Excess iron load: Physiological responses and Oxidative stress

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Abstract**—** *Water in Niger-Delta region, Nigeria is either red or yellows due to the high concentration of iron. Hence, the physiological responses and oxidative stress biomarkers on Clarotes laticep, one of the dominant fish species in this region exposed to iron was investigated. Environmental concentration of the metal was evaluated in the fish caught from one of the major Rivers in the region. The fish were exposed to the ranges of concentrations observed in the field. The physico-chemical characteristics of the text media were similar to the ones observed in the field. Fish were starved 48 hours to clear the gut contents before starting the experiment. At the end of each experimental period, blood was collected and fish transferred into a clean aquaria containing de-chlorinated tap water for 28 days to allow depuration and returned to the wide. Lipid peroxidation level, Catalase activity, glutathione (GSH) level and Superoxide dismutase activity were measured using respective analytical methods. There was an induction and inhibition of the activity of these enzymes, an indication of oxidative stress. The concentration of iron recorded in C. laticep was above the recommendation limit for human and aquatic life. A call for holistic treatment of water in this region become imperative, and fish from this region needs to be depurated before consumption or sales to the public. Though, iron is an essential nutrient, is one of the most common nutritional deficiencies. However, iron overload can damage internal organs and may increase the risk of diabetes, heart attack and cancer.*

Keywords**—** ***Iron, Clarotes laticep, Lipid peroxidation, Catalase, GSH, and Superoxide dismutase.***

I. INTRODUCTION

Iron overload means an excess of total body iron, most of which is located in the storage compounds ferritin and hemosiderin [1]. Aside from the pathologic forms of primary and secondary iron overload moderately elevated iron stores may be of concern because of a possible association with several chronic diseases, such as heart disease [2,3], cancer [4,5], and diabetes [6]. Metals are one of the main inducers of oxidative stress in aquatic organisms, promoting formation of reactive oxygen species through two mechanisms. Redox active metals generate reactive oxygen species through redox cycling, while metals without redox potential impair antioxidant defenses, especially that of thiol-containing antioxidants and enzymes [7]

Oxidative stress occur when there is an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses in living organisms [8], and toxicity of transition metals is often due to their great participation and action as catalysts in the production of the ROS through the Fenton/Haber-wiess reactions, which are highly reactive chemicals containing oxygen (e.g. hydroxyl free radical OH) that reacts easily with other molecules, resulting in potentially damaging modifications.

In biological systems, oxidative stress has become of significant interest issue for environmental toxicology studies; especially oxidative damage induced by different classes of chemical pollutants [9] Toxic consequences of oxidative stress at the subcellular level include lipid peroxidation, oxidative damage to DNA and proteins as well as alteration of the antioxidant enzymes responses [10] Antioxidants such as lipid peroxidation, catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) are good biomarkers of contaminant induced oxidative stress, especially by transition metals such as iron, mercury and copper in a variety of marine and freshwater organisms and their induction reflects a response to pollutants [11,12]. The current knowledge that processes of oxidation damage occur in aquatic organisms gave the impetus to extend environmental and ecotoxicological studies to aquatic organisms as sentinels of environmental contamination by toxic chemicals [13]

Iron can be a troublesome chemical in water body. It is one of the earth's most plentiful resources, making up at least 5 percent of the earth's crust. Rainwater as it infiltrates the soil and underlying geologic formations dissolves iron, causing it to seep into aquifers that serve as sources of

groundwater. The recommendation by various regulatory limit of iron in drinking water is 300 µg/L [14], which is insignificant compared to what was observed in Brass River at Onuebu town. Iron is mainly present in water in two forms: either the soluble ferrous iron or the insoluble ferric iron. Water containing ferrous iron is clear and colorless because the iron is completely dissolved. When exposed to air in the pressure tank or atmosphere, the water turns cloudy and a reddish brown substance begins to form. This sediment is the oxidized or ferric form of iron that will not dissolve in water.

Fish give one of the essential nutrients for healthy growth, and those from polluted sites present a potential risk to human health. Since fish occupy the top of the aquatic food chain, they are suitable bio-indicators of health status of aquatic environment. Metals are well-known inducers of oxidative stress, and assessment of oxidative damage and antioxidant defenses in fish can reflect metal contamination of the aquatic environment. However, in the wider environment other factors my work in synergy with the metals to induce stress. Thus the aim of this research is to assess the effect of iron concentrations (hyper dominant metal in South-south Nigeria) observed in the field on the oxidative stress enzymes of the dominant fish species in this region.

II. MATERIALS AND METHODS

Field Analysis of Iron

Study Area: Brass River is in southern Nigeria that is considered the direct continuation of the Niger River After the Niger bifurcates into the Brass and Forcados rivers about 20 miles (32 km) downstream from Aboh, the Brass flows through sparsely settled zones of freshwater and mangrove swamps and coastal sand ridges before completing its 100-mile (160-km) south-southwesterly course to the Gulf of Guinea, a wide inlet of the Atlantic Ocean, at Elepa (Fig. 1). The River flows through Onuebu, a town which lies between latitudes 4.6901ºN of the equator and longitudes 6.3213ºE of the Greenwich Meridian. It is a wet terrain with a fairly good road network. The prevalent climatic condition in the area is marked by two main regimes: the rainy and the dry seasons. The wet season is from April to October while the dry season starts in November to March. The average monthly temperature is high throughout the year. A mean annual temperature of 32°C is typical of the area. The area lies within the swamp rain forest belt of Nigeria.

