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Alterations in Water quality, Enzyme levels and Haematology of *Oreochromisniloticus* (Nile Tilapia) from River Ogun at Abeokuta, Nigeria

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Abstract

Natural aqua-systems sometimes receive pollutants resulting from human, domestic, and industrial activities. Fish being sensitive to contaminants, are excellent indicators of pollution. Contamination of River Ogun at Abeokuta was determined by assessment of the water quality, enzyme and haematological indices of O. niloticusfrom three sites on the river while Agodi fish farm served as control site. Haematology and enzyme levels were determined from blood and serum of 72 experimental and 20 control fishes. Water quality differed significantly (p>0.05) at the three sites. Temperature and pH were within permissible limits while DO, BOD and water transparency were not. The weight and length of O. niloticus ranged from 58.74-304.2g and 14.8-25.17cm respectively. There were significant (P<0.05) decreases in RBC, Hband PCV, indicative of anaemia in the fishes while WBC was significantly (P<0.05) elevated compared to the control fish. Enzyme levels in experimental fish were also significantly (P<0.05) elevated. The altered haematological and enzymatic indices indicate the fish's response to environmental stress. Alterations in fish health are important because such changes may impact human health on consumption of these fishes. Adequate protective legislation and remedial practices are needed to protect the river and fish from continuous pollution.

Keywords: Haematology, liver enzyme, pollution, *Oreochromisniloticus*.

Introduction

In the last few decades, the contamination of freshwater by a wide range of pollutants has become a major concern[1-3]. Various organic and inorganic wastes in industrial and domestic effluents discharged intentionally or unintentionally into water bodies are responsible for water pollution. Most of these effluents contain toxic substances which have been reported to enter and accumulate in aquatic fauna and flora causing several physiological changes in them[4]. Aquatic animals live in very intimate contact with their environment thus; absorb pollutants from the surrounding contaminated water which ultimately affect their health. Among the aquatic animals, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants[5] and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components[6].

In order to evaluate the adverse effects of pollutants on aquatic organism, there is a worldwide trend to complement chemical and physical parameters with biomarkers in aquatic pollution monitoring[7]. Fish blood is known to exhibit pathological changes even before the onset of external symptoms of toxicity and it may truly reflects the physical and chemical changes occurring due to pollutant accumulation in the fish body[8]. In fish, exposure to chemical pollutants can induce either increase or decrease in haematological levels. Studies shows that fish blood provide the possibility of knowing the physiological conditions within the fish long before there is an outward manifestation of pathology/disease[9]. This is because under stressful condition as well as environmental imbalances some blood parameters change to reflect the changes in the environment.

