HAEMATOLOGICAL INDICES AND ENZYMATIC BIOMAKER OF BLACK JAW TILAPIA (*SAROTHERODON MELANOTHERON)* FROM LAGOS LAGOON

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ABSTRACT

The increases in industrial activities and rapid urban development which occur along the shores of the lagoon have resulted in serious pollution problems. The release of wastes containing hazardous substances and dumping of waste indiscriminately into water bodies could lead to environmental disturbance that might be considered a potential source of stress to the aquatic organism. A toxicological study was carried out to ascertain the effects of aquatic pollutants in the blood of Sarotherodon melanotheron at the Lagos lagoon for a period of three months. Four enzymatic biomarkers were analysed which include protein, superoxide dismutase, Malondialdehyde and Reduced Glutathione. Water sample were collected from Lagos Lagoon and analyzed.

The differential counts of Haematological indices such as Haemoglobin (Hb), Packed cell volume (PCV), Red blood cell (RBC), White blood cell (WBC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin content (MCHC), were investigated. The lipid peroxidation and antioxidant biomarkers in the blood of S. melanotheron showed a significant difference (P < 0.05). The mean value recorded for Haemoglobin was 39.78±14.43g/L, PCV was 0.16±0.16L/L, RBC was 2.13±1.47T/L, WBC 6.22±2.66G/L while the Mean corpuscular volume was 73.39fl, MCH was 24.57±11.07pg, MCHC was 344.19±24.04L/L. Also the mean value Lymphocyte was 70.78±6.53% and Monocyte was 0.39±0.69% while 0% was recorded for Eosinophil and Basophil. Air temperature, water temperature, Salinity, pH, conductivity, Turbidity and dissolved oxygen had a mean value of 25.75±1.44°c, 25.17±0.75°c, 0.15±0.1‰, 6.13±0.82, 3.60±1.49mS/cm, 31.0±8.37FTU, 1.60±0.26mg/l respectively. Also the mean value of alkalinity, nitrate, phosphate, sulphate, chemical oxygen demand and biological oxygen demand are 68.07±22.92mg/l, 6.77±3.90mg/l, 0.53±0.55mg/l,0.84±0.37mg/l, 307.67±27.30mg/l and117.19±14.61mg/l respectively. Environmental factors such as low dissolved oxygen, high Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) values of this study may be implicated as the possible cause of alteration in the haematological characteristics of S. melanotheron in this study. This study indicates that there was an alteration in haematological profile, antioxidant enzyme and lipid peroxidase activities in S. melanotheron blood which may cause biochemical dysfunction in this specie. All these result provide a useful tool in monitoring the condition and state of health of fish by knowing the normal value with respect to their responses to stress which affects body metabolism.

Keywords: Biomarker, *Sarotherodon melanotheron,* Haematology, Lagos Lagoon

INTRODUCTION

Water pollution has become a menace in recent times, causing a great damage to the aquatic ecosystems. Waste from industries and domestic uses gradually find their way into the aquatic environment. Most of the water bodies have become polluted due to haphazard and extravagant pouring of wastes into them and making it unfavourable for aquatic organism (Chindah *et al.,* 2008). Aquatic contamination by industrial and domestic sewage outlets are a constant sources of public health concern. The series of contamination may vary between organic pollutant such as polycyclic aromatic hydrocarbons from oil explorative activities, resin acids, and heavy metals from industries and also akylphenols deriving from domestic activities (Chindah 2004). The effluents from industries are a complex mixture of a number of chemical constituents (Nestmann *et al.,* 1980) and have huge amount of oxidant potential (Ellis *et al.,* 2003). According to Kummerer (2001), most industrial effluents contain a wide range of anthropogenic substance including toxic industrial chemicals and pharmaceutical residues.