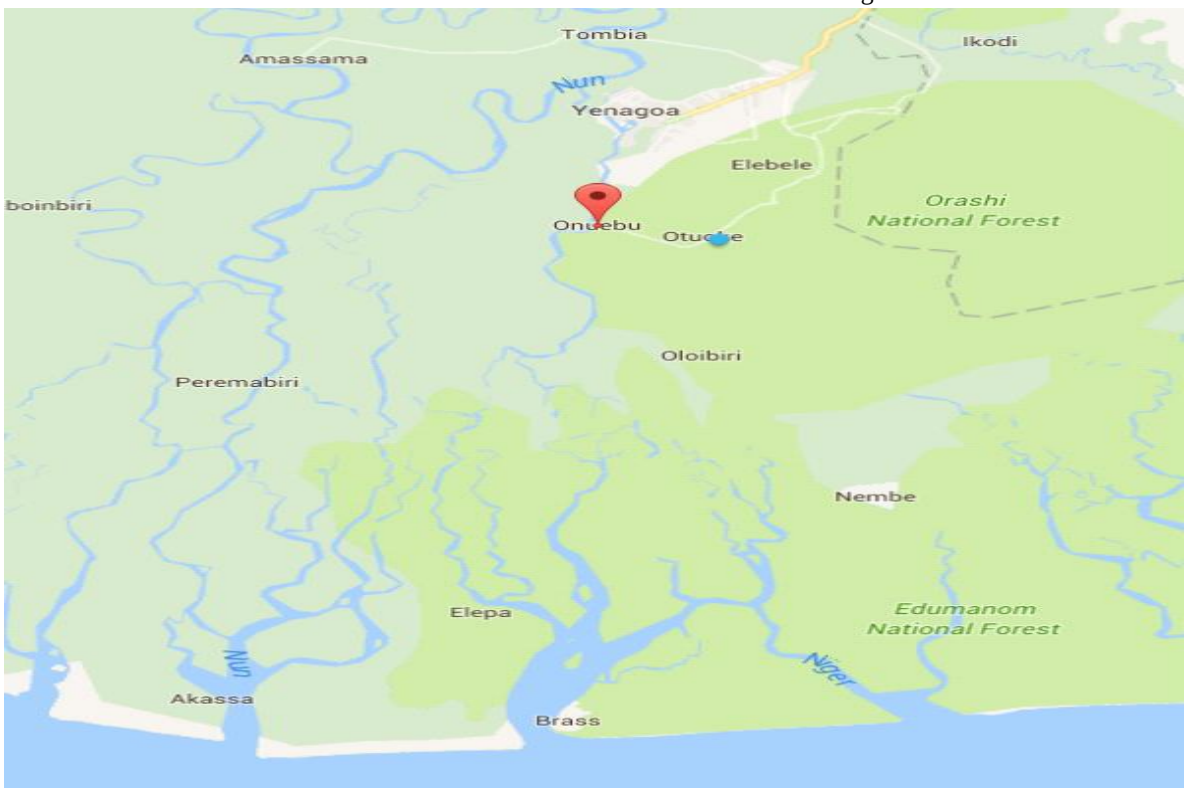

Fig.1: Map showing sampled stations at Onuebu town

Samples Collection, Extraction and Analysis: Samplings were carried out on monthly basis; January, 2016 to June, 2016 and were taken at Onuebu town. Three stations were chosen within the town. Three post-juvenile of *C. laticeps* mean weight (1.20 + 0.2kg) and length (54.20 + 1.3m) were caught using cast nets from each sample station. The fish were kept together in labeled plastic bags and placed on ice (<6 h) and then stored at −20 °C in the laboratory until analysis. Fish samples were freeze-dried and ground into fine powders to obtain homogenous composite. Five (5) gram of a dried sample was placed in a Teflon vessel and 4 ml of analar nitric acid was added. The vessels were tightly covered and were allowed to predigest at room temperature overnight. The digestion vessels were placed on a preheated hotplate at 80°C for 3 hours. The samples were cooled at room temperature, thereafter transferred to 25 ml volumetric flask. The digested solutions were analyzed for Fe concentrations using Flame Atomic Absorption Spectroscopy (Perkin Elmer Analyst 300, USA). Standards and reagents utilized for the blanks and calibration curves were all of analytical grade.

Laboratory Experimentation

Eighty post juveniles of *C. laticeps* were raised in a serene environment for this investigation. The weights and the sizes were similar to the fish caught in the field (1.22 $\pm$ 0.1kg) and length (53.70 $\pm$ 0.1m). The fish were acclimatized for 14 days in aquaria supplied with de-chlorinated tap water. They were fed daily to satiation on a commercial fish feeds (Taiyo feeds). The water was cleaned daily; the uneaten and faecal wastes were siphoned daily. Water replenished twice a week.