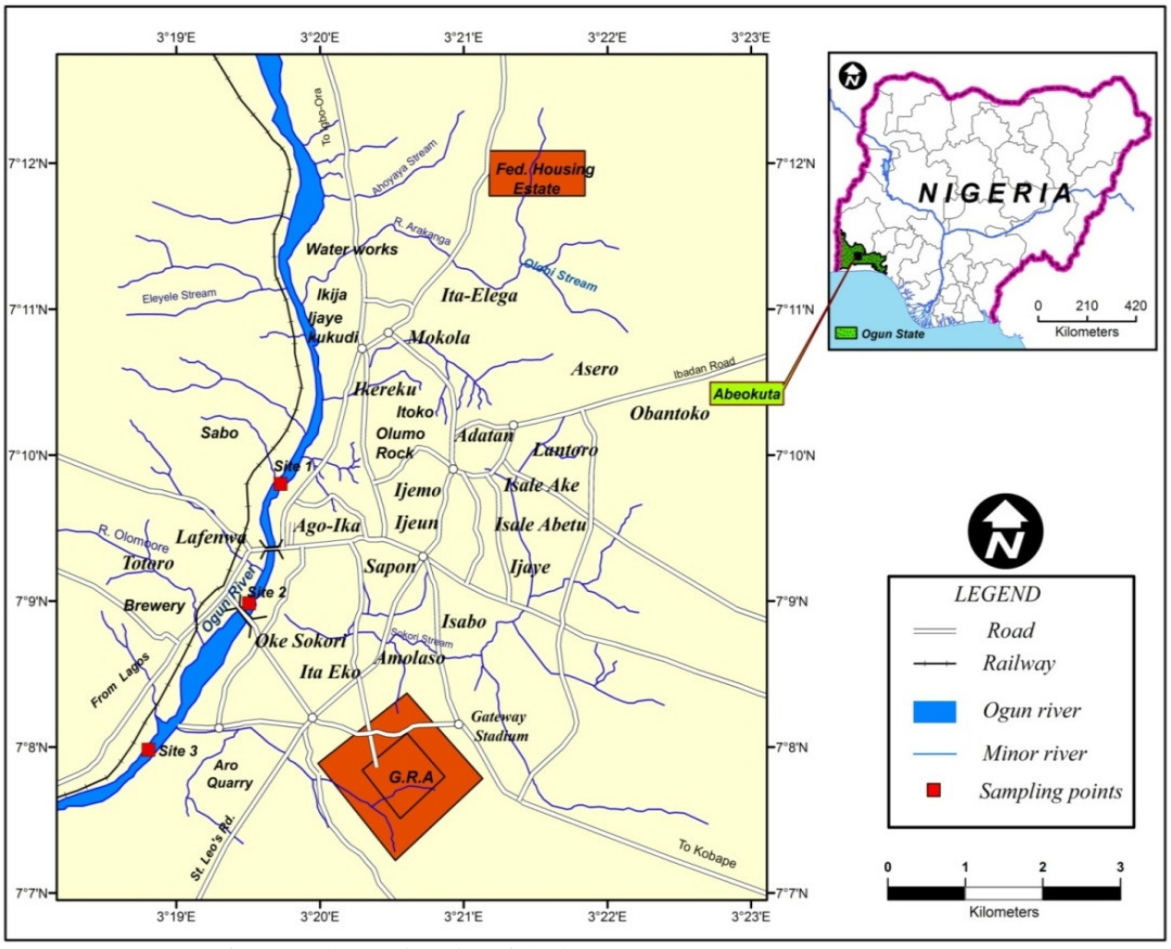
The fish liver plays an important role in metabolism and it is the major organ of accumulation, biotransformation and excretion of contaminants in fish[10]. The measurement of suitable biomarkers in the liver is useful and can provide information about the health and state of fish. Toxicological studies have shown that the impact of contaminants on aquatic ecosystems can be evaluated by measuring biochemical parameters in the liver of the fish[11]. Liver enzyme analyses have been used as biomarkers for environmental pollution[12] and histopathological changes have been reported in livers of fish exposed to a wide range of organic compounds.

The Ogun River at Abeokuta while serving as a source of fish for human consumption runs through urban areas exposing it to anthropogenic contaminants. Therefore the study was carried out to determine the state of the river by assessing some physico-chemical parameters, Haematologicalindicies and enzyme activity (Aspartate Aminotransferase, Alanine Aminotransferase, Alkaline Phosphatase and Gamma-glutamyltransferase) of *Oreochromisniloticus* at three sampling points along Ogun River at Abeokuta.

Material and Methods

Study Area and sampling sites: River Ogun is one of the major rivers in south western Nigeria with a total area of 22.4 km^2 and a fairly large flow of about 393 m^3secG^1 during the wet season. Ogun river rises in Oyo state near Shaki at coordinates 8^0 41`0"N/8.6833^0 N, 3.46667^{0}Eand flows into Lagos State through Ogun state. In this study, the three sampling sites chosen along the course of Ogun River at Abeokuta Ogun State, were based on their potential for contamination by domestic, industrial, municipal and agricultural wastes, and also based on easy accessibility to the people. Site 1 was located at Latitude 7.16232^{0}N and Longitude 3,33209^{0}E along the river (figure-1). At this site, there were few residential buildings and agricultural activities. Site 2 is at latitude 7.14913^{0}N and Longitude 3.32449^{0}E along Ogun River and it is located close to a major road with a few industries (Tower roofing industry, Arco foam factory, Gateway Portland cement) and some small scale block making industries. Also at this site there were mechanic workshops and car wash shops. Site 3 which is at latitude 7.13296^{0}N and Longitude 3.31325^0 E had agricultural activities involving the use of pesticides and fertilizer at the bank of the river. The surrounding community dumps domestic waste into the river, and also carries out laundry and processing of agricultural products at this site.

