MEDWIN PUBLISHERS
Committed to Create Value for Researchers

International Journal of Oceanography & Aquaculture
ISSN: 2577-4050

Alterations in Immunology in Three Sizes of Black Jaw Tilapia (*Sarotherodon melanotheron*) Exposed to Dimethoate in the Laboratory

Onwujiariri CE[1]*, Okeke PA[2], Onwujiariri CA[1] and Nwankwo AK[3]

[1]African Regional Aquaculture Centre/Nigerian Institute for Oceanography and Marine Research, Aluu, P.M.B. 5122, Port Harcourt, Rivers State, Nigeria

[2]Department of Fisheries and Aquaculture, Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

[3]Department of Mariculture, Nigerian Institute for Oceanography and Marine Research, Buguma, Rivers State, Nigeria

Research Article

Volume 8 Issue 2

Received Date: April 11, 2024

Published Date: May 14, 2024

DOI: 10.23880/ijoac-16000314

***Corresponding author:** Onwujiariri CE, African Regional Aquaculture Centre/ Nigerian Institute for Oceanography and Marine Research, Aluu. P.M.B. 5122, Port Harcourt, Rivers State, Nigeria, Email: emmanuelonwujiariri@gmail.com

Abstract

Three different sizes of Black Jaw Tilapia (*Sarotherodon melanotheron*) were subjected to dimethoate exposure in the laboratory, and changes in their immunology were observed. The fish were treated with the chemical for 15 days at five different concentrations: 0.00 (control), 0.05, 0.10, 0.15, and 0.20 mg/L. The trial involved the assessment of six water quality variables, including temperature, pH, salinity, dissolved oxygen, nitrite, and ammonia. After the experiment, blood samples from the fish were obtained, and standard laboratory procedures were used to look for immunological profiles in them. Based on all the parameters evaluated, the findings indicated that the chemical had a detrimental effect on the organism even at the lower concentration (0.05 mg/L) over the duration of time (15 days). The findings also showed that the values for ammonia and nitrite increased in proportion to the chemical's concentration. At the same time, the dissolved oxygen levels dropped ($P<0.05$). While temperature, pH and salinity were all within the same range for every chemical concentration. However, the chemical significantly distorted the immunological profiles of *S.melanotheron*, with increasing leucocytosis. Following exposure to this chemical, there are substantial decreases (*monocytopenia*) and significant increases ($P<0.05$) in the number of each cell type as well as in the number of lymphocytes (*lymphocytosis*), eosinophils (*eosinophilia*), and neutrophils (*neutropenia*). The study showed that the chemical has a dose-dependent effect on the fish's immune system. Therefore, care should be exercised when applying this chemical in close proximity to an aquatic environment.

Keywords: Pollution; Contaminants; Stress; Fish; Immunology

Abbreviations: EDTA: Ethylene Diamine Tetra-acetic Acid; WBC: White Blood Cells; APHA: American Public Health Association; ANOVA: Analysis of Variance.

Introduction

Unwanted substance additions to water bodies alter the aquatic system's biological, chemical, and

physical properties, creating an ecological imbalance [1]. Pesticides and other chemicals are major sources of water contamination, endangering aquatic life [2,3]. A larger portion of the contaminants have the ability to bio-magnify and bio-accumulate, which can have a variety of negative effects and stressors on aquatic life [4]. Pollution is having a severe impact on fish populations as well as those of other aquatic animals. This is due to the fact that every organism has distinct life stages, some of which are more susceptible than others to changes in their surroundings and pollutants. Research has indicated that the most vulnerable phases in the life cycle of fish are the early developmental stages, including the embryonic and larval stages [5]. In addition, to extended exposure to low concentrations of contaminants that may have detrimental effects on the organism, it has been highlighted that brief exposure may also have an adverse effect on the organism's ability to feed and float, regulate its osmotic balance, grow, and survive, all of which may have an adverse effect on population recruitment [6]. Since these parameters were used as an effective and sensitive index to track the physiologic and pathologic changes brought on by anthropogenic or natural factors like bacterial or fungal infection or water resource pollution, hematologic studies in fishes have taken on more significance [7]. As a result, blood parameters are thought to be helpful tools or indications for determining how well the body is functioning in response to different stressors [8]. Stressors known as toxicants build up in fish through the food chain or are absorbed through the fish's skin and seriously impair the biological and molecular functions of the life-supporting system. Fish immune properties typically alter quite quickly as a result of pollution [9-11].