The release of chemicals into the aquatic environment results in some changes, which may threaten functional attributes, the integrity and existence of aquatic organisms, especially fish (Chindah and Hart, 2000). Recently, haematological parameters have become promising biomarkers in measuring the effects of chemical pollutant in fish. Blood samples can regularly be obtained from test organisms, thus allowing the use of non- destructive approach in effect assessment (Akinrotimi *et al.,* 2010). Typically, haematological parameters are non-specific in their responses towards chemical stressors. Nevertheless, they may provide important information in assessment studies, by providing an indication as to the general physiology and health status of the organism under investigation (Beyer, 1996).

Several researcher have investigated the toxicity, uptake and tissues distribution and haematological changes of pollutants in fish (Chindah *et al.,* 2008, Akinrotimi *et al.,* 2007 and Gabriel *et al.,* 2007a), and the use of hematological techniques in fisheries research is growing rapidly, as it is very important in toxicological research which result in monitoring and predicting health conditions of the fish (BittenCourt *et al.,* 2003, Akinrotimi *et al.,* 2009). Since fish are so intimately associated with the aqueous environment, the blood will reveal measurable physiological changes in the fish more rapidly than any physiological assessment parameters (Ezeri *et al.,* 2004). Pollutants such as herbicides, pesticide and industrial effluent are known to alter the haematological indices of fish (Houston 1990; Gabriel *et al.,* 2007b; Akinrotimi *et al.,* 2007). Long term exposures of fish to effluents and sewages have been reported to altered haematological parameters by disrupting haematopoiesis, consequently resulting in anemia condition (Nikinmaa and Oikari, 1992; Ellis *et al.,* 2003).

The Lagos Lagoon is one of the meandering networks of lagoons and creeks found along the coastline of southern Nigeria. Its serves as waste disposal point for the entire community in Lagos, one of the most highly populated coastal cities in West Africa. Sources of pollution into the Lagoon include effluents from brewery, industries (such as food, organic chemical, textile), solid wastes from slaughter houses, sawmills as well as domestic and untreated sewage (Akpata and Ekundayo, 1978). Brewery and textile industries are highly concentrated in Lagos area (Akpata and Ekundayo, 1978). The Lagos Lagoon also serves as a major source of fish and economically

important crustaceans and molluscs for the inhabitants of Lagos (Fagade and Olaniyan, 1974). The discharge of sewage into the Lagoon has health implications in human (Akpata and Ekundayo 1978). According to Nwankwo and Akinsoji (1989), the Lagos lagoon is under pressure from pollution and most of the pollutants are; untreated sewage, sawdust, petrochemical materials, detergent and industrial effluents.

Fish is in close contact with their environment, and are very susceptible to physical and chemical changes which may be seen in their blood components (Wilson and Taylor, 1993). Blood reflects physical and chemical changes occurring in organism. Biomarkers range from general to specific, reflecting general stress or exposure to specific classes of environmental contaminants. Since changes in the organism could affect population and community levels, biomarkers can be used as early warning signals of environmental disturbance (Walker *et al.,* 2006). Therefore, biomarkers are considered useful tools in determining the health condition of a fish. Haematology is used as an index of fish health status in many fish species to detect physiological changes following different stress condition such as exposure to pollutants, diseases, heavy metals, hypoxia etc. According to Svobodova *et al.,* (1996) study of haematological parameter are carried out on the fish to ascertain the normal range of blood parameter, find out the variation with age, sex, season, and determine the effects of disease condition on the fish. This study is aimed at assessing the enzymatic biomarkers and haematological indices of Tilapia specie (*Sarotherodon melanotheron*) of the Lagos lagoon.

MATERIALS AND METHODS

Description of Study Site
The Lagos lagoon is the largest of the four lagoon system of the gulf of Guinea and also the largest of the eight lagoons systems of Nigeria (Webb, 1958). It stretches for about 257km from Cotonou in the republic of Benin to the western edge of the Niger delta. Sandison and Hill (1966) documented that in the harbor, the annual range of surface water temperature is only 4°C.Light intensity allowing for differences in cloud cover is always of the same order and the length of day is also fairly constant being approximately 12hr at all times.