Fish collection: A total of ninety-two (92) Nile tilapia (*Orechromisniloticus*) were collected over a period of 3months (April-June 2014), twenty four (24) from each of the three sites and 20 control fishes from Government owned fish farm in Agodi, Ibadan, Nigeria. These samples were collected between 0700 and 1000 hours in the morning as recommended by Adebisi[13]. Fishes were randomly caught by fishermen using cast nets and transported to the laboratory in containers with the river water. Sexes of fishes were determined by the presence or absence of an intermittent organ on the ventral side just before the anal fin. This was later confirmed by the presence or absence of testes or ovaries during dissection. Morphometric information (length and weight) were obtained using standard methods.



Source: Department of Geography, University of Ibadan

Figure-1
Map of Abeokuta showing sampling points along Ogun River

Water Quality Analysis: Water samples were collected at the time of fish collection at each site into pre-cleaned plastic containers. The water sampling was done midstream by dipping each sample bottle into the water at approximately 20-30 cm below the water surface, projecting the mouth of the container against the water flow. The collected water quality was determined by assessing the following parameters: water temperature, pH, dissolved oxygen (DO), biological oxygen demand (BOD) and transparency. These parameters were measured using water checker U-10 Horiba. The values obtained were compared to WHO and NESREA permissible limits for rivers.

Blood Analysis: Blood Collection and Haematological Assessment: Blood (5ml) was collected from the caudal blood vessel of each *O. niloticus*and 2ml of the blood was introduced into EDTA bottles for haematological analysis while 3ml was placed in Lithium heparin bottles for enzyme assays. The bottles were quickly placed in iced chests and transported to Clinical Pathology Laboratory, College of Veterinary Medicine University of Ibadan Nigeria. Standard haematological procedures were employed[14,15] in the assessment of haemoglobin, packed cell volume, red blood cell, lymphocytes, Eosinophil, monocytes and platelets.

Estimation of Liver Enzyme in Serum: The blood in the lithium heparin bottles were centrifuged at 3000 rpm for 10 minutes to separate out the serum for enzyme analysis. The activities of AST, ALT and ALP were analysed with Randox assay kits using the procedure described by Reitman and Frankel[16]. The method described by Szasz[17] was employed for the assay of Gamma Glutamyl Transferase (GGT).

Statistical Analysis: Data were analyzed using one way analysis of variance (ANOVA) test and principal component analysis (PCA) and presented as mean ± standard deviation. All statistical analyses were done using SPSS (version 17.0 for Windows), differences between means were considered significant at $p \leq 0.05$.

Results and Discussion

Water Quality parameters: There were variations in the water quality at the three sites (table-1). Though the pH and temperature recorded in all three sites were within WHO and NESREA permissible limits for rivers, there were significant differences ($P<0.05$) in the values recorded at site 1 compared to sites 2 and 3. The BOD values also differed significantly ($P<0.05$) at the 3 sites and these values were significantly higher than the WHO and NESREA limits. The DO values recorded were also higher than NESREA limit but below the WHO limit for rivers, though there were no significant difference ($P>0.05$) in the levels of dissolved oxygen amongst the sites.

Morphometrics of O. niloticus: The mean weight and length of *O. niloticus* collected from different sampling sites of River Ogun and fish farm showed significant variations (table-2). The mean weight (127.31±21.41) and length (21.90±4.62) recorded from the control fish were highest compared to fish from the three experimental sites on Ogun River while, the mean weight and length of fish from site 2 were lowest.

