

HEPATIC ENZYME PROFILE OF BLACK JAW TILAPIA *Sarotherodon melanotheron*, IN RELATION TO WATER QUALITY IN THE LAGOS LAGOON

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ABSTRACT

Pollution of the aquatic environment is a serious and growing problem. Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment led to various deleterious effects on the aquatic organisms. The hepatic enzyme of black jaw tilapia, *Sarotherodon melanotheron* collected from the Lagos Lagoon were evaluated in relation to water quality parameters for a period of 3 months (July, August, September 2012). The physico-chemical parameters of water samples were monitored at both high and low tides. Serum from blood samples of *S.melanotheron* were analyzed for Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), and Aspartate Transaminase (AST). Results indicate that Mean SD ALT values were 6.112.21, 17.004.33 and 25.674.45, AST values were 19.934.69, 71.9422.87 and 98.2917.00 while ALP values were 23.4615.46, 45.549.76, and 66.7916.80 for July, August and September respectively. There was no significant difference ($P>0.05$) in enzyme level within the three months of study. AST showed strong positive correlation with ALT ($r=0.795$) and a weak positive correlation with ALP ($r=0.53$) while ALP showed weak positive correlation with ALT ($r=0.51$). The physico-chemical parameters of water were optimal at both high and low tides. There was a weak positive correlation between the condition factor and length ($r=0.32$), as well as condition factor against weight ($r=0.218$) and the correlation of length against weight showed a positive correlation ($r=0.928$). In this study, the results suggest that environmental stress was noticed in the Lagos lagoon indicating the increasing level of biochemical stressors at varying levels which had sub-lethal effects on the health of the fishes.

Keywords: Hepatic enzyme, *Sarotherodon melanotheron*, Water quality, Lagos Lagoon

INTRODUCTION

Persistent contamination of water bodies through continuous discharge of industrial effluents is common and can produce varying degree of physiological and biochemical changes in fish species (Gabriel *et al.*, 2010). Such disruptions have been monitored by assessing change in the activities of enzymes and electrolytes in the functional organs (liver, kidney, gill, spleen and muscle) in the exposed fish (Akinrotimi *et al.*, 2009). Enzymes of common interest include the Transaminase: Alanine transaminase (ALT) Aspartate transaminase (AST) and alkaline phosphatase (ALP). Whereas the commonly assayed

electrolytes include the anions Na^+ , K^+ , Ca^{2+} Mg^{2+} and cations Cl^- and HCO_3^- (Akinrotimi *et al.*, 2007). Aquatic pollution has been a major issue of concern in many parts of the world. The adverse effect of anthropogenic substances on the aquatic organisms range from lower to higher biota in the ecosystem (Akinrotimi *et al.*, 2007).

Effects at higher hierarchical levels are always preceded by earlier changes in biological processes, allowing the development of early warning enzymes and electrolytes. In an environmental content, enzymes and electrolytes promise as sensitive indicators demonstration that toxicants have entered organisms, have

distributed between tissues, and are eliciting a toxic effect at critical targets (McCarthy and Shugart, 1990). In this respect, it is also interesting to study the development and application of sensitive laboratory bioassays, based upon the responses of electrolytes and enzymes. Therefore, biochemical parameters have been used in order to assess exposure to and effects of environmental contaminants. Bioassays offer many advantages for comparing the relative toxicity of specific chemicals or specific effluents. However, toxicity tests also have serious limitations for biological monitoring, because most studies do not account for the effect of chemical specification in the environment. Kinetics and adsorption of chemical to sediment accumulation through food chains and modes of toxic action which are not readily measured as short term effects, Li and Faudak (2009), suggested the use of enzymes in toxicity tests as an attempt to line enzymes' responses to effects on life-history characteristics (e.g. survival and reproduction), which will provide a further foundation for the use of biomarkers in environmental assessment.

Fish can be found virtually everywhere in the aquatic environment and they play a major ecological role in the aquatic food webs because of their function as carriers of energy from lower to higher trophic levels. The understanding of toxicant uptake, behavior and enzymes and electrolytes responses in fish may, therefore, have a high ecological relevance. As demonstrated in many studies of relevance in fish species.