Experimental Design

Fifteen glass aquaria were also used with 3 replicates per treatment. All experiments conducted at room temperature, tanks were properly aerated and environment well ventilated. The physico-chemical characteristics, such as temperature, pH, DO, hardness, chloride and conductivity of experimental were similar to the ones observed in the field. Fish were starved 48 hours to clear the gut contents before starting the experiment. Ten fish per test concentration were exposed to different concentrations of dietary treated iron for 28 days. These iron concentrations were within the ranges observed in the field, while the Taiyo feeds (Ingredient composition - fish meal, wheat flour, soybean meal, corn meal, yeast, vitamins and mineral salt : Proximate analysis - crude protein mineral; 32%, crude fat mineral;4% ,crude fibre, maximum;5% and moisture, maximum; 10%). They were fed with the treated feeds twice daily. Tank clean daily, the uneaten and faecal wastes were siphoned and water replenished thrice weekly.

Blood was collected from the caudal vessel (*a. et. v. caudalis*) with a heparinized syringe on day 7, 14, 21 and 28th. Immediately, after blood collection, the fish were transferred into a clean aquaria containing de-chlorinated tap water for 28 days to allow depuration [15]. After this period, they were returned to the wide. During this period of investigation 7 fish died, about.

The whole blood was divided into two parts.

Some of the whole blood was used immediately to determine CAT and SOD activities and GSH level. Remaining whole blood was centrifuged at 5,000 rpm for 10 min, plasma separated and stored at -80^0 C for MDA analysis

All chemicals used in this study were obtained from Sigma or Merck (Germany). Glutathione was measured following the method of Beutler [16],CAT was determined using the method of [17] and SOD activities according to the methods of [18] and [19]. MDA level was assayed by the method of [20]

Statistical Analysis

T-test and one way analysis of variance was used to analyze the differences between the control and various treatments and within the treatments [21]. All data are presented as mean ± SE. A value of $p< 0.05$ was considered statistically significant.

Lipid peroxidation Determination

Lipid peroxidation was measured by determination of malondialdehyde (MDA) concentrations in the plasma using Thiobarbitoric acid (TBA) assay. TBA reagent (composition: 20% TCA, 0.5% TBA, and 2.5 N HCl) was added to the plasma and heat steadily for 20 min in a boiling water bath. After cooling, the solution was centrifuged at 2000 rpm for 10 min and the precipitate obtained decanted. The absorbance of supernatant was measured at 535nm against a blank that contained the all the reagents excluding the plasma. The MDA equivalents of the sample were calculated using an extinction coefficient of 1.5×10^5 $M^{-1}cm^{-1}$ and expressed per milligram of protein (nmol mg/protein).

GSH Determination

A 50 µL sample of whole blood was added to a microcentrifuge tube containing 100 µL cold 5% Metaphosphoric acid (CAS 37267-86-0). The

microcentrifuge tube was then vortexed, centrifuged at 600xg at 4°C for 10 minutes and the supernatant collected and placed on ice. The precipitate (erythrocytes) was discarded. A 2.5ml of Na2PO4 (0.3M) "phosphate buffer 0.5ml of above supernatant, and 0.25ml of DTNB-Reagent was added and the mixture incubated for 10min at 37°C. The absorbance was read at 412nm using spectrophotometer.

Catalase Determination

Catalase (EC.1.11.1.6) catalyses the decomposition of hydrogen peroxides to give water and molecular oxygen. The activity of the enzyme was determined according to the method described by [22] as modified by [23]. The decomposition of H_2O_2 is directly proportional to the decrease in absorbance at 240 nm. A 50 µL sample of whole blood was in a glass tube was allowed to clot at room temperature. Serum was rapidly separated by centrifugation at 700-1,000 x g for 10 minutes at 4°C. The pale yellow plasma was carefully aspirated off plasma without disturbing the white buffy layer which was discarded. Due to the high concentration of catalase in erythrocytes their hemolysis was checked by serum hemoglobin determination, and it was 80 mg/l (which was within the range needed for the analysis). 0.2 ml serum was incubated in 1.0 ml substrate (65 pmol per ml hydrogen peroxide in 60 mmol/l sodium-potassium phosphate buffer, pH 7.4) at 37°C for 60 s. All dilutions of the substrate and enzyme preparations were made with M/15 phosphate buffer. The enzyme concentration was adjusted by proper dilution to approximately the same range of activity in order that the ratio of substrate to enzyme concentration was fairly constant. Preliminary investigations were performed with erythrocyte and solutions in order to select a substrate to enzyme concentration which showed a constant proportionality with enzyme concentration even with appreciable variations of substrate. The proportionality between enzyme activity and concentration, at any reaction rate over a limited range, makes it possible to express enzyme activity in terms of arbitrary units. The arbitrary unit is defined as that amount of catalase which will liberate 1 ml of oxygen per second from a 1.0 N H_2O_2 solution at 0°C. Enzyme and nitrogen determinations were performed in triplicates, the average values of the results obtained being used to calculate the unit enzyme content. The usual rate of oxygen evolution corresponded to approximately 2 ml. per minute, and calculation of the enzyme activity was obtained by determining the maximum initial reaction rate. The catalase activity was determined by measuring the rate of oxygen evolution from H_2O_2 manometrically and was expressed as mmol H_2O_2 /min/ mg protein. An enzyme unit was defined as the amount of enzyme that catalyzes the release of one µmol of H_2O_2 per min at 20 °C. Specific activity was calculated in terms of units per mg of protein.