Table-1
Physico-chemical Parameters of the Different Sampling Site in River Ogun

Parameter	Sampling Sites			Regulatory Limit	
	1	2	3	WHO	NESREA
Surface Water Temperature (^{0}C)	25.8±2.30^a	26.3±1.60^b	26.2±2.30^b	20-30	20-33
pH	7.2±0.20^a	6.8±0.50^b	6.9±0.50^b	6.5-9.5	6.5-8.5
Dissolved Oxygen (mg/l)	4.70±0.90^a	4.5±0.40^a	4.4±1.00^a	6.0	≥4
Biochemical Oxygen Demand (mg/l)	42.70±5.30^a	44.1±5.30^b	43.1±5.90^c	<4	6.0
Seechi Water Transparency (cm)	18.90±1.30^a	18.9±1.80^a	19.7±1.80^b	>80	nil

Results are expressed as Mean of the triplicate samples ± standard deviation. Mean values in same row with different superscript are significantly different ($P>0.05$)

Table-2
Morphometrics of *O. niloticusfrom* 3 sampling sites on River Ogun and the Agodi fish farm

Site	Number	Variable	Mean	Standard Deviation	Minimum values	Maximum values
Site 1	24	Weight (g) Length (cm)	106.41 18.20	14.32 5.71	65.80 16.70	146.87 22.30
Site 2	24	Weight (g) Length (cm)	83.47 18.80	11.53 6.74	58.74 15.40	126.20 23.70
Site 3	24	Weight (g) Length (cm)	94.72 17.26	23.96 7.14	67.10 14.80	112.90 19.20
Control (fish farm)	20	Weight (g) Length (cm)	127.31 21.90	21.41 4.62	92.00 17.74	304.20 25.17

Haematological Parameters: The haematological indices of fish from the control farm were significantly different from those obtained at the 3 experimental sites. Significantly ($p<0.05$) reduced PCV, Hb, RBC, platelet, lymphocyte, monocyte and eosinophil levels were recorded at the 3 sites compared to values in control fish (table-3). Also, the indices of fish from site 1 were significantly different from values obtained from fish at sites 2 and 3. The PCV of *O. niloticus* from Sites 2 (25.20±6.70) and 3 (25.70±4.10) were significantly ($P<0.05$) lower than values (29.70±2.20) obtained at Site 1. Significantly ($P<0.05$) lower Hb, RBC and Platelet values were recorded for *O. niloticus* from the 3 sites compared to the control. The result of the total WBC count showed a significant ($P<0.05$) elevation in the experimental fish compared to the controls. Fish from Sites 2 and 3 also had significantly ($P<0.05$) higher WBC ($17.00\pm2.90x10^3$µl and 16.80 ± 2.10 $x10^3$µl respectively) compared to fish from Site1 (11.34±1.20x103µl).

The differential WBC analysis showed significantly ($P<0.05$) elevated lymphocyte counts (62.10±1.70%) in *O. niloticus* collected from Site 1 compared to samples from Sites 2 (51.20±2.30%) and 3 (52.80±4.40%). The monocytes, eosinophil and basinophil levels of *O. niloticusfrom* Site 1 were also significantly ($P<0.05$) higher than the values obtained from sites 2 and 3 (table-3).

Table-3
Haematological Parameters of *O. niloticusfrom* the Different Sampling Sites