Pollution of the aquatic environment is a serious and growing problem (Sasaki *et al.*, 1997). Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment led to various deleterious effects on the aquatic organisms (McGlashan and Hughies, 2001). The varying degree of physiology and biochemical changes in fish species are being

monitored by assessing change in the activities of enzymes and electrolytes in the functional organs (liver, kidney, gill, spleen and muscle) in the exposed fish (Akinrotimi *et al.*, 2009). The Lagos lagoon is one of the meandering networks of lagoons and creeks found along the coastline of southern Nigeria. It has continued to be under intensifying pressure from pollution such as sawdust and petrochemical materials, untreated sewage, detergents and industrial effluents, petroleum products (Amund, 2000); sawdust and faecal pollution (Akpata, 2002).

Sarotherodon melanotheron is an curyhaline species of tilapia commonly found in the creeks, lagoons and estuaries, in the coastal regions of Nigeria and fish farms close to various industries, where effluents are continuously released into the aquatic environment which can be contaminated through run offs and channeling of water into the farms. This study was therefore carried out to assess the possible effects of effluents from manufacturing industries on the activities of enzymes and in the liver of *S. melanotheron* as a means of monitoring aquatic contamination.

MATERIALS AND METHODS

Description of study site

The Lagos Lagoon, a tropical estuarine lagoon with a surface area of 208km² is located between latitudes 6° 26'N and 6° 39'N and longitudes 3° 29'E and 3° 50'E (Figure 1). It is an open tidal estuary. The lagoon is fed in the north by Ogun River. The lagoon opens into the Atlantic Ocean via the Lagos Harbour. River Ogun its major source of water, discharges a large volume of water into the lagoon and as a result of this, the salinity is very low during the rainy season.

Lagos Lagoon is shallow in depth and in most places it is a little more than 1.5 metres deep. The lagoon borders the forest belt and receives input from a number of important large rivers draining more than 103,626 km of the country.