The immunological index is a widely used technique to identify the sub-lethal effects of the pollutant and may be used successfully to monitor fish responses to different toxicants reflecting the ecologic status of the habitat [7,12]. As a result, blood parameters-particularly those related to toxicology-are employed as markers to forecast the health and symptoms of organisms, especially fish [13]. According to Akinrotimi, et al. [14], fish immune parameters are affected by extrinsic elements like water temperature, diet, stress, and seasonal dynamics, as well as internal factors like sex, age, size, reproductive stage, and health. The alterations in blood parameters could indicate a physiological reaction in fish against external stressors [15]. It has been demonstrated that blood variables provide information on fish physiologic responses to changing external environments and are stress indicators [16]. Numerous haematological indices have been employed to identify contaminants in the aquatic environment, including neutrophils, leucocrit, white blood cells, lymphocytes, monocytes, and thrombocytes [17].

Since fish lymphocytes are thought to be immunocompetent, they are in charge of producing antibodies as a result of an immune response [18]. White blood cells, which primarily consist of lymphocytes, monocytes, and granulocytes, are important components of the immune system [19]. The immune system of the fish will be impacted when lymphocytes and monocytes are impacted. As a result, the fish weaken and become more susceptible to infection from various infections. It is believed that the species *C. gariepinus*' immunity may be impacted by the careless use of dimethoate. Throughout evolution, many of the components of the innate immune system appear to have changed very little. Differences in the integrity of disease resistance and the immunological response are particularly sensitive markers of toxic damage in mammalian systems, at least in part because of the complexity of the immune system [20]. As a result, immune system susceptibility to a given toxin may be species-specific. There has long been discussion regarding the connection between disease in fish populations and environmental toxicity. Thus, the goal of this study was to find out how exposure to various dimethoate concentrations affected the immune profiles of *C. gariepinus*.

Materials and Methods

Experimental Location and Fish

The study was conducted at the African Regional Aquaculture Center in Buguma, Rivers State, Nigeria, which is a branch office of the Nigerian Institute for Oceanography and Marine Research. During low tide, a total of 180 *S. melanotheron*, used for the experiment were sourced from the recruitment ponds in the centre. They were later sorted and grouped into three based on their sizes. Group 1(Juveniles) were of the size (mean length 12.45cm±1.98SD and mean weight 66.23g±3.04SD), Group 2 (Sub-Adults) were of the size (mean length 15.22cm±3.03SD and mean weight 100.34g±11.00SD) and Group 3 (Adults) were of the size (mean length 19.04cm±6.09SD and mean weight 142.05g±12.54SD). The fish were brought to the lab in six open, 50-liter plastic containers, where they acclimated for seven days.

Preparation of Test Solutions and Exposure of Fish

In the present study, dimethoate was used. Dimethoate is a white crystalline solid, with a camphor-like odor, white to grayish crystals for technical product. This material is a contact and systemic organophosphate insecticide effective against a broad range of insects and mites when applied on a wide range of crops, was used to make the stock solutions. The pesticide was purchased from a commercial outlet in Port Harcourt, Nigeria. *T.guineemsis* were exposed to the chemical at the concentrations of 0.00 (control), 0.05, 0.10, 0.15, and 0.20 mg/L in triplicates. Five fish were randomly

distributed into each test tank. The experiment lasted for a period of 15 days. The water in the tanks was renewed daily. The fish were fed twice daily at 3% body weight with a commercial feed.