	Sampling Site			
Parameter	Site 1	Site 2	Site 3	Control
Packed Cell Volume (%)	29.70 ± 2.20^a	25.20 ± 6.70^b	25.70 ± 4.10^b	34.10 ± 3.70^c
Haemoglobin (g/dl)	9.30 ± 3.20^a	8.20 ± 2.30^b	8.60 ± 1.50^b	11.40 ± 1.20^c
Red Blood Cell (10^6µl)	3.40 ± 0.20^a	2.10 ± 0.60^b	2.20 ± 0.40^b	3.60 ± 0.40^c
Platelet (10^3µl)	158.20 ± 32.30^a	132.30 ± 24.20^b	139.20 ± 19.40^b	162.40 ± 49.20^c
White Blood Cell (10^3µl)	11.34 ± 1.20^a	17.00 ± 2.90^b	16.80 ± 2.10^b	9.30 ± 1.70^c
Lymphocyte (%)	62.10 ± 1.70^a	51.20 ± 2.30^b	52.80 ± 4.40^b	62.70 ± 10.10^c
Monocytes (10^3µl)	3.10 ± 0.80^a	2.30 ± 0.80^b	2.70 ± 0.50^b	4.00 ± 0.50^c
Eosinophil (10^3µl)	1.80 ± 0.20^a	2.40 ± 0.50^b	2.30 ± 1.00^b	1.90 ± 0.80^c
Basinophil (10^3µl)	0.70 ± 0.02^a	0.20 ± 0.09^b	0.30 ± 0.25^b	0.80 ± 0.05^c

Results are expressed as Mean of the triplicate samples ± standard deviation, Mean values in same row with different superscript are significantly different ($P>0.05$)

Liver Enzymes Analysis: The liver enzymes values of *O. niloticus* differed significantly (P<0.05) at all the sites. The values from all 3 experimental sites were significantly elevated (P<0.05) compared to values obtained from fish at the control site (table-4). The highest values for AST (205.70±55.20 IU/L), ALT (5.40±1.30 IU/L), ALP (244.40±140.50 IU/L) and GGT (2.10±1.40 µmol/ml) were recorded at site 3 while samples from Site 1 had the lowest AST (152.80±21.50 IU/L), ALT (5.00±1.60 IU/L), ALP (213.20±87.20 IU/L) and GGT (1.20±0.20 µmol/ml) values.

Principal Component Analysis (PCA) of Data: The Eigenvector and variance proportions of the physico-chemical, haematology and enzyme parameters are shown in table-5. The variables analyzed were grouped as components PC 1 (Haematology factor) and PC 2 (Enzyme factor) and these factors occur independently. The data obtained showed an association between temperature, pH, BOD and haematological parameters while DO and water transparency showed associations with enzyme level alteration. There were positive inter-relationships between most haematological parameters analyzed. The WBC and Eosinophil showed negative relationship with the other haematology parameters. There was a positive relationship between the analysed liver enzymes.

Discussion: Water quality parameters: Changes in water quality of an aquatic environment could have an impact on the aquatic life in such water bodies. Dissolved oxygen is an important indicator of water quality, ecological status, productivity and health of a reservoir. Decreased DO levels in rivers are reported to be partially due to displacement of dissolved oxygen by dissolved solids within effluents[18] while low levels of DO are suggested to be caused by organic imputes from domestic and industrial wastes[19,20]. The lower DO levels recorded in the study may be attributed to high levels of organic input from human activities such as agricultural, manufacturing and domestic activities occurring along River Ogun.

Biological Oxygen Demand (BOD) is useful in determining the relative waste loading in an aquatic system and higher levels in water is therefore an indication of the presence of large amounts of organic pollutants and a relatively higher level of microbial activities. Shakirat K.T. et. al. suggested[21] will consequently deplete the oxygen content of the water body. The highly elevated BOD values recorded for the three sampling sites on River Ogun at Abeokuta suggest gross organic pollution exceeding the recommended maximum allowable concentration (RMC) (3.0-6.0 mg/L) set by the European Union for good quality water for fisheries and other aquatic life[22]. Similar reports of high BOD from Rivers Oni, Ona, Ogun, Ogunpa and Lagos Lagoon in Nigeria were indications of the high level of organic pollution in major Nigerian rivers[23].

The decrease in seechi water transparency obtained in the present study was comparable with previous studies on some selected rivers in South Western Nigeria[24,25]. This study was carried out during the rainy season; therefore the low seechi water transparency values recorded could be attributed to surface runoff carrying soil/silt and partially dissolved or undissolved organic matter into River Ogun at Abeokuta. Decreased water transparency is associated with rainy seasons, where run-offs bring in clay, organic inputs and other particles from the water shed into the water body[26].