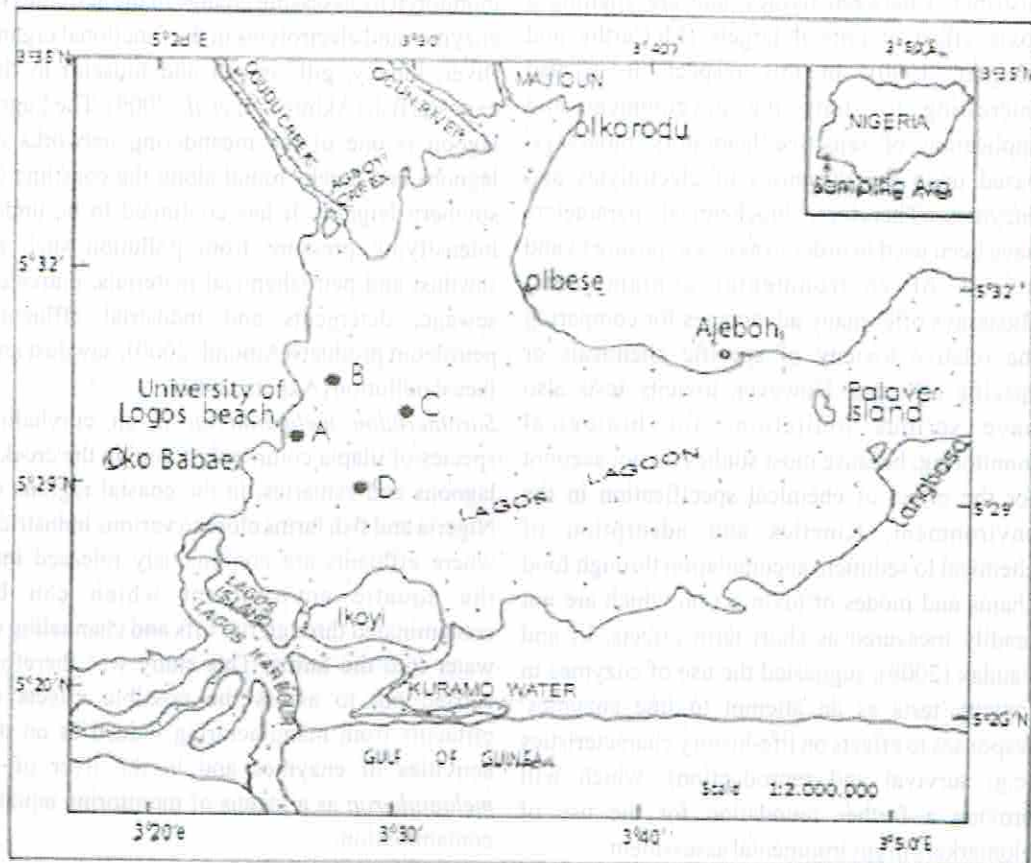


FIG 1: Map of Lagos Lagoon

FIELD PROCEDURES

Collection of sample organisms

Test organism collected for this study was the black jaw tilapia, *Sarotherodon melanocheilus*. Ten samples were collected from the Lagos Lagoon with weight and length between 15.98-22.27g and 8.403-7.0 respectively. They were collected in 25 litre bowl using a net and a portable aerator is placed in the bowl to preserve the fish.

Determination of physico-chemical parameters in study sites

Water samples used for this study were collected from the Lagos lagoon using a 1litre water sampling bottles. The bottles were then preserved in a cool dry place and transported to

the laboratory for further analysis of water quality parameters that could not be measured at the sampling site. Parameters measured in-situ using a handheld multi-parameter probe (Horiba Water Checker Model U-10) include; surface water temperature, air temperature, hydrogen ion concentration (pH), conductivity, dissolved oxygen, salinity, turbidity while others were measured in the laboratory. The pH of the water samples was determined on the field with the aid of a pH meter. This was done by dipping the probe in 1 litre of water sample and reading off the values from the meter. The surface water temperature of the water samples was determined in-situ by using a mercury-in-glass thermometer calibrated in degrees centigrade

(°C). The thermometer was immersed in the water sample for about four (4) minutes and the level of the mercury was read on the graduated glass tube.

Air temperature (°C)

The air temperature was determined in-situ using the mercury in glass thermometer calibrated in degree centigrade (°C). The thermometer was held in the open air for about three (3) minutes for equilibration then the values were determined. The conductivity of the water samples were measured using the Horiba water quality checker, model U-10. Water samples were put in sample cup and coupled with the probe, and readings were recorded from the meter. The salinity of the water samples was determined in-situ using a hand held refractometer. A drop of the water sample was placed on the prime of the meter of the refractometer and the meter was adjusted to 0‰ marks and viewed through the eye piece. The daylight plate was closed and the salinity of the water sample read on the scale. The turbidity of the water samples at the study site were measured using the Horiba water checker, model U-10 probe. The Dissolved oxygen level was, measured in-situ using a DO meter. The probe was placed in the water sample and the reading was recorded from the meter.

LABORATORY PROCEDURES

The Alkalinity (mg L^{-1}) was determined by titration method. Two drops of methyl orange was added to 50 cm^3 of the sample or approximately diluted sample in a conical flask over a white surface, and then titrated with 0.02 N HCl to faint orange equivalent point. The surface water Phosphate-phosphorus was determined using the ascorbic acid method. Sulphate (mg L^{-1}) was determined using titration method. Surface water Nitrate-nitrogen was determined gravimetrically. The Biochemical Oxygen Demand (BOD) for the water samples from the study area were determined taking the difference between the DO content of the samples on the day of the sample collection and then 5 days after the samples were collected. The water samples were incubated at 2°C for five days. Measurement of the 5-day DO was carried out using the same Horiba water

checker (Model U-10) used to measure the DO during the sampling period.