SOD Determination

A 50 µL sample of whole blood was separated by centrifugation into blood plasma -and erythiocyte fractions. Erythrocyte fractions were washed with saline three times ready for assay. The Hb content of each haemolysate was measured cyanmethemoglobin method [24]. SOD activity in red-blood-cell lysates was assayed in chloroform/ethanol extracts by the method described by [18] with some modifications. The assay was based on the inhibition of the conversion of Nitro Blue Tetrazolium by SOD into a blue tetrazolium salt, mediated by superoxide radicals that were generated by xanthine oxidase. The assay was performed in 3 mi of sodium carbonate buffer (50 mmol/l; pH 10.2) containing xanthine (0- 1 mmol/l), Nitro Blue Tetrazolium (25 pmol/l) and xanthine oxidase (5-8 nmol/l). The amount required to inhibit the rate of reduction of Nitro Blue Tetrazolium by 50% was defined as 1 unit of activity. The assay was performed at 25°C and the rate of reduction was followed at 560 nm with a Hitachi (Tokyo, Japan) model 200-20 spectrophotometer.

III. RESULTS

Iron is the dominant metal in the Niger-Delta ecological zone. It status in the brass river revealed that the metal is highly concentrated in this region (Table 1). Statistical analysis of data revealed that there was a significant decrease in the iron concentrations in the fish tissues as the year progresses ($p < 0.05$). Similarly, significance was observed between the three stations in the month of June ($p < 0.05$).

Table.1: Concentrations Fe (ppm) (mean ± SEM) of Brass River at Onuebu town, means with different superscript in the columns and rows varies significantly ($p < 0.05$)

Months	Station 1	Station 2	Station 3
January	1860 ± 2.40 ^{a}	1830 ± 1.90 ^{a}	1780 ± 2.10 ^{a}
February	1820 ± 2.20 ^{a}	1800 ± 1.20 ^{a}	1740 ± 4.20 ^{a}
March	1780 ± 3.10 ^{a}	1690 ± 2.50 ^{a}	1650 ± 3.10 ^{a}
April	1740 ± 2.10 ^{a}	1640 ± 2.30 ^{a}	1630 ± 2.60 ^{a}
May	1660 ± 3.10 ^{b}	1620 ± 2.40 ^{b}	1600 ± 1.40 ^{b}
June	1640 ± 2.10 ^{a}	1600 ± 1.70 ^{b}	1580 ± 1.60 ^{c}

Catalase activity was relatively constant in the control group irrespective of the exposure duration. The enzyme activity increases with increased in the concentration of the toxicant, and it ranges between 18.10 – 41.00 U/mg protein (Figure 1). On day 7and 14, the activity of the enzyme showed no significant difference between the control and various treatments and within the various treatments ($p > 0.05$). However, there was significant difference between the control and various treatments except the fish exposed to 1700ppm Fe ($p < 0.05$).

In the same manner, the blood lipase peroxidase that was measured by determination of malondialdehyde (MDA) concentrations and expressed in nmol/ml, showed a significant ($P < 0.05$) increase in a dose- time - dependent manner (Figure 3). The lowest MDA level 2.32 nmol/ml was observed on day 7th at 1600ppm Fe concentration, while the highest concentration of 4.5 nmol/ml was observed on day 28th at 1900 ppm Fe concentration. The activity of the enzyme varies significantly ($p < 0.05$) between the control and various treatments, but not within the treatments (Figure 2)

The GSH levels decreased with increase in Fe concentration and exposure duration. In the fish exposed to 1600ppm Fe, the enzyme levels range between 1.2 – 4.4 nmol./mg protein, with the highest inhibition recorded on day 28th . At 1700ppm Fe, it ranges between 0.80 – 4.10 nmol./mg protein, at 1800ppm Fe, 0.40 – 4.0 nmol./mg protein, at 1900ppm Fe, 0.2 – 4.00 nmol./mg protein (Figure 3). There were no significant different between the GSH levels and the control on day 7 and 14 ($p > 0.05$), but was highly significance between the control and various treatments on day 21 and 28th ($p < 0.01$). Equally, there was significant different between the GSH levels within the various treatments ($p < 0.05$)

Similarly, the SOD activities was significantly ($p < 0.05$) decrease in blood in the iron treated fish compared to those in the control group except on day 7 and 14th (Figure 4). There was progressive decreased in the inhibition of the enzyme as the day progresses, with the highest inhibition of 1.6units/g of Hb observed on day 28th at 1900 Fe concentration. The least inhibition of 6.9units/g of Hb was observed on day 14th at 1600 Fe concentration.