Experimental Procedures
This experiment was carried out at University of Lagos Lagoon front and the study organisms were black jaw tilapia (*Sarotherodon melanotheron)* which were collected from the last week in July to the first week of September with one week interval totaling six weeks. The weight of the organisms ranged between 68 to 119kg and about 14 to 21cm in length. Gill nets (50-120mm stretch mesh size) were set over night prior to collection. The ten (10) fishes were washed in clean water to remove adhering dirt and transported in polythene bags to the laboratory for analysis. Ten (10) cultured fishes of *S. melanotheron* were obtained from Aquaculture unit of Dept. of Marine Sciences, University of Lagos and were used as control for the experiment.

Collection and Analysis of Water Sample
Water samples were collected using a liter water sampling bottles. The bottles were preserved in a cool dry place and transported to the laboratory for further analysis of water quality parameters.

Parameters measured in-situ using a handheld multi-parameter probe (Horiba water Checker model U-10) includes salinity, Hydrogen ion concentration (pH), turbidity, conductivity, dissolved oxygen. The air temperature was read with the aid of mercury in glass thermometer and the temperature of the surface water of the study site was also measured with the aid of a mercury glass thermometer by dipping the thermometer into the water body and the reading of the temperature from the scale was noted. The water sample was also taken to the laboratory to measure the phosphate, nitrate, alkalinity, Biological Oxygen Demand (BOD).

BIOCHEMICAL PARAMETERS

Malondialdehyde (MDA)

The levels of homogenized tissue MDA, as an index of lipid peroxidation were determined by thiobarbituric acid reaction (TBARS Assay). In this method, malondialdehyde is measured spectrophotometrically at absorbance levels of 535 nm to assay for the extent of lipid peroxidation in a sample.

ENZYMES ACTIVITY ASSAYS

Superoxide Dismutase (SOD)

The SOD enzyme assay determined the difference between superoxide anion decomposition and production i.e. its ability to inhibit the autoxidation of epinephrine. Enzyme activity was monitored at absorbance level of 450 nm. Concentrations are expressed as SOD Unit/ mg protein or U/mg, where one unit is defined as the amount of enzyme needed to inhibit 50% epinephrine reduction per minute and per milligram of protein at 25^0C and pH 7.8.

Reduced Glutathione (GSH)

Glutathione (GSH) was determined in the 10, 000 g supernatant fraction of the liver and gills homogenates of two fishes accordingly at 412 nm using 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB).

Total Protein Estimation

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

BLOOD ANALYSIS

Blood Collection and Haemological Examination

Blood samples were collected through the gill with the use of 5ml syringe and needle that has been treated with anti-coagulant such as heparin to prevent clotting into small sampling bottles containing Ethylene diamine tetra-acetic acid (EDTA). After the collection, the blood samples were taken to the haematological laboratory of Nigerian Institute for Medical Research (NIMR), Yaba, Lagos where the haematological analysis was carried out.

Determination of Erythrocyte Count (RBC)

This was done by using colorimetric methods using spectrophotometer (spectrum lab S23A). Pawinski solution was used, which was prepared from the following; Anhydrous sodium sulphate (Na_2SO_4)-26.7g, Sulphosalicyclic acid (SSA) - 2.0g, Distilled water-1000ml. This test was done within 20minutes to 4hours. 10microlitre of the blood sample was mixed with 5ml of the diluting

fluid and was mixed thoroughly. It was read at wavelength 600nm. Normal Range is within 1.1 to I.8T/l.

Determination of Haemoglobin (Hb)
Haemoglobin content was analyzed using the cyanohaemoglobin method. 20 microlitre of blood was mixed with the diluting fluid. It was read after 3minutes at 540-546nm against the diluting fluid as blank. Extra polated from the standard curve expressed in g/1.Ranges in 60-100g/l.