Evaluation of Immune Systems of Fish

The fishes were taken out individually using a small hand net and placed belly upward on a table. Blood samples of about 2.0 mL was collected from the caudal peduncle with the aid of a 2 mL plastic syringe, 2 mL of the blood was dispensed into Ethylene Diamine Tetra-acetic Acid (EDTA) anticoagulant for haematological studies .Leukocyte count (WBC) were determined using the improved Neubauer haemocytometer after appropriately diluted. Differential leukocyte counts were determined by scanning Giemsa's stained slides in the classic manner [21]. The leucocytes count was made using improved Neubauer haemocytometer after diluting the blood 1:100 with Shaw's solution.

Evaluation of Water Quality Parameters

Water quality parameters in the experimental tanks during the study were evaluated: Water temperature was measured with mercury in glass thermometers, pH with pH meter (Model 3013, Jenway, China), and Salinity was determined with hand held refractometer (Atago products, Model H191, Japan). The values of dissolved oxygen, nitrite and ammonia were evaluated using the method described by APHA (1998).

Data Analysis

The results were analysed with SPSS version 22, using two way analysis of variance (ANOVA) followed by F-LSD post hoc test. The significance level was taken as $P < 0.05$.

Results

Table 1 shows the results of the physico-chemical parameters in the experimental tanks throughout the exposure time. All the parameters were within the same range for temperature, pH, and salinity values. While, nitrite and ammonia levels considerably rose, though. However, as the chemical concentration increased, the amount of dissolved oxygen decreased. The immune profiles in juveniles, sub-adults and adults of *S. melanotheron* exposed to different concentrations of dimethoate are presented in Tables 2-4 respectively. The results indicated how the chemical affected the total leucocyte and differential white blood cell counts in the juvenile, sub-adults and adult sizes of *S. melanotheron*. The result showed that the total leucocyte in the treatment groups were significantly higher ($P < 0.05$) than the control. Also the leucocyte counts in the treatment groups were significantly different ($P < 0.05$).

The findings also demonstrated that *S. melanotheron* peripheral blood included three distinguishable types of white blood cells: lymphocytes, neutrophils, and monocytes. Depending on whether or not granules were present in their cytoplasm, these were categorized as either granulocytes or a granulocytes. The most prevalent leucocyte type in the blood of *S. melanotheron* is lymphocytes. When compared to the treated groups, the leucocyte levels in the control group were considerably greater ($P< 0.05$). As exposure time increased, lymphocytosis developed. The second type of a granulocyte found in blood is the monocytes, which are spherical cells with oval nuclei and clumped chromatin.

The monocytes in the exposed fish decreased significantly ($P 0<05$) when compared to the control group. As exposure time increased, *monocytopenia* developed. The granulocytes in the blood of *S. melanotheron* were the neutrophils and eosinophils. When compared to the control, the treated groups' neutrophil and eosinophils counts were considerably higher ($P< 0.05$). The fish that had been exposed to the chemical displayed the most severe *neutropenia*.

Table 1: Physico-chemical Parameters of Water in Experimental Tanks (Meant SD).

Parameters	Concentrations of Dimethoate (mg/L)				
	0	0.05	0.1	0.15	20
Temperature (^{0}C)	28.87±1.77^a	28.91±1.82^a	28.71±1.66^a	28.44±1.46^a	28.89±1.87^a
pH	6.65±1.11^a	6.67±1.07^a	6.66±1.88^a	6.67±1.02^a	6.04±1.77^a
Ammonia (mg/l)	0.17±0.01^a	0.38±0.01^{ab}	0.44±0.17^b	0.49±0.08^b	0.58±0.45^c
DO (mg/l)	6.65±0.07^c	6.40±0.57^c	5.50±0.69^b	4.05±0.33^b	3.79±0.55^a
Nitrite (mg/l)	0.02±0.01^a	0.06±0.01^b	0.07±0.01^b	0.08±0.01^b	0.13±0.02^c
Salinity (ppt)	11.45±1.04^a	11.45±3.89^a	11.47±1.03^a	11.46±3.67^a	11.47±2.04a

Means within the row with different superscripts are significantly different ($p<0.05$).