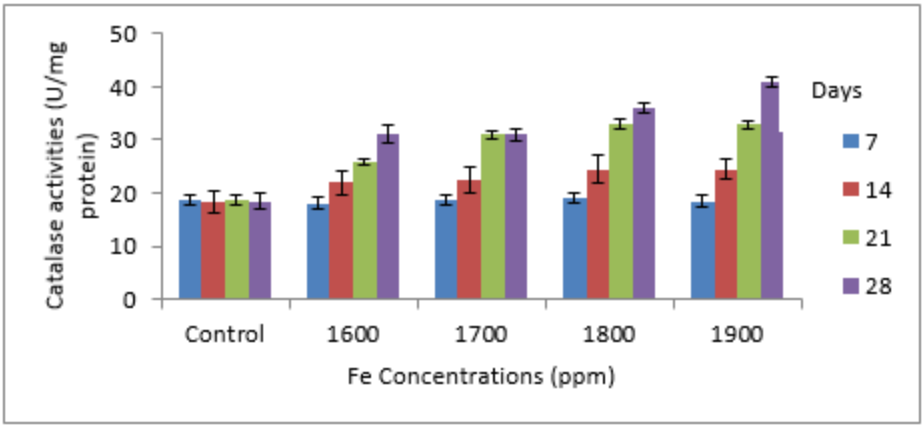

Fig.2: Catalase activities in the blood tissue of C.laticept exposed to the different concentrations of iron

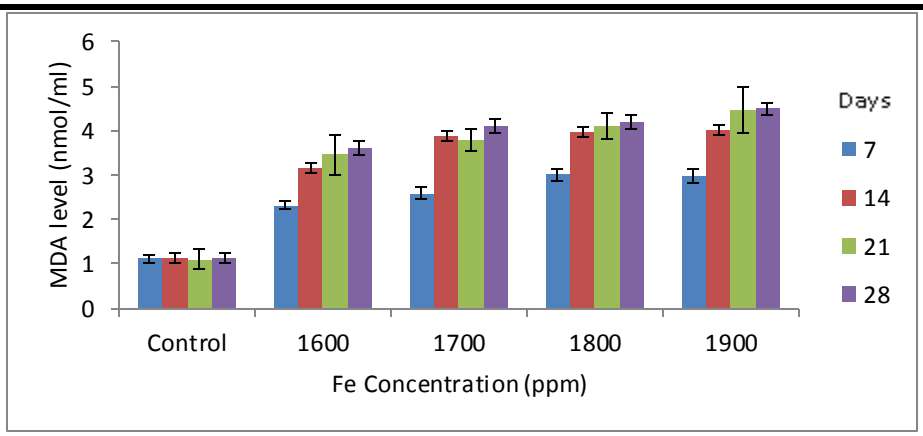

Fig.3: MDA level in the blood tissue of C. laticept exposed to the different concentrations of iron

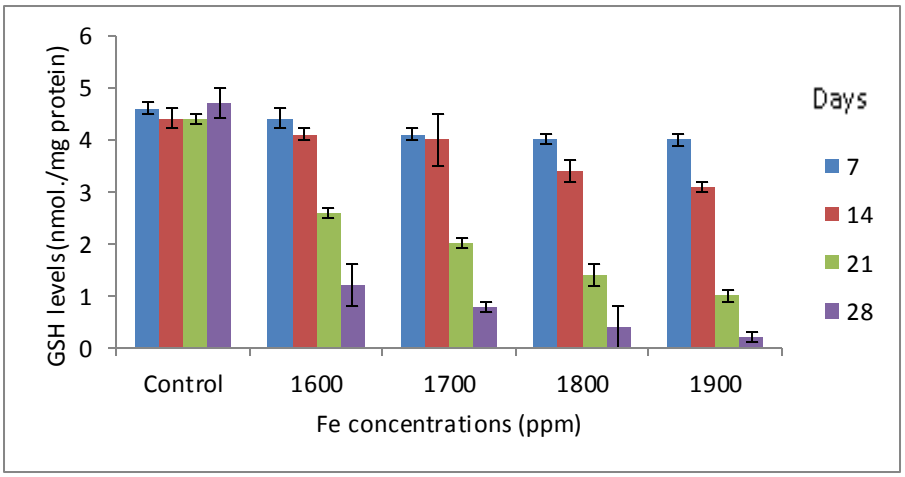

Fig.4: GSH level in the blood tissue of Clarotes laticept exposed to the different concentrations of iron

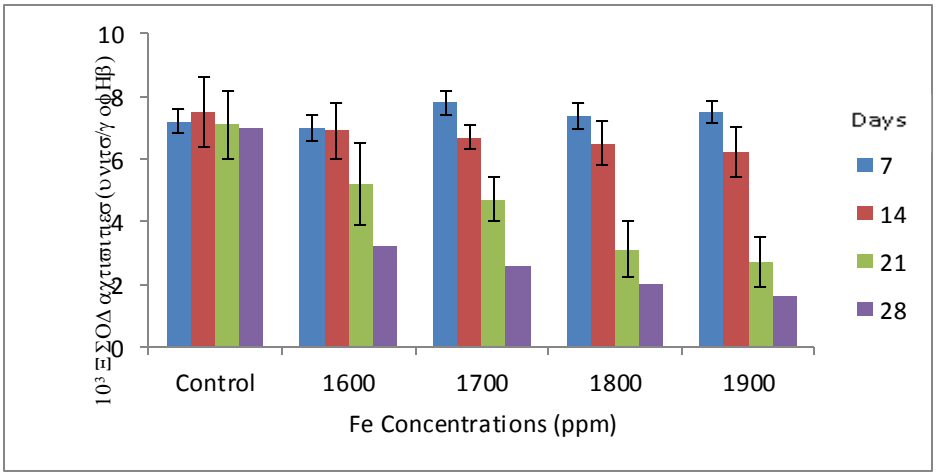

Fig.5: Superoxide dismutase activities in the blood tissue of Clarotes laticept exposed to the different concentrations of iron

