl and azinphos methyl on juvenile rainbow trout (*Oncorhynchus mykiss*) detoxifyingenzymes. Pest. Biochem. Physio., 88(2): 134-142.

Fulton, T.W., 1902. Rates of Growth of Sea-Fishes. Neill and Co., Edinburgh, pp: 114.

Hasspielar, B.M., J.V. Behar and R.T. Di Giulio, 1994. Glutathione-dependent defense in channel catfish (*Ictalurus punctatus*) and brown bullhead (*Ameriurus nebulosus*). Ecotoxocol. Environ. Saf., 28(1): 82-90.

Jollow, D.J., J.R. Michell, N. Zampaglione and J.R. Gillete, 1974. Bromobenzene induced live necrosis: Protective role of GSH and evidence of 3, 4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology, 11: 151-169.

Kohen, R. and A. Nyska, 2002. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions and methods for their quantification. Toxicol. Pathol., 30(6): 620-650.

Li, F., L. Ji, Y. Luo and K. Oh, 2007. Hydroxyl radical generation and oxidative stress in *Carassius auratus* liver as affected by 2, 4, 6-trichlorophenol. Chemosphere, 67(1): 13-19.

Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Poll. Bull., 42(8): 656-666.

Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol Chem., 93(1): 193-265.

Lucky, Z., 1977. Methods for the Diagnosis of Fish Diseases. Amerind Publishing, New Delhi, pp: 140.

Magwere, T., Y.S. Naik and J.A. Hasler, 1997. Effects of chloroquine treatment on antioxidant enzymes in rat liver and kidney. Free Rad. Biol. Med., 22(1-2): 321-327.

McCord, J.M., 1996. Effects of positive iron status at a cellular level. Nutri. Rev., 54(3): 8-85.

Miller, G., N. Suzuki, L. Rizhsky, A. Hegie, S. Koussevitzkys and R. Miller, 2007. Double mutants deficient in cytosolic and thylakoid ascorbate peroxidase reveal a complex mode of interaction between reactive oxygen species, plant development and response to abiotic stresses. Plant Physiol., 144(4): 1777-1785.

Monterio, D.A., J.A. Almedia, F.T. Rantin and A.L. Kalinin, 2007. Oxidative stress biomarkers in the freshwater Characid fish, *Brycon cephalus*, exposed to organophosphorus insecticides Follisuper 600 (methyl parathion). Compar. Biochem. Physiolog. Toxicol. Pharmacol., 143(2): 141-149.

Nesrea, 2011. National Environmental (Surface and Groundwater quality) Regulations. National Environmental Standards and Regulations Enforcement Agency, S.I., pp: 22.

Nwabueze, A.A. and J.K. Ekelemu, 2011. Growth and survival of *Clarias gariepinus*, (Bruchell, 1822) fingerlings in different concentrations of domestic leachate. ARPN J. Agric. Biol. Sci., 6(5): 25-29.

Nwadozie, J.M., 1998. The determination of heavy metals pollutants in fish samples from River Kaduna. J. Chem. Soc. Nigeria, 23: 21-23.

OECD, 2002. OECD Guidelines for the Testing of Chemicals OECD Guidelines for the Testing of Chemicals: Fourteenth Addendum. OECD Publishing, Paris, pp: 87, ISBN: 9264198334.

Ozmen, I., 2005. Evaluation of effect of some corticosteroids on glucose-6-phosphate dehydrogenase and comparative study of antioxidant enzyme activities. J. Enzyme Inhib. Med. Chem., 20(1): 19-24, In Press.

Pandey, S., S. Parvez, I. Sayeed, R. Haque, B. Bin-Hafeez and S. Raisuddin, 2003. Biomarkers of oxidative stress: a comparative study of river Yamuna fish Wallago attu (Bl. & Schn). Sci. Total Environ., 309(1-3): 105-115.

Reish, D.L. and P.S. Oshida, 1987. Manual of Methods in Aquatic Environment Research. Food and Agriculture Org, Rome, pp: 56, ISBN: 9251025177.

Rodriguez-Ariza, A., J. Peinado and C. Pueyo, 1993. Biochemical indicators of oxidative stress in fish from polluted littoral areas. Can. J. Fisheries Aquat. Sci., 50(12): 2568-2573.

Ross, D., 1988. Glutathione, free radicals and chemotherapeutic agents. Mechanisms of free-radical induced toxicity and glutathione-dependent protection. Pharmacol. Ther., 37(2): 231-249.

Sahan, A., E. Belge and T. Altun, 2010. The determination of biochemical indicators (Biomarkers) in the common Carp (*Cyprinus carpio*) to the Physico-chemical parameters of Ceyhan river (Adana-Turkey). Ekoloji, 19(76): 8-14.

Shams, T. and M. Ahmad, 2011. Some enzymatic/non enzymatic antioxidants as potential biomarkers of trichloroethylene, heavy metals mixture and ethyl alcohol in rat tissues. Environ. Toxicol., 26(2): 207-216.

Pavlović, S.Z., S.S. Borković Mitić, T.B. Radovanović, B.R. Perendija, S.G. Despotović, J.P. Gavrić and Z.S. Saicić, 2010. Seasonal variations of the activity of antioxidants defense enzymes in the Red mullet (*Mullus barbatus*) from Adriatic sea. Mar. Drugs, 8(3): 413-428.

Vijayavel, K., R.D. Gomathi, K. Durgabhavani and M.P. Balasubramanian, 2004. Sublethal effect of naphthalene on lipid peroxidation and antioxidant status in the edible marine crab *Scylla serrata.* Mar. Poll. Bull., 48(5-6): 429-433.

Wang, W.N., L.A. Wang, L. Chen, Y. Lui and R.Y. Sun, 2002. Effects of pH on survival, phosphorus concentration, adenylate energy charge and Na+-K+ ATPase activities of *Penaeus chinensis* (Osbeck) juveniles. Aquatic Toxicol., 60(1-2): 75-83.

Wendelaar, S.B., 1997. The stress response in fish. Physiol. Rev., 77(3): 591-625.

Yildirin, N.C., F. Benzer and D. Danabas, 2011. Evaluation of environmental pollution at Munzur River of tunceli applying oxidative stress biomarkers in capoeta trutta (Heckel, 1843). J. Anim. Plant Sci., 21(1): 66-71.

Zhu, X., L. Zhu, Y. Lang and Y. Chen, 2008. Oxidative stress and growth inhibition in the freshwater fish *Carassius auratus* induced by chronic exposure to sublethal fullerene aggregates. Environ. Toxicol. Chem., 27(9): 1979-1985.