Determination of Haematocrit Value (PCV)
The freshly collected blood of *Sarotherodon melanotheron* were sucked with capillaries to about 2/3% of their weight and the clean end were sealed over a burner. The capillaries were placed into centrifuge (at speed of 1400rpm) and then left to centrifuge for 3minutes. After centrifuging, the haematocrit percentages were directly read on the haematocrit meter which is a part of the haematocrit centrifuge set. The percentage value obtained in this way is multiplied by co-efficient of 0.01 and the result value is the Haematocrit (PCV) value in liter.

Mean Corpuscular Volume (MCV)
The value of the MCV can be obtained from the haematocrit value (PCV) expressed in L/L and from the erythrocyte count (Er) expressed in T/L.

$$MCV = \frac{PCV \times 1000}{RBC\ T/L}$$

MCV is expressed in fentolitres (FL)
Ranges 200-300FL or 350-400FL.

Mean Corpuscular Haemoglobin
Mean corpuscular haemoglobin expresses the average haemoglobin concentration in individual erythrocytes and is given in picogrammes Pg ($10g^{-12}$). It is calculated from the haemoglobin value in g/1 and from the erythrocyte count (Er)/RBC in T/L according to the following formula: ffb(gil)

$$MCH = \frac{Hb\ (g/l)}{RBC'(T/L)}$$

Mean Corpuscular Haemoglobin Concentration (MCHC)
The mean corpuscular haemoglobin concentration expresses the concentration haemoglobin in unit volume of erythrocytes. It is calculated from the haemoglobin value (Hb) g/1 and from the haematocrit value (PCV), expresses in L/L according to the formula below:

$$MCHC = \frac{Hb\ (g/l)}{PVC\ (L/L)} \times 1000$$

Determination of white blood cell (Leucocytes)
This is done with the use of a counting chamber (Weubauer counting chamber). The counting chamber has a 4 horizontal and 4 vertical boxes when viewed under the microscope.
The solution is allowed to stand for at least 2 weeks and should be filtered before used. 25µl of blood is diluted in 4975µl of the diluting fluid (1:200 dilution), using the counting chamber, the cells are counted and multiplied by 0.5 to give the leucocyte count.

Determination of Differential leucocyte Count

This examined the total lymphocytes, monocytes, neutrophil granulocytes, eosinophil granulocytes and basophil granulocytes.

The proportion of each leucocyte is of diagnostic importance. A drop of blood was spread thinly (Smear) and evenly. It was then fixed and stained with Giemsa - Romanowski staining agent was used at a dilution ratio of 1:40 (1 part of stains, 40 parts neutral distilled water) for 15 - 20 minutes. The stain was then washed off. Using dropper, a film of oil was dropped and allowed to smear evenly over the slide. The slide was then mounted on a microscope and was viewed using X 100 objective lens. The differential leucocyte cells were counted in hundred and the number of the difference cells was expressed in their percentages.

STATISTICAL ANALYSIS

All Data obtained from the study were subjected to descriptive statistics to establish means and standard deviations, one-way analysis of variance for identification of significant variation and Pearson correlation (at 0.05) for identification of useful relationship, using SPSS 18.0.

RESULTS

PHYSICO-CHEMICAL PARAMETERS OF WATER

The physico-chemical analysis of water sample from the Lagoon is shown in Table 1.

Air temperature had a range of 23- 27°c with a mean value of 25.75±1.44°c throughout the period of study(table 1). Water temperature values showed a slight difference from air temperature with a mean value of 25.17±0.75°c with a minimum and maximum value of 24°c and 26°c between week 1 and week 6 of the study respectively. Salinity had a mean value of 0.15±0.1‰ with a maximum value of 0.3‰ in week 5. pH had a mean value of 6.13±0.82 while conductivity showed a mean of 3.60±1.49ms/cm 1.50 and 5.20ms/cm as minimum and maximum values for week 1 and week 2 respectively. Turbidity had a maximum value of 46FTU in week 4 and a minimum value of 26FTU in week 1, 2 and 5, mean value was 31.00±8.37FTU.