IV. DISCUSSION

Pollution decrease water quality and affects all living organisms in that ecosystem, it become imperative to identify and manage these pollution sources and to monitor their impact on the health of aquatic ecosystems. Fishes are use as sentinel agents for health status of water body, and have been considered as good model for biomonitoring of the aquatic environment [25]. In the present study, Fe

treated fishes showed a significant increase in the mucous secretion that is proportional to the exposure duration without significant changes in the body weights when compared with that of control fishes. Mucous cells are considered efficient in seizing the toxic agents and thus help in the hindrance of the entrance of these agents in to the gills [26]. Hyper secretion of mucous may be as a result of a chronic defensive mechanism of the fish against the exposure to the toxicant.

When the fish kept in serene environment were exposed to the ranges of concentrations observed in the field, (the examination was not done in the field, to eliminate the synergic effects of other pollutants) various oxidative responses were observed. Oxidative stress induced by excessive production of catalase and an imbalance in other antioxidant enzymes, which may be detrimental.

There was proportionate increase in catalase with increased in the concentration of the toxicant with time. The increased in the erythrocyte catalase could be related to the oxidative damage of membrane protein and lipid by increased oxygen free radicals in the body. Excess iron load may also elevate the sugar level in the fish, since elevated iron stores is associated with diabetes [6]. Similar observation was reported in rat treated with streptozotocin, a diabetes inducer [23]

Malondialdehyde (MDA), one of the products of lipid peroxidation, has been studied widely as an index of lipid peroxidation and as a marker of oxidative stress [27]. In this study, the amount of MDA formed in the fish blood is directly proportional to the concentration of Fe used for induction, indicating the higher the concentration of the toxicant, the more the magnitude of the stress experienced by the fish. Similar observation was reported by [28], when rainbow trout was in vitro exposed to ozone for 5 min. He observed that the fish plasma and red blood cells resulted in marked increased in TBARS (thiobarbituric acid-reactive substances, products of lipid peroxidation). Corroborate this finding is the work of [29], who observed Lipid peroxidation and alterations in antioxidant enzyme activity in embryonic and adult medaka *Oryzias latipes* exposed to nano-iron. Dose-dependent inhibition of SOD activity and increased production of malondialdehyde (MDA) was observed in medaka embryos. Activity of hepatic and cerebral SOD in adult medaka was initially reduced following nano-iron exposure but subsequently increased with exposure time. Also, according to [30], an iron-enriched diet in the African catfish *Clarias gariepinus* induced LPO in the liver and heart. Significant increases in SOD activity and higher levels of LPO were observed in erythrocytes of cichlid fish from a metal-contaminated river, with the highest levels in spring, when the concentration of iron in water was elevated [31]. [32]observed increases in protein carbonyls, a marker of protein oxidation, in the goldfish GSH is produced naturally by the liver, and is the most abundant intracellular thiol an important antioxidant preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides.

In this study, GSH decreases significantly compared to the control and were concentration and time dependent. The decreased GSH content during exposure to Fe may be due to an increased utilization of GSH, which can be converted into oxidized glutathione, and inefficient GSH regeneration [33]. The depletion could also be an aggravation status due to reduced cell protection ability [34]. [35]suggested that a severe oxidative stress may inhibit GSH levels due to the impairment of adaptive mechanisms. Thus, the decrease in the GSH level in Fe treated fish may be construed as inabilities to protect against toxicants. Similar to our finding is the work of [36]where lower GSH level were observed in *Cyprinus carpio* caught in polluted Sitilce site of Ataturk Dam Lake. Also, [37] showed a decline in GSH level in tissues of *C. trutta* caught from contaminated site in Munzur River.

Similar to GSH was the SOD activity, which decreases with time and increased in the concentration of the iron. Inhibition of SOD activity could be as a result of oxidative damage to the hepatic tissue, or that the fish have been exposed to pollutants for a short time during which the fish did not have time to adapt to the environment. SOD activity could also be depleted by prolonged exposure to pollutants at low concentrations [38]. Kong *et al*. [39]showed that, in response to oxidative stress, SOD enzyme may be consumed to prevent oxidative damage since it was shown that the overproduction of ROS exhausts the SOD capacity. Similar observation was recorded by [40]regarding the antioxidant defense system in general *Carassius auratus* liver and kidney after waterborne ferrous sulphate exposure.