Estimation of liver enzyme in serum

Sample preparation

Blood samples were extracted from individual fish randomly. The blood samples were collected in heparin sample bottles to prevent the blood from clotting. The bottles containing the blood samples were then transported to the laboratory as soon as the blood was extracted but in cases when the blood could not be taken to the lab immediately, the bottles containing the samples are put in a container covered with ice block to serve as a form of preservation for a few hours till it is taken to the laboratory. In the laboratory, the blood was centrifuged at 3000rpm for ten minutes leaving a distinct separation in the blood sample with the supernatant which is the serum and the residue which is the plasma. The serum is then separated from the plasma for analysis of the activities of Aspartate aminotransaminase (AST) and Alanine aminotransaminase (ALT) using the Reitman and Frankel method, with reagents in the RANDOX assay kit.

STATISTICAL ANALYSIS

After the study, all the data obtained were analysed using ANOVA. The statistical methods used during this study include; mean and standard deviation, one-way analysis of variance for identification of significant variation and Pearson's correlation for identification of useful relationship.

RESULTS

Physico-chemical parameters

The mean of the result of the Physico-chemical parameters is presented in Table 1. The values in the table show the difference in low tides and in high tides. Correlation matrix (Table 2) shows that the correlation between salinity and conductivity was positive with high value ($r=0.643$). BOD showed positive correlation with DO ($r=0.493$). Alkalinity showed positive correlation with pH ($r=0.010$), water temperature showed negative correlation with Air temperature ($r=-0.817$).

Table 1: Mean \pm SD (Standard deviation) values for water quality parameters.

WATER PARAMETERS	MEAN VALUES	
	HIGH TIDE	LOW TIDE
AIR TEMPERATURE (°C)	25.19 \pm 0.71	26.58 \pm 2.56
WATER TEMPERATURE (°C)	25.67 \pm 1.21	26.83 \pm 1.72
SALINITY (‰)	0.18 \pm 0.15	0.16 \pm 0.12
PH	6.45 \pm 0.74	6.82 \pm 1.32
CONDUCTIVITY (mS/cm)	4.32 \pm 2.31	4.25 \pm 1.70
TURBIDITY (FTU)	27.67 \pm 7.53	32.67 \pm 12.11
DISSOLVED OXYGEN (mg/l)	1.75 \pm 0.15	1.77 \pm 0.69
ALKALINITY(mg L ⁻¹)	69.60 \pm 12.94	68.05 \pm 22.91
NITRATE (mg L ⁻¹)	6.15 \pm 3.34	6.31 \pm 4.04
PHOSPHATE (mg L ⁻¹)	0.54 \pm 0.52	0.53 \pm 0.57
SULPHATE (mg L ⁻¹)	0.79 \pm 0.34	0.77 \pm 0.28
BIOLOGICAL OXYGEN DEMAND (mg L ⁻¹)	120.50 \pm 18.88	114.17 \pm 12.38

Table 2: Correlations Matrix quality parameters

	Air temp	Water temp	Salinity	pH	Conductivity	Turbidity	DO	Alkalinity	Nitrate	Phosphate	Sulphate	BOD
Air temp	1											
Water temp	-0.817	1										
Salinity	-0.384	0.748	1									
pH	0.077	-0.179	-0.507	1								
Conductivity	-0.472	0.732	0.643	-0.089	1							
Turbidity	-0.188	-0.146	-0.511	0.018	-0.681	1						
DO	-0.373	0.762	0.941	-0.188	0.711	-0.613	1					
Alkalinity	0.020	0.265	0.671	0.010	0.205	-0.544	0.771	1				
Nitrate	0.210	-0.022	0.398	0.103	-0.185	-0.278	0.491	0.922	1			
Phosphate	0.672	-0.743	-0.447	0.531	-0.539	-0.149	-0.287	0.335	0.568	1		
Sulphate	-0.799	0.672	0.468	0.0419	0.171	0.179	0.510	0.459	-0.392	-0.240	1	
BOD	-0.584	0.831	0.457	-0.060	0.770	-0.134	0.493	-0.139	-0.430	-0.809	0.208	1

* Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

HEPATIC ENZYMES PROFILE OF

Sarotherodon melanotheron

Aspartate Aminotransaminase (AST)

The mean of AST, ALT and ALP recorded in the liver tissue of *Sarotherodon melanotheron* is presented in Table 3. Statistical analysis of variance (ANOVA) shows that there was no significant difference ($P > 0.05$) in the values of AST recorded. Further analysis on AST using LSD (Least square difference) with $N=10$ reveals that there was significant difference ($P > 0.05$) between July and September.