Dissolved oxygen for the study period had a mean value of 1.60mg/l. Week 6 had the lowest value of 1.1mg/l while the highest value of 1.8mg/l for the period of the study was recorded in week 5. Alkalinity had a mean value of 68.07±22.92mg/l. Nitrate, phosphate and sulphate had mean values of 6.77±3.90mg/l, 0.53±0.55mg/land 0.84±0.37mg/l respectively. COD had a maximum value of 333mg/l in week 4, with a mean value of 307.67±27.30mg/l. BOD values ranged between 97.80 and 133.00mg/l. It had a mean value of 117.19±14.61mg/l.

Table 1: Mean and standard deviation of the Physico-Chemical parameters of the Lagos Lagoon from July to September

	Mean±SD	Minimum	Maximum
Air temp (°c)	25.75±1.44	23.00	27.00
Surface Water temp (°c)	25.17±0.75	24.00	26.00
Salinity(‰)	0.15±0.10	0.00	0.30
pH	6.13±0.82	5.20	7.20
Conductivity (mS/cm)	3.60±1.49	1.50	5.20
Turbidity (FTU)	31.00±8.37	26.00	46.00
Dissolved Oxygen (mg L^{-1})	1.60±0.26	1.10	1.80
Alkalinity (mg L^{-1})	68.07±22.92	45.20	96.40
Nitrate (mg L^{-1})	6.77±3.90	2.44	13.58
Phosphate (mg L^{-1})	0.53±0.55	0.12	1.65
Sulphate (mg L^{-1})	0.84±0.37	0.46	1.35
COD (mg L^{-1})	307.67±27.30	271.00	342.00
BOD (mg L^{-1})	117.19±14.61	97.00	133.00

CORRELATION BETWEEN PHYSICO-CHEMICAL PARAMETERS

From the correlation matrix Table (as shown in Table 2), dissolved oxygen correlates negatively with water temperature (r = -0.408). COD showed a negative correlation with dissolved oxygen (r =-0.337) likewise a negative correlation existed between BOD and dissolved oxygen at (r = -0.189). A positive correlation (r = 0.367) occurred between turbidity and dissolved oxygen. Dissolved oxygen correlate positively with pH at (r =0.132). Water temperature correlated negatively with pH (r =-0.194). Conductivity showed a positive correlation with salinity = 0.633). pH and phosphate correlated negatively (r = 0.451).

BIOCHEMICAL PARAMETERS

MALONDIALDEHYDE - (MDA) (N mol/ml)

The mean MDA in the blood of *Sarotherodon melanotheron* caught from the Lagos lagoon at different weeks within six (6) weeks (July - September) ranged from 45.26 to 139.79nmol/ml (Table 2). The analysis of variance (ANOVA) results of the lipid peroxidation assay indicates that the level of MDA in the blood of the fish at the Lagos Lagoon shows that there was a significant difference (P < 0.05). Further ANOVA post-hoc test using DMRT showed there was a significant difference (P < 0.05) in the MDA of the fish.

SUPEROXIDE DISMUTASE – (SOD) (Min/mg/pro)

The mean SOD in the blood of *Sarotherodon melanotheron* caught from the Lagos lagoon at different weeks within (6) weeks (July - September) ranged from 26.84 to 39.94 min/mg/pro (Table 2). The analysis of variance (ANOVA) result of the Superoxide Dismutase assay indicated that the level of SOD in the blood of the fish at the Lagos lagoon showed that there was no significant difference ($P > 0.05$). Further ANOVA post-hoc test using DMRT showed there was no significant difference ($P > 0.05$) in the SOD of the fish.

ESTIMATION OF REDUCED GLUTATHIONE - (GSH) (µmol/ml)

The mean GSH in the blood of *Sarotherodon melanotheron* caught from the Lagos lagoon at different weeks within six (6) weeks (July - September) ranged from 0.53 to 0.82µmol/ml (Table 2). The analysis of variance (ANOVA) result of the reduced Glutathione assay indicated that the level of GSH in the blood of the fish at the Lagos lagoon showed that there was no significant difference ($P > 0.05$). Further ANOVA post-hoc test using DMRT showed there was no significant difference ($P > 0.05$) in the GSH of the fish.