V. CONCLUSION

The pollutants use blood as routes of transportation to various organs in the body. Most of the pollutants and their metabolites induce toxicity via oxidative stress arising from the increased production of free oxygen radicals. The antioxidant defense enzymes involved in the removal of ROS are the principal candidates for biomarkers of oxidative stress. The antioxidant defense enzymes,

however, have great variability and are dependent on the organisms, organ tissues and the nature of the pollutants involved [41]

Iron, an essential nutrient, is one of the most common nutritional deficiencies. In decades past, many parents worried that children who were picky eaters would develop iron-deficiency anemia. However, these days, more attention is being paid to the opposite problem: iron overload, which studies indicate can damage internal organs and may increase the risk of diabetes, heart attack and cancer, particularly in older people [42]. The reference range of values of iron is considered to be 15–300 µg/L and above 300 µg/L, an indicative of abnormally high iron in the body [43]. There was induction and inhibition in the activity of these enzymes, an indication of oxidative stress. The concentrations of iron recorded in *Clarotes laticep* was above the recommendation limit for human and aquatic life. A call for holistic treatment of water in this region become imperative, and fish from this region needs to be depurated before consumption or sales to the public

REFERENCES

[1] J.W. Halliday,G.A. Ramm , and L.W. Powell, Cellular iron processing and storage: the role of ferritin. In: Brock JH, Halliday JW, Pippard MJ, Powell LW, eds. Iron metabolism in health and disease. London: WB Saunders Co Ltd, 1994,pp 97–121.

[2] L.W. Powell, E.Jazwinska , and J.W. Halliday, Primary iron overload. In:Brock JH, Halliday JW, Pippard MJ, Powell LW, eds. Iron metabolism in health and disease. London: WB Saunders Co Ltd, 1994,227–70,

[3] J.T. Salonen, K.Nyyssonen, H. Korpela, J.Tuomilehto,R. Seppanen,and R. Salonen, High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. Circulation, 86:803–11,1992

[4] G.Klipstein, K. Koster, and J.F.Grobbee, Serum ferritin and risk of myocardial infarction in the elderly: the Rotterdam Study. Am J Clin Nutr,69:1231–6,1999

[5] R.G.Stevens, D.Y.Jones, M.S.Micozzi, and P.R.Taylor, Body iron stores and the risk of cancer. N Engl J Med.,319:1047–52,1999

[6] T.P.Tuomainen, K. Nyyssonen, and R. Salonen, Body iron stores are associated with serum insulin and blood glucose concentrations. Population study in 1,013 eastern Finnish men. Diabetes Care, 20:426–8,1997.

[7] M.H.Sevcikova, A. Modra, Z. Slaninova, and Z. Svobodova, Metals as a cause of oxidative stress in fish: a review; *Veterinarni Medicina, 56,(11):* 537–546,2011

[8] Y.Nishida, The chemical process of oxidative stress by copper (II) and iron (III) ions in several neu-rodegenerative disorders. Monatshefte fur Chemie 142, 375–384,2011

[9] A. Valavanidis, T. Vlahogianni, and M. Dassenakis, Ecotoxicol. Environ. Safety 64,78–189,2006

[10] S.A. Kelly, C.M. Havrilla, and T.C. Brady, Environ. Health Perspect. 106,375–384,1998

[11] F. Lucesoli, and C.G. Fraga, *Arch. Biochem. Biophys.* 316, 567–571,1995

[12] S.S.Borković, J.S.Šaponjić, S.Z.Pavlović, D.P.Blagojević, and S.M.Milošević, The activity of antioxidant defence enzymes in the mussel *Mytilus galloprovincialis* from the Adriatic Sea. Comp Biochem Physiol.,141C:366–374,2005

[13] A.Celso,D. Bainy, and M.Narques, Global analysis of biomarker responses in aquatic organisms exposed to contaminants. Commun. Toxicol . 9,271, 2003

[14] USEPA US Environmental Protection Agency., The Long Island Sound Study: the comprehensive conservation and management plan. Stony Brook (NY): University of New York; Long Island Sound Office of the US EPA, 1994a, 168 p

[15] T.O. Ikpesu, Depuration Technique of Xenobiotics with Reference to Accumulation and Elimination of *Paraquat dichloride* in *Clarias gariepinus*, *Iranian Journal of Toxicology*,Vol.(7) 894-899, 2013

[16] E.Beutler , Red cell metabolism: a manual of biochemical methods. 2. New York: Grune and Stration Company,1975pp 102

[17] S. Lartillot, and P. Kedziora, Athias, A simple method for clinical assay of superoxide dismutase. [Clin Chem. 1988] Sun Y, Oberley LW, Li Y Clin Chem., Mar; 34(3):497-500,1988.

[18] C.C.Winterbourn, R.E. Hawkins, M. Brian and R.W.Carrell, The estimation of red cell superoxide dismutase activity. *Journal of laboratory and clinical medicine*, 85, 337-341, 1975

[19] Y.Yin, H. Jia, Y.Sun,H. Yu,X. Wang, J.Wu, and Y.Xue, Bioaccumulation and ROS generation in liver of *Carassius auratus*, exposed to phenanthrene. Comp Biochem Physiol.,145- 153,2007

[20] I.M.Dubovskiy,V.V. Martemyanov,Y.L. Vorontsova, M.J.Rantala, E.V.Gryzanova, and V.V.Glupov, Effect of bacterial infection on antioxidant activity and lipid peroxidation in the midgut of *Galleria mellonella* L.

larvae (Lepidoptera, Pyralidae) Comp Biochem Physiol. 2008;148:15.

[21] R.G. Steel, and J.H. Torrie, Principles and Procedures of Statistics, second ed., McGraw Hill, New York, USA, 1980

[22] H.Abei, Catalase in the Method of Enzymatic Analysis. Vol. 2. New York. USA: Academic Press, pp. 673–684, 1974.