Table 2: Immune Profiles in Juveniles of *S. melanotheron* Exposed to Different Concentrations of Dimethoate (Mean±SD).

Parameters	Concentrations of Dimethoate (mg/L)				
	0	0.05	10	0.15	0.2
WBC (cellsx10^9)	15.99±2.99^a	18.32±1.99^a	23.77±4.02^b	29.21±3.56^b	33.04±2.93^c
Leucorit (%)	13.66±1.77^c	10.01±1.03^b	9.02±1.44^b	6.02±1.11^b	4.04±0.66^a
Thrombocytes (%)	148.02±9.03^c	132.66±3.03^c	110.55±9.54^b	90.55±5.51^a	80.12±7.34^a
Neutrophils (%)	10.42±3.05^a	13.98±1.54^b	16.78±1.44^b	20.59±1.54^a	37.23±2.05^a
Lymphocytes (%)	71.44±4.87^c	67.52±1.88^c	63.50±1.67^{ab}	51.18±1.01^a	43.87±1.04^a
Monocytes (%)	17.14±2.23^a	15.47±1.04^a	13.52±1.66^b	11.22±1.34^c	10.79±1.77^c
Eosinophils (%)	1.00±0.11^a	3.03±0.44^a	6.20±0.81^a	7.11±0.66^a	8.11±1.98^a

Means within the row with different superscripts are significantly different ($p<0.05$).

Table 3: Immune Profiles in Sub-Adults of *S. melanotheron* Exposed to Different Concentrations of Dimethoate (Mean±SD).

Parameters	Concentrations of Dimethoate (mg/L)				
	0	0.05	10	0.15	0.2
WBC (cellsx10^9)	15.99±2.99^a	18.32±1.99^a	23.77±4.02^b	29.21±3.56^b	33.04±2.93^c
Leucorit (%)	13.66±1.77^c	10.01±1.03^b	9.02±1.44^b	6.02±1.11^b	4.04±0.66^a
Thrombocytes (%)	148.02±9.03^c	132.66±3.03^c	110.55±9.54^b	90.55±5.51^a	80.12±7.34^a
Neutrophils (%)	10.42±3.05^a	13.98±1.54^b	16.78±1.44^b	20.59±1.54^a	37.23±2.05^a
Lymphocytes (%)	71.44±4.87^c	67.52±1.88^c	63.50±1.67^{ab}	51.18±1.01^a	43.87±1.04^a
Monocytes (%)	17.14±2.23^a	15.47±1.04^a	13.52±1.66^b	11.22±1.34^c	10.79±1.77^c

Means within the row with different superscripts are significantly different ($p<0.05$).

Table 4: Immune Profiles in Adults of *S. melanotheron* Exposed to Different Concentrations of Dimethoate (Mean±SD).

Parameters	Concentrations of Dimethoate (mg/L)				
	0	0.05	10	0.15	0.2
WBC (cellsx10^9)	19.11±6.87^a	24.90±1.04^a	28.98±6.11^b	35.01±7.65^b	39.66±7.99^c
Leucorit (%)	18.09±1.00^c	15.23±1.05^b	12.87±3.09^b	10.08±1.55^b	8.07±1.66^a
Thrombocytes (%)	189.08±9.90^c	160.88±9.05^c	149.99±9.05^b	126.07±7.54^a	119.97±11.88^a
Neutrophils (%)	12.02±5.09^a	17.02±5.09^b	20.20±4.09^b	36.42±1.88^a	50.22±5.90^a
Lymphocytes (%)	70.55±9.77^c	64.02±1.88^c	57.80±3.90^{ab}	46.83±6.98^a	31.88±4.98^a
Monocytes (%)	15.43±6.07^a	13.76±1.60^a	14.00±3.09^b	8.04±1.99^c	7.77±0.75^c
Eosinophils (%)	2.00±0.44^a	5.20±0.36^a	8.00±2.99^a	8.71±1.77^a	10.12±2.88^a

Means within the row with different superscripts are significantly different ($p<0.05$).