TOTAL PROTEIN (g/l)

The mean total protein in the blood of *Sarotherodon melanotheron* caught from the Lagos lagoon at different weeks within six (6) weeks (July - September) ranged from 33.85 to 42.67g/l (Table 2). The analysis of variance (ANOVA) result of the total protein assay indicated that the level of GSH in the blood of the fish at the Lagos lagoon showed that there was no significant difference ($P > 0.05$). Further ANOVA post-hoc test using DMRT showed there was no significant difference ($P > 0.05$) in the total protein content in blood of the fish.

RESULTS OF HAEMATOLOGICAL INDICES

The results of the haematological indices obtained from week 1 to week 6 during the study are shown in table 3. Mean haemoglobin value was 39.78±14.43g/l with minimum and maximum values of 16.0 and 73.0g/l in week 4 and week 1 respectively. PCV value showed a mean of 0.16±0.16L/L while RBC mean value was 2.13±1.47T/L. Minimum and maximum values were 0.70 and 5.30T/L respectively. WBC values ranged between 2.88 and 11.22G/L with a mean value of 6.22±2.66G/L. For the period of sampling MCV values ranged from 28.26 to 114.28f/L with a mean value of 73.39±30.19f/L.

MCH values ranged from 9.35pg in week 3 to 44.20pg in week 2. MCHC ranged between 228.37 and 333.33L/L with mean value of 324.19±24.04L/L for the period of study. Neutrophils and Lymphocytes showed mean values of 28.83±6.46% and 70.78±6.53% respectively, monocyte counts showed a mean value of 0.39±0.69% with a maximum value of 2.00% in week 1. Eosinophils and Basophils count had zero mean value with no range value.

Table 2: ENZYMATIC BIOMARKERS OF BLACK JAW TILAPIA (*Sarotherodon melanotheron*) IN THE LAGOS LAGOON FROM JULY TO SEPTEMBER

	Enzymatic Biomarker in Black Jaw Tilapia (*Sarotherodon melanotheron*)			
	PRO (g/l)	SOD (Min/mg/pro)	MDA (Nmol/ml)	GSH (µmol/ml)
WEEK 1	36.65±2.13[abc]	26.84±1.71[a]	139.79±16.27[d]	0.53±0.06[a]
WEEK 2	35.55±1.85[ab]	32.85±6.16[a]	115.95±1.80[c]	0.57±0.05[a]
WEEK 3	42.24±0.94[bc]	29.02±1.58[a]	119.27±4.98[cd]	0.72±0.03[bc]
WEEK 4	33.85±1.00[a]	37.60±3.98[a]	99.85±4.52[c]	0.81±0.06[c]
WEEK 5	42.67±2.77[c]	39.94±6.32[a]	45.26±1.25[a]	0.66±0.02[ab]
WEEK 6	40.87±3.05[bc]	37.88±6.35[a]	74.61±2.40[b]	0.82±0.05[c]
MEAN±SD	38.64±1.97	34.02±4.35	99.12±5.20	0.67±0.05

Pooled standard error; Means in the same column with the same superscript are not significantly different from each other.

Table 3: Mean and standard deviation of the Haematological Indices of Tilapia *(Sarotherodon melanotheron)* from July to September, 2012

Parameters	Mean±SD	Minimum	Maximum
Hb (g/l)	39.78±14.43	16	73
PCV (L/L)	0.16±0.16	0.07	0.80
RBC (T/L)	2.13±1.47	0.70	5.30
WBC (G/L)	6.22±2.66	2.88	11.22
MCV (fl)	73.39±30.19	28.26	114.28
MCH (pg)	24.57±11.07	9.35	44.20
MCHC (L/L)	324.19±24.04	228.57	333.33
NEUT (%)	28.83±6.46	17.00	45.00
LYMPH (%)	70.78±6.53	55.00	82.00
MONO (%)	0.39±0.69	0.00	2.00
EOS (%)	0.00±0.00	0.00	0.00
BAS (%)	0.00±0.00	0.00	0.00