Alanine Aminotransaminase (ALT)

Statistical analysis of variance (ANOVA) showed no significant difference ($P > 0.05$) in the values of ALT recorded. Further analysis using

LSD on ALT (Least square difference) with $N=10$ revealed significant difference ($P > 0.05$) between July and September.

Alkaline Phosphate (ALP)

Statistical analysis of variance (ANOVA) showed no significant difference ($P > 0.05$) in the values of ALP recorded. Further analysis using LSD on ALP (Least square difference) with $N=10$ revealed significant difference ($P > 0.05$) between July and September.

CORRELATION OF HEPATIC ENZYME

AST showed strong positive correlation with ALT ($r=0.795$) as shown in Figure 2 and also showed a weak positive correlation with ALP ($r=0.53$) as shown in Figure 3. ALP showed weak positive correlation with ALT ($r=0.51$) as indicated in Figure 4.

Table 3: Mean Hepatic enzyme activity

PARAMETERS	JULY	AUGUST	SEPTEMBER
AST	19.93±4.69 ^a	71.94±22.87 ^a	98.29±17.00 ^a
ALT	6.11±2.21 ^a	17.00±4.33 ^a	25.67±4.45 ^a
ALP	23.46±15.46 ^a	45.54±9.76 ^a	66.79±16.80 ^a

Mean (SD, standard deviation) with the same superscript letter in a row are statistically the same in LSD test.