The mean surface water temperature and pH recorded were within the permissible regulatory limits because temperature and pH of rivers are adjusted along the river course, since the water bodies have self-adjusting abilities[27]. The mean temperature and pH values for the three sampling sites of River Ogun studied were comparable with previous studies of some selected rivers in the south-western Nigeria[23, 28-30].

Table-4
Serum Enzymes levels of *O. niloticus* from Different Sampling Sites of River Ogun

	Sampling Sites			
Parameters	**Site 1**	**Site 2**	**Site 3**	**Control**
Aspartate Aminotransferase (IU/L)	152.80±21.50^{a}	171.30±42.60^{b}	205.70±55.20^{c}	106.90±70.90^{d}
Alanine Aminotransferase (IU/L)	5.00±1.60^{a}	5.20±1.10^{b}	5.40±1.30^{c}	4.90±1.60^{d}
Alkaline Phosphatase (IU/L)	213.20±87.20^{a}	234.40±125.10^{b}	244.40±140.50^{c}	123.50±57.90^{d}
Gamma Glutamyltransferase (µmol/ml)	1.20±0.20^{a}	1.90±0.70^{b}	2.10±1.40^{c}	0.60±0.08^{d}

Results are expressed as Mean of the triplicate samples ± standard deviation, Mean values in same row with different superscript are significantly different (P>0.05)

Table-5
Eigenvector and variance proportions of physico-chemical, haematology and enzyme parameters

Variables	PC 1	PC 2
Temperature	-.905	.425
Ph	.926	-.376
DO	.572	-.820
BOD	-.991	-.137
Transparency	.106	.994
PCV	.864	-.503
Haemoglobin	.966	-.258
RBC	.848	-.531
Platelet	.933	-.360
WBC	-.827	.563
Lymphocyte	.881	-.473
Monocytes	.994	-.105
Eosinophil	-.890	.455
Basophil	.905	-.425
AST	-.244	.970
ALT	-.407	.914
ALP	-.583	.812
GGT	-.666	.746
Eigenvalues	15.241	2.759
Variance (%)	84.671	15.329
Cumulative variance (%)	84.671	100.000

Haematology indices: Haematology is a useful index of fish health, detecting physiological changes following stress conditions such as exposure to pollutants, diseases, hypoxia[31]. The low levels of packed cell volume and haemoglobin concentration in the studied fishes are indicative of anaemic condition of the fishes. Such anaemic conditions have been reported to result from disruption in erythrocyte production[32,33], haemodilution[34], and destruction of intestinal cells involved in the production of vitamin B12 used in the production of the haemoglobin portion of the red cells[35]. The pollutants from the manufacturing, industrial and other human activities dumped into the river at the sampling sites may be responsible for such disruptions. The low value of PCV in fish exposed to stress could also be attributed to osmotic changes[36]. Similar observations of decreased haemoglobin concentration and packed cell volume as obtained in this study were reported In *Clarias gariepinus* and *Labeorohita* examined from various locations in Bisalpur reservoir in India[37]. The findings of this study was also consistent with the report of observed decreased PCV and Hb in some freshwater fishes *(Tilapia zilli, Labeocoubie and Synodontis membranaceous)* resulting from organic and inorganic wastes discharged into River Manyara, Nigeria[31].

The lower RBC and platelets values would also be attributable to the destructive action of pollutants released into the river, because decreased RBC values are reported to be indicative of accelerated destruction of the cells and hemolysis which occur in response to toxicity[38]. Such destructions results in alteration of the selective permeability of the cell membrane. Pollutants are reported to alter the properties of erythrocyte membranes and render them more fragile and permeable, which probably might result in cell swelling, deformation (erythrocyte elongation, roundness) and damage[39]. The industrial and automobile effluents discharged into the river at the sampling sites would most probably contain heavy metals which are known constituents of automobile and agricultural products. These could be partly responsible for the destructive activity on fish RBC. Similarly decreased RBC count in *Clarias gariepinus* exposed to metal fishing company effluent were reported [40] while exposure to pollutants were suggested to result in decreases in the total RBC count, and Hb content due to impaired intestinal absorption of iron[41,42].