DISCUSSION

Mean water temperature of 25.73±1.44°C in the study was in accordance with the range (20-30°C) suggested by the Federal Environment Protection Agency (FEPA, 1999) for optimum physiological state of fish. The trend in the relationship of blood characteristics of fishes with environmental variables is specie-specific especially with respect to tilapia species as many researchers have reported the effect of environmental influences on haematological characteristics of fish. Some of the most important factors are temperature which causes changes which were observed in fish blood (Houston, 1980), day-length which was shown to affect oxygen transport system. Dissolved oxygen is required for the metabolism of aerobic organisms and it is often used as an indicator of water quality. The amount of dissolved oxygen depends highly on temperature. Dissolved oxygen values generally showed a decreased value with a mean value of 1.60±0.26 mg/l which is quite below the 3-5mg/l of oxygen for survival of fish. The reason for this hypoxic condition may not be far-fetched as this could likely be as a result of contaminants. This is depicted in the mean values of BOD and COD which were

recorded as 117.19±14.81 mg/l and 307.67±14.62mg/l respectively. These values are higher than the range for water suggested by (FEPA, 1999).

The lipid peroxidation and antioxidant biomarkers in the blood of *Sarotherodon melanotheron* showed a significant difference ($P < 0.05$). Reduced glutathione (GSH) is considered one of the most important antioxidant agents involved in protection of cell membranes from lipid peroxidation by scavenging oxygen radicals (Meister,1983). Moreover, glutathione is the cofactor of many enzymes catalyzing the detoxification and excretion of several toxic compounds. The low concentration of the antioxidant biomarker (SOD and GSH) observed in the blood of *S. melanotheron* in this study might be the reason for elevated concentration of lipid peroxidation found in the blood of the fish. Several studies have shown enhanced lipid peroxidation in aquatic organisms exposed to high concentrations of pollutants and of pollutants in contaminated sediments (Livingstone, 1993; Sole *et al.,* 1996). Elevated lipid peroxidation was observed by Wilhelm Filho *et al.* (2001) in cichlid fish taken from polluted sites, compared to clean sites. Oakes and Van Der Kraak (2003) and Oakes *et al.,* (2004) have reported increase in lipid peroxidation in gonads of white suckers exposed to pulp and paper mill effluents as well as municipal sewage treatment plant effluents. Increase in lipid peroxidation was observed in naphthalene exposed marine crab *Scylla serrata* by Sole e*t al.,* (1996).

Lipid peroxidation expresses the oxidative damage in a biological system. Oxidative damage set in when there is no equilibrium between the reactive oxygen species (ROS) generated as a result of bioaccumulation of contaminant and the antioxidant biomarker response. Alternatively, the ROS overwhelm the production of antioxidant biomarkers. The elevated lipid peroxidation concentration observed in this study is due to pollutants exposure which might be due to the microsomal metabolism of xenobiotic and microsome mediated redox cycling which gives rise to oxyradicals capable of oxidizing membrane lipids. Superoxide dismutase appears to be an important agent of toxicity of oxygen and this provides a defense against this aspect of oxygen toxicity (Kadar *et al*., 2005). The apparent increase in glutathione levels in the organs suggests an adaptive and protective role of this biomolecule against oxidative stress induced by the anthropogenic activities. Oxidation of proteins is of importance in regulating protein function within the cell. Many proteins undergo regulatory steps altering their oxidative status prior to release from the endoplasmic reticulum or Golgi apparatus. Protein modifications can also take place in the cytoplasm, either as a result of the action of regulatory enzymes or ROS. Protein kinases may bind or unbind regulatory proteins, thereby altering activity (Thannickal and Fanburg 2000).