Discussion

The creation of more antibodies is linked to an increase in WBC count, and this aids in the survival and recovery of fish exposed to pesticide concentrations below the fatal threshold [22]. Similar investigations by Akinrotimi, et al. [23] revealed how detergents affected *Clarias gariepinus* and revealed notable drops in WBC. Additionally, Akinrotimi, et al. [24] have found changes in the immunological indices of fish (*S.melanotheron*) during the period and methods of acclimation. They stated that there was variance in the study's immunological index values, namely in the values of WBC, thrombocytes, neutrophils, lymphocytes, monocytes, and eosinophils; notably, a marked decline in values was observed in females. Akinrotimi, et al. [25] observed comparable changes in immunological values in

Tilapia guineensis males and females exposed to various water pH environments, which were linked to variations in immunological concentration in the exposed fish.

A decrease in leucocyte count was found in *C. gariepinus* following chronic exposure of freshwater teleosts to cypermethrin in the laboratory [26]. Akinrotimi, et al. [27] found a substantial drop in leucocyte count after exposing *Tilapia guineensis* to industrial effluents. Gabriel, et al. [28] found a substantial increase in leucocyte concentration in tilapia, *Oreochromis niloticus*, after adaptation to captivity. A considerable decrease in lymphocyte count and a marked increase in neutrophils in *Tilapia guineensis* subjected to handling stress were documented [29]. In this study, the number of lymphocytes reduced dramatically while neutrophils increased. Nte, et al. [30] reported this in *S. melanotheron* that had been exposed to industrial wastewater. In addition, freshwater fish treated with fenvalerate and malathion have shown an increase in the number of neutrophils [31]. A large increase in neutrophils was also detected as a result of toxic stress caused by the pulp mill effluent [32]. Monocrotophos reduced neutrophil counts while increasing lymphocyte counts in *Channa punctatus*, a freshwater fish [33].

The drop in lymphocyte and increase in neutrophil content could be attributed to the death of haematopoetic tissue, as well as a decrease in non-specific immune system due to elevated concentrations of protective toxin. A study was done to assess the haematological parameters of *C. punctatus* after exposure to Rayon industry effluents. The effluents reduced monocyte content in *C. punctatus* [34]. When *H. fossilis* was exposed to lindane, the number of monocytes decreased significantly [35]. Monocrotophos had an influence on C. punctatus's erythropoetic activity and haematological parameters, resulting in a drop in monocyte count [34]. In this study, sublethal quantities of the synthetic pesticide dimethoate increased the percentage of monocytes in *S. melanotheron*. The decline in monocyte count in the current study could be attributed to dimethoate's toxic effects on the kidney and spleen (haematopoetic tissues). Chemical exposure has been associated with an increase in eosinophil concentration in fish [36].

The toxicity of diazinon increased the quantity of eosinophils in Cyprinus carpio [37]. When the experimental fish were subjected to malathion, the number of eosinophils decreased [38]. After 60 days of exposure to carbon tetrachloride, *C. batrachus* had more eosinophils and fewer basophils [39]. In this study, *S. melanotheron* exposed to sublethal quantities of dimethoate revealed a decrease in thrombocyte count as well as a slower blood coagulation time. Heteropneustes fossilis exhibited a significant decrease in thrombocyte count following heptachlor treatment [40]. Endosulfan therapy in *C. batrachus* resulted in a considerable increase in circulating thrombocytes [41]. In the current study, a significant decrease in thrombocyte count due to carbofuran toxicity could be attributed to defective haemopoiesis and thrombocyte disintegration via capillary hemorrhage.