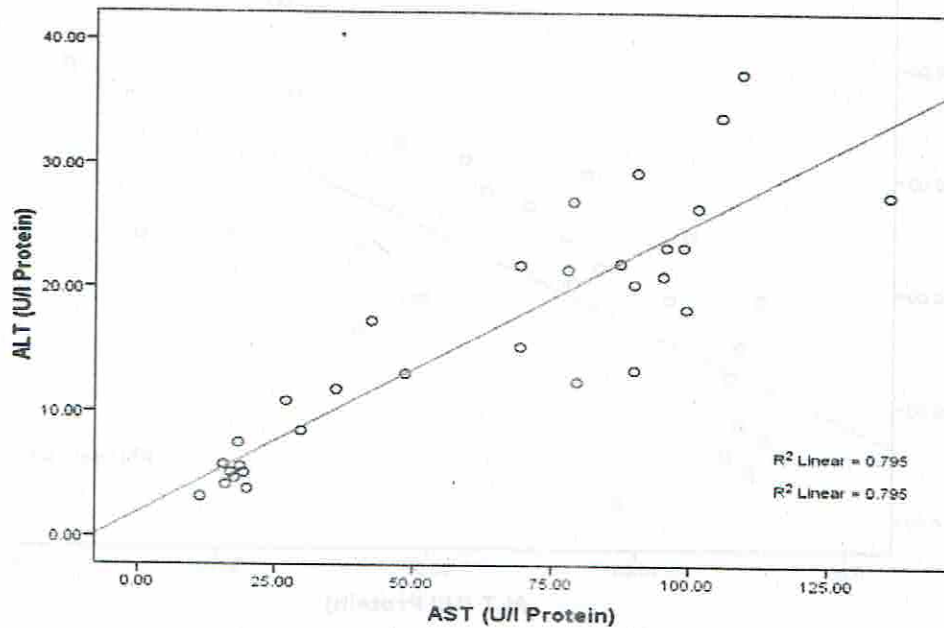


FIG 2: Relationship between ALT and AST

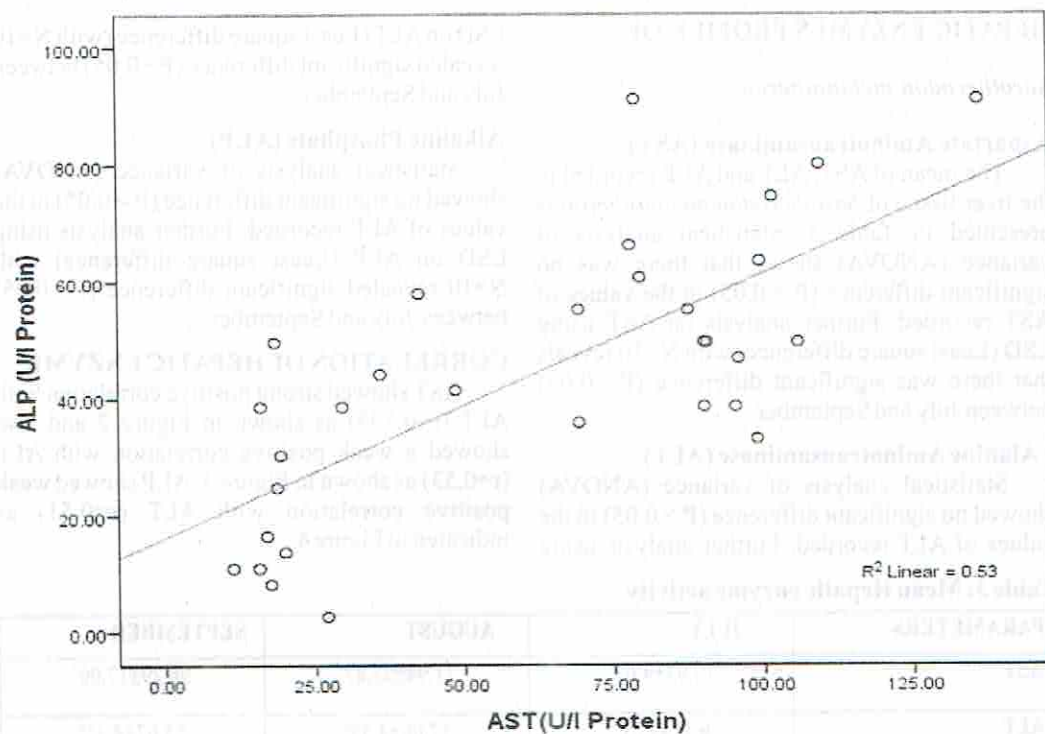


FIG 3: Relationship between ALP and AST

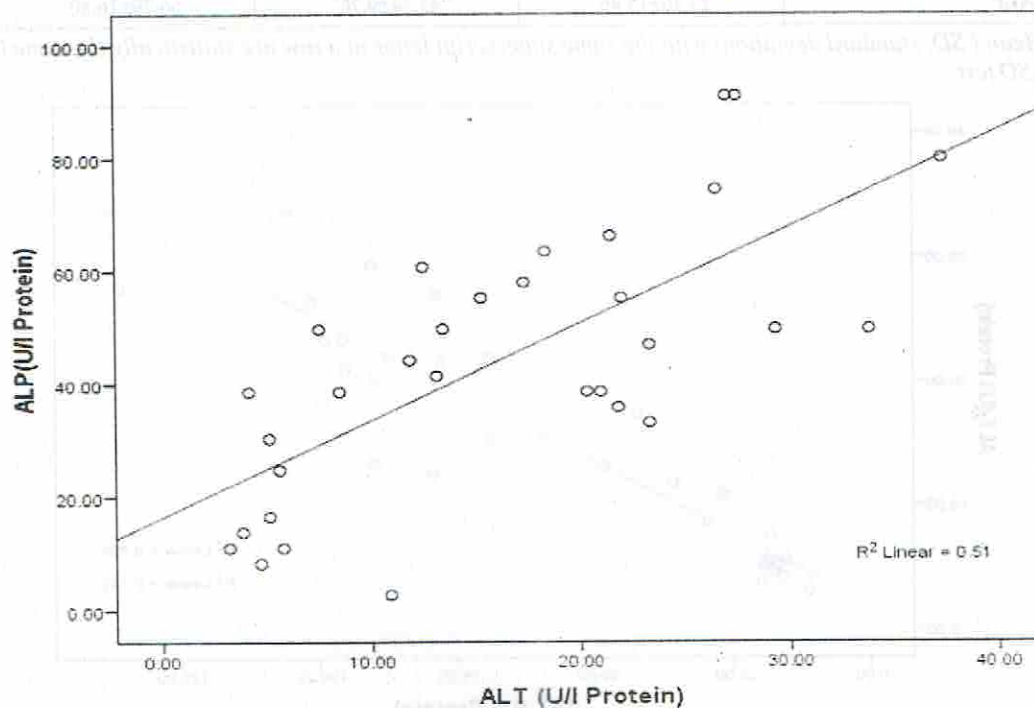


FIG 4: Relationship between ALP and ALT

DISCUSSION

The result of this work showed that temperature ranged from 24.00-30.00C which is optimal for growth of the fish under study (*S.melanotheron*). (Boyd, 1979)

Fish and other vertebrates have an average blood pH of 7.40 (Boyd, 1979). The desirable pH of Lagos lagoon was essentially neutral within a range (7.00-9.00) at the beginning of the study between July and August. The pH values throughout the sampling period varied slightly it ranged (5.2-8.8) between July and September. Fish may become stressed and die if the pH drops below 5.00(e.g. acidic) or rises above 10.00 (e.g. Low alkalinity combined with intense photosynthesis by dense algal bloom-phytoplankton or filamentous algae (Boyd, 1979).

Low oxygen level is usually the result of high oxygen demand caused by allochthonous input, mainly fallen leaves of the riparian vegetation (Welcome, 1979). High dissolved oxygen concentration is usually associated with peak rainfall season when nutrient and debris are brought into the lagoon with the influx of fresh water from inland rivers. Concentrations below 5 mg/l may adversely affect function and survival of biological communities and below 2 mg/l can lead to death of most fishes. D.O values ranged from 1.2-1.7 mg/l which is below the range reported by Water Quality Assessments (1996) indicating that the function and survival of the fishes have been adversely affected. The effect of toxicants on enzymatic activity is one of the most important biochemical parameters, which is affected under stress. When an organ is diseased due to the effect of a toxicant, enzymes activity appears to be increased or it may be inhibited due to the active site being either denatured or disturbed. Since some enzymes catalyze a few steps in the metabolism of carbohydrates and protein, they are present in most tissues. The increase or decrease in their level may be sufficient to provide information of diagnostic value (Valarmathi and Azariah, 2003).