Haematological parameters are closely related to the response of the animal and to the environment, an indication that environment where the fish lives exert some influence on the haematological characteristics (Gabriel *et al*., 2004). Haematology and clinical chemistry analysis, although not used regularly, can provide substantial diagnostic information once reference values are established. Unfortunately, reference values are not used on a routine basis in fish and the number of studies in which reference intervals have been determined for fish is limited (Hrubec *et al.*, 2000).

However, it is well known that blood sampling, laboratory techniques, seasoned variation, size, genetic properties, sex, population density, lack of food supply, environmental stress and transportation could affect haematological data (Arnold, 2005). Hence, comparison of reference interval should be done with caution in respect to variation in environmental condition. With respect to the haematological value of *S. melanotheron* from Buguma Creek reported by Gabriel *et al*., (2007b), it is apparent that a marked deviation of haematological indices for this study is evident. This is in resonance with the position of Mulcittrick and Leatherland (1983) who stated that a change in the water quality characteristics in specific area where there is high fish population could affect their haematological indices.

S. melanotheron depicts its haematological requirements of high oxygen demand to meet up with high metabolic rate. However, the reduction in the mean value of haemoglobin (Hb), haematocrit (PCV) and red blood cell (RBC) recorded for *S. melanotheron* in this study could be attributed to the environmental stress as revealed from the values of dissolved oxygen, BOD, COD and pH. Although a low correlation existed between pH and haematocrit (PCV) for this study, changes in pH of aquatic environments are of great concern following the declining catch of fin and non-fish species which often times been attributed to altered water quality. Some studies have implicated nutrient enrichment, increased heavy metals and presences of pesticides to reduced pH of aquatic medium (FAO, 1997).

Throughout the period of the study, pH values ranged from 5.2-7.20. Chindah *et al*., (2008) observed a gradual increase in whiteblood cells of *S. melanotheron* at pH 3.8, 4.0, 5.0 and 6.0. Significant increase in value for haematological and mucus secretion of the gills of *S. melanotheron* is attributed to low pH condition. Secretion of mucus by the gills is an evidence suggesting irritation due to stress conditions (Omoregie *et al*., 1994). Low correlation between dissolved oxygen and haematocrit is evidently depicted in this study. The mean haematocrit (PCV) value of *S. melanotheron* recorded in this study is low when compared with that of *S. melanotheron* from Buguma Creek reported by Gabriel *et al*., (2007). This situation is similar to that experienced by Akinrotimi *et al*., (2010) on *T. guinensis*. Low PCV value can be attributed to reduction in red blood cell caused by osmotic changes (Alwan *et al.,* 2009). Comparison of white blood cells and differential counts in *S. melanotheron* in this study with those reported in *S. melanotheron* from Buguma Creek (Gabriel et al., 2007a) reveal marked reduced deviation of these indices. Reduction in neutrophil, lymphocytes and monocyte counts in *S. melanotheron* indicate a stress condition of the environment as shown by Gabriel *et al*., (2011). The mean corpuscular values are concerned with volume of the average erythrocyte indices; MCV and MCH mean values were below baseline value for *S. melanotheron* reported by Gabriel et al.,(2007b). Environmental factors such as low dissolved, high BOD and COD values recorded in this study may be implicated as the possible cause of alteration in the haematological characteristics of *S. melanotheron* in this study.

CONCLUSION

This study indicated that there was an alteration in antioxidant enzyme and lipid peroxidase activities in *S. melanotheron* blood which may cause biochemical dysfunction in this specie. In

addition, the results provided evidence that enzymic and non enzymic biomarkers of oxidative stress can be sensitive indicators of aquatic pollution.

It is however recommended that antioxidant biomarkers should be used for environmental monitoring in aquatic environment, since it gives an early warning signal of effect of xenobiotics on aquatic organisms at molecular level which helps to prevent the effect at organismal level. As the aquaculture industry expands, evaluation of haematological indices of *S. melanotheron* will enhance its culture by facilitating early detection of infectious disease and identification of sublethal conditions affecting production performance.

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