Conclusion

In conclusion, the findings of this study indicate the stress to which fish are subjected as a result of unregulated chemical discharge into the aquatic environment, such as pesticides. The results of fish exposure to various toxicants can be used to forecast the fate of wild fish populations exposed to contaminants in their natural habitats. Also, caution should be exercised when applying this chemical near an aquatic medium.

References

1. Adhikari S, Sarkar B, Chatterjee A, Mahapatra CT, Ayyappan S (2004) Effect of cypermethrin and carbofuran on certain hematological parameters and prediction of recovery in a freshwater teleost, *Labeo rohita* (Hamilton). Ecotoxicology and Environmental Safety 58(2): 220-226.

2. Akinrotimi OA, Abu OMG, George OS, Ucdeme NB, Aranyo AA (2010) Haematological characteristics of *Tilapia guineensis* from Buguma creek Niger Delta, Nigeria. League of Researchers in Nigeria 9(8): 1415-1422.

3. Akinrotimi OA, Gabriel UU (2012) Haematological profile of *Clarias gariepinus* broodfish raised in water recirculating system. Advanced Journal of Agricultural Sciences and Engineering Research 2: 97-103.

4. Gabriel UU, Akinrotimi OA, Ariweriokuma SV (2012) Alterations of selected electrolytes in organs of African catfish, *Clarias gariepinus* treated with cypermethrin. Advances in Students Research 2(1): 53-60.

5. Gabriel UU, Amakiri EU, Ezeri GNO (2007). Haematology and gill pathology of Clarias gariepinus exposed to refined petroleum oil under laboratory conditions. Journal of Animal and Veterinary Advances 6(3): 461-465.

6. Ghaffar A, Ashraf S, Hussain R, Hussain T, Shafique M, et al. (2014). Clinico haematological disparities induced by triazophos (organophosphate) in Japanese quail. Pakistan Veterinary Journal 34(2): 257-259.

7. Nte ME, Edun OM, Akinrotimi OA (2018) Biochemical Changes in Mudskipper (*Periophthalmus papilio*) exposed to sodium bromide. International Journal

of Advanced Research in Medical & Pharmaceutical Sciences 3(2): 1-6.

8. Okomoda J, Ayuba VO, Omeji S (2010) Haematological changes of *Clarias gariepinus* (Burchell, 1822) fingerlings exposed to acute toxicity of formalin. PAT Journal 6(1): 92-101

9. Akinrotimi OA, Gabriel UU, Orokotan OO (2013) Changes in Enzymes activities of *Clarias gariepinus* brood fish exposed to anaesthtics metomidate. Applied Ecology and Environmental Science 1(3): 37-40.

10. Ariweriokuma SV, Gabriel UU, Deekae SN, Akinrotimi OA (2016) Haematological charcacteristics of African catfish (*Clarias gariepinus*) fed dietary inclusion levels of green leaf (*Amaranthus cruentus*). International Journal of Innovative Studies in Aquatic Biology and Fisheries 2(2): 11-22.

11. George ADI, Akinrotimi OA (2017) Influence of Sex on Haematological Response of *Clarias gariepinus* Juveniles Treated with Atrazine and Metalochlor. Trends Green Chemistry 3(1): 1-6.

12. George ADI, Akinrotimi OA, Nwokoma UK (2017) Haematological Changes in African Catfish (*Clarias gariepinus*) Exposed to Atrazine and Metolachlor in the Laboratory. Journal of Fisheriesscience.com 11(3): 48-54.

13. Akinrotimi OA, Wilfred EPC, Ukwe OIK (2018) Changes in lymphocytes in three sizes of African catfish (*Clarias gariepinus*) exposed to different chemicals in the laboratory. International Journal for Research under Literal Access 1(4): 1-6.