[23] O.Durdi and R.Timur, Catalase (antioxidant enzyme) activity in streptozotocin-induced diabetic rats. Int J Diabetes & Metabolism 15: 22-24,2007.

[24] E.J.Van Kampen, and W.G. Zulstra, standardization of hemoglobinometry. clinica Chimica, A.C.,1961

[25] E. Lopez-Lopez , J.E.Sedeno-Diaz,C. Soto, and L. Favari, Responses of antioxidant enzymes, lipid peroxidation, and Na^+ /K^+ -ATPase in liver of the fish *Goodea atripinnis* exposed to Lake Yuriria water. Fish Physiol Biochem.,;37:511 522. 2011

[26] S.F.Perry,. The chloride cell: Structure and functions in the gills of freshwater fishes. Annu. Rev. Physiol., 59: 325-347, 1997.

[27] M.Janero, Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury, *Free Radic. Biol. Med. 9* , 515–540, 1990

[28] B.J.Ewa, The Effect of Aquatic Cadmium and Lead Pollution on Lipid Peroxidation and Superoxide Dismutase Activity in Freshwater Fish. *Polish J. of Environ. Stud. Vol. 19, No. 6, 1139-1150, 2010*

[29] H.C.Li, Q. Zhou,Y. Wu, J. Fu,T. Wang, and G.Jiang, Effects of waterborne nano-iron on medaka (*Oryzias latipes*): Antioxidant enzymatic activity, lipid peroxidation and histopathology. Ecotoxicology and Environmental Safety 72, 3684–3692, 2009

[30] R.T.M.Baker,P. Martin,S.J. Davies, Ingestion of sub-lethal levels of iron sulphate by African catfish affects growth and tissue lipid peroxidation. Aquatic Toxicology 40, 51–61.Bull 42(3):215–223, 1997

[31] C.B. Raus, C.D.Carvalho, H.S.Araujo, E.L.Espindola, M.N. Fernandes, Oxidative stress biomarkers of exposure in the blood of cichlid species from a metal-contaminated river. Ecotoxicology and Environmental Safety 71, 86–93,2008.

[32] T.V.Bagnyukova, O.I. Chahrak, V.I. Lushchak, Coordinated response of gold fish antioxidant defenses to environmental stress. Aquatic Toxicology 78, 325–331,2006

[33] S. Kojima, H. Shimomra and S. Matsumoi, Effect of irradiation with low dose gamma-rays on chemically induced hepatotoxicity and glutathione depletion.Anticancer Res. 20(3A): 1583-1588, 2000

[34] Yin, H. Jia, Y.Sun, H. Yu, X.Wang, J.Wu,and Y. Xue, Bioaccumulation and ROS generation in liver of *Carassius auratus*, exposed to phenanthrene. Comp Biochem Physiol.;145- 153,2004

[35] J.Zhang, H.Shen, X.Wang, J.Wu, Y.Xue, Effects of chronic exposure of 2,4-dichlorophenol on the antioxidant system in liver of freshwater fish Carassius auratus. Chemosphere.55:167-174,2003.

[36] V.L. Maria,I. Ahmad ,M. Oliveira ,A. Serafimb, M.J.Bebianno,M. Pacheco, and M.A. Santos , Wild juvenile *Dicentrarchus labrax* L. liver antioxidant and damage responses at Aveiro Lagoon, Portugal. Ecotoxicol Environ Safe. 2009;172:186

[37] N.C.Yildirim, F. Benzer ,D. Danabas, 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, 2011.

[38] S. Pandey,. I.Ahmad, S.Parvez, B. Bin-Hafeez, R. Haque and S. Raisuddin, Effect of endosulfan on antioxidants of freshwater fish *Channa punctatus* Protection against lipid peroxidation in liver by copper preexposure. *Arch Environ Contam Toxicol* 41:345–352, 2001.

[39] Q.Kong, J.A.Beel, and K.O. Lillehei,"A threshold concept for cancer therapy". Medical Hypotheses. *55 (1): 29–35*, 2011

[40] K.Onodera, N.O. Omoi, K. Fukui, T. Hayasaka, T. Shinkai, S.Suzuki, K. Abe and S.Urano, Oxidative damage of rat cerebral cortex and hippocampus, and changes in antioxidative defense systems caused by hyperoxia. Free Radic Res 37: 367–372, 2003

[41] M. Haiam, E.Aboul, A. Abi,r, B. Saad, M.A. Amany, C. El-Sikaily, I. Taha, Oxidative stress and DNA damage in relation to transition metals overload in Abu-Qir Bay, Egypt. Journal of Genetic Engineering and Biotechnology, 9, 51–58, 2011

[42] J. Diana, F. Paul,L. Katherine, J.M. Tucker, R. Massaro, AND W.F. Peter Wilson,and Richard J Wood. Am J Clin Nutr, 73:638–46,2001

[43] C.Datz, T. Haas,H. Rinner, F. Sandhofer, W. Patsch, B. Paulweber, Heterozygosity for the C282Y mutation in the hemochromatosis gene is associated with increased serum iron, transferrin saturation, and hemoglobin in young women: a protective role against iron deficiency? Clin Chem, 44:2429–32, 1998.