Under stress conditions the body mechanism are altered to combat the effect of the pollutants/stressor in order to maintain equilibrium in the organism (Siva, 1980). Fish under stress mobilizes triglycerides and protein

to meet an increased demand for energy resulting from increased physical activity, bio-transformation and excretion of xenobiotic (Alhakem *et al.*, 1998).

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities suggest a decrease in energy demand, metabolic pathway and amino acids. Decrease in ALP activity in the organs (gill, kidney and liver) and muscle tissue could be attributed to a fall in the synthesis of glycogen imbalance caused by tissues over hydration. Through the 3 months of study, there was increase in the value of AST and ALT; an increase in the activities of AST and ALT recorded indicates that there was an increased demand for energy due to the tissue impairment (Ayalogu *et al.*, 2001; Tiwari and Singh, 2004), but there was no increase in ALP concentration through the project period. The ALP decreased is a measure of damaged or diseased liver. It is found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, and pancreas. Low amount of ALT are normally found in the blood. Increased ALT found in this study is indicative of hepatic dysfunction. Elevation of ALP, AST and ALT reflect hepatic disease, some inflammatory disease or injury to the liver hepatocellular damage (Ayalogu *et al.*, 2001)

CONCLUSION

The enzyme activity of *S. melanotheron* in Lagos lagoon seems to be increasing per month; this was enough to show that the environment was stressed although increase or decrease in enzyme activity could be caused by other factors such as energy demand, metabolic pathway and amino acids. The water quality parameters were good enough for growth and survival of the fish. This implies that the Lagos Lagoon is a good environment for the culture of *S. melanotheron*.

REFERENCES

- Amund O .O (2000). *The oil-eating microbes: a remedy to the menace of oil pollution*. An Inaugural Lecture delivered at the University of Lagos, Nigeria. Pp70
- Akinrotimi, O.A.; Abu O.M.G, Ansa E.J, Edun O.M. and George OS (2009) Haematological responses of *Tilapia guineensis* to acute stress. *Int.J Nat Appi Sd.* 5:338 - 343.

- Akinrotimi, O. A.; Ansa, E. J.; Owhonda, K. N.; Onunkwo, D. N.; Anyanwu, P. E.; Edun, O. M.; Opara, J. Y. & Cliffe, P. T. (2007b). Effects of transportation stress on haematological parameters of black chin tilapia *Sarotherodon melanotheron* *Journal of Animal and Veterinary Advances* 6(7):841-845.
- Akpata, T.V.I., (2002). Aquatic microbes: Impact on man and environment. Inaugural lecture. University of Lagos Press Publication. 40pp.
- Alkahem, H.F., Ahmed, Z., Al-Akel, A.S. and Shamsi, M.J.K., (1998). Toxicity bioassay and changes in the haematological parameters of *Oreochromis niloticus* induced by trichloform. *Arab Gulf. J. Sci. Res.* 16: 581-593.
- Ayalogu, O.E., Igbon, N.M. and Dede, E.B., (2001). Biochemical changes in the serum and liver of albino rats exposed to petroleum samples (gasoline, Kerosene and crude petroleum). *J. Appl. Sci. Environ. Mgt.* 5(1):97-100.
- Boyd, C. E., (1979). Water Quality in Warm water Fish Ponds. Auburn University Agricultural Experiment Station Alabama. 356pp
- Gabriel, U.U.; Obomanu, F.G and Etori, O.S (2010). Biochemical changes in hybrid cat fish (*Heterobranchius bidorsalis*, *Clarias gariepinus*). Treated with Nuracron. *Chinese J. Appl. Environ. Biol.* 16 (3): 353-357.
- Li, Z.H, and Faudak T. (2009). Residual pharmaceutically active compounds (phacs) in Aquatic environment status, toxicity and cinetics: A Review *Vet. Med* 52: 295-314.
- Mc Carthy, J.F. and Shugart, L.R., (1990). Biological Markers of environmental contamination. In: Mc-Carthy, J.F., Shugart, L.R. (Eds). *Biomarkers of Environmental Contamination*. Lewis Publishers, Boca Raton, FL. USA. 3-16pp.
- McGlashan D. J., Hughies J. M., (2001). Genetic evidence for historical continuity between populations of the Australian freshwater fish *Craterocephalus stercusmuscarum* (Atherinidae) east and west of the Great Diving Range. *J. Fish Biol.*, 59: 55-67.
- Sasaki, Y. F., Izumiyama, F., Nishidate E, Ishibashi S, Tsuda S, Matsusaka N, Asano N, Saotome K, Sofuni T, Hayashi M (1997). Detection of genotoxicity of polluted sea water using shellfish and alkaline single-cell gel electrophoresis (SCE) assay: A preliminary study. *Mutat. Res.*, 393: 133-139.
- Siva, P.R.K., (1980). Studies on some aspects of metabolic change with emphasis on carbohydrate utility in the cell free system of the teleost, *Tilapia mossambicus* (Peters) under methyl parathione exposure Ph.D. Thesis, S.V. Univ., Tirupati, India.
- Valarmathi, S. and Azariah J., (2003). Effect of copper chloride on the enzyme activities of the crab *Sesarma quadratum* (Fabricus) *Turk.J.Zool.* 27: 253-256.
- Water Quality Assessment (1996). A Guide to Use of Biota, Sediments and Water in Environmental Monitoring. Chapman & Hall press. 651pp.
- Welcome, R.L., (1979). Fisheries Ecology of Flood Plain Rivers. London Longman press. 377pp.