14. Akinrotimi OA, Wilfred EPC, Ukwe OIK (2018) Effects of 2,2-Dichlorovinyl Dimethyl Phosphate (DDVP) on leukocyte profiles in juveniles and adult sizes of *Tilapia guineensis*. MOJ Immunology 6(5): 173-176.

15. Alkinson J, Judd FW (1978) Comparative hematology of *Lepomis microlophus* and *Chiclasoma cyanoguttarum*. Copeia 12: 230-237.

16. Banaee M, Mirvagefei AR, Rafei GR, Majazi AB (2008) Effect of sublethal diazinon concentrain on blood plasma biochemistry. Int. J. Environ. Res 2(2): 189-198.

17. Alalibo OO, Gabriel UU, Akinrotimi OA (2019). Changes in Metabolites of African Catfish (*Clarias gariepinus*) Exposed to Different Salinity Levels. International Journal of Innovative Studies in Aquatic Biology and Fisheries 5(4): 1-7.

18. Akinrotimi OA, Godwin OS, Okenwa U, Lawrence AW (2021) Haematology of *Clarias gariepinus* under Multiple Exposures to Syszygium aromaticum as Anaesthetics. Journal of Agriculture and Aquatic Science 1: 60-66.

19. Jemal A, Graubard B, Devesa SS, Flegal KM (2002) The association of blood lead level and cancer mortality among whites in the United States. Environ. Health Prespect 110(4): 325-329.

20. Ghosh K, Banerjee V (1993) Alterations in blood parameters in the fish Heteropneustes fossilis exposed to dimethoate. Environcol 11: 979-981.

21. Hrubec TC, Cardinale JL, Smith SA (2000) Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis hybrid*). Veterinary Clinical Pathology 29(1): 7-12.

22. Adedeji OB, Adedeji OA, Adeyemo OK, Agbeda SA (2009) Acute effects of diazinon on blood parameters in the African catfish (*Clarias gariepinus*). Internet J Haematol 5: 708-715.

23. Akinrotimi OA, Amachree D (2016) Changes in haematological parameters of *Tilapia guineensis* exposed to different concentrations of detergent under laboratory conditions. Journal of Aquatic Science 31(1): 95-103.

24. Akinrotimi OA, Gabriel UU, Anyanwu PE, Anyanwu AO (2007) Influence of sex, acclimation methods and period on haematology of *Sarotherodon melanotheron*. Research Journal of Biological Sciences 2: 348-352.

25. Akinrotimi OA, Opara JY, Ibemere IF (2012) Changes in haematological parameters of *Tilapia guineensis* exposed to different water pH environment Innovation in Science and Engineering 2: 9-14.

26. Akinrotimi OA, Gabriel UU, Ariweriokuma SV (2012) Haematotoxicity of cypermethrin to African catfish *Clarias gariepinus* under laboratory conditions. Journal of Environmental Engineering and Technology 1(2): 20-25.

27. Akinrotimi OA, Orlu EE, Gabriel UU (2013) Haematological Responses of Tilapia guineensis treated with industrial effluents. Applied Ecology and Environmental Sciences 1(1): 10-13.

28. Gabriel UU, Akinrotimi OA, Eseimokumo F (2011) Haematological responses of wild Nile tilapia *Oreochromis niloticus* after acclimation to captivity. Jordan Journal of Biological Sciences 4(4): 225-230.

29. Akinrotimi OA, Abu OMG, Ansa EJ, Edun OM, George OS (2009) Haematological responses of *Tilapia guineensis*

to acute stress. Journal of Natural and Applied Sciences 5(4): 338-343.

30. Nte MD, Hart AI, Edun OM, Akinrotimi OA (2012) Effect of industrial effluent on haematological parameters of Black jaw tilapia *Sarotherodon melanotheron*. Continental Journal of Environmental Science 5(2): 29-37.

31. Mukhopadhay PK, Dehadrai PV (1980) Biochemical changes