The Increase in total WBC count observed in *O. niloticus from* sampling sites may be a result of direct stimulation of WBC production for defense due to the presence of pollutants. The changes in total white blood cells and differential WBC counts (neutrophils, lymphocytes and monocytes) recorded are indications that the fishes live in a stress condition similar increase in WBC were recorded in *Limandalinianda* which were believed to be the result of migration of white blood cells from the spleen to the blood circulation[43]. The elevated WBC levels reported By Saravanan and Harikrishnan[44] in *Sarotherodon mossambicus* exposed to sublethal concentration of copper were attributed to alteration in blood parameters as a result of the direct effects of pollutants. These observations are in agreement with the reports of Karuppasamy R et al[45] and Hardikar B.P. et al[46]. Progressive increased levels of total WBC count reported in *Clariasgariepinus* exposed to metal finishing company effluents [40] led to leukocytosis and the level of leukocytosis was directly proportional to severity of stress condition in fish. Increased WBC levels are therefore a result of direct stimulation of immunological defense in fish due to the presence of pollutants in their aquatic environment.

Reduction in lymphocytes, monocytes, eosinophils and basinophils counts in *O. niloticus* from the experimental sites are indicative of a stress condition. These lowered values might be associated with re-trafficking of cells to the lymphoid tissues which consequently leads to clearance of these cells from the blood stream as suggested by Harris and Bird[47]. It is known that cortisol secreted during stress reactions shorten the life span of lymphocytes promotes their apoptosis[48], and reduces their proliferation[49]. Therefore decreases in lymphocyte count, as well as activity are often observed effects of stress, irrespective of the stressing agent.

Liver enzymes: The elevated levels of AST, ALP, ALT and GGT recorded for *Oreochromis niloticus* from study sites suggests exposure of fish to pollutants. GGT catalyses the transfer of gamma glutamyl group of glutathione to acceptors and has been observed to be critical in detoxification process[50]. The high levels of GGT observed may therefore be due to an increase in the antioxidant status of the liver by recycling nutrients and amino acids, as well as conjugation of toxins with glutathione to detoxify harmful substances[51]. AST and ALT activities are stimulated by induced hepatic cell injuries which led to their leakage into circulation or their increased synthesis by the liver[52]. As in this study, significant increase in AST and ALT levels in *O. niloticus* were recorded from highly polluted rivers[53]. Also elevated activities of AST, ALT and ALP were reported in carps exposed to nitrite toxicity[54] and it was suggested that the elevation of the transferases are a result of the diversion of the alphamino acids in the TCA cycle to keto acids to augment energy production. Also the increases in activities of AST and ALT reported in the muscle and liver of common carp exposed to cyfluthrin may be due to a disturbance in the Kreb's cycle[55]. Decreased activity in the Kreb's cycle causes decreases in Kreb's cycle intermediates which are compensated for AST and ALT by the provision of α– ketoglutarate. Also Yildirim inferred that observed increase in enzyme activities (AST and ALT) in *Oreochromisniloticus* from exposed to deltamithrin increased the role of proteins in the energy production during toxicant stress[53].

Conclusion

Toxicants and pollutants cause physiological dysfunctions in fish which could be monitored by changes in blood parameters and enzymes activity. These make them predictive biomarkers for monitoring aquatic environmental pollution. The present investigation suggests that pollution induced stress does create enzymes level alteration and hematological disturbances in fish population. These subsequently affect the immune system, making the fish vulnerable to diseases. Therefore, the use of enzyme and haematological parameters as biomarkers might be an important approach in aquatic environmental health assessment.

It is also important that legislation be enforced to ensure that untreated domestic and industrial waste/effluents are not discharged into the river. This will prevent bioaccumulation of pollutants in aquatic fauna and subsequently in human consumers of fishery resources from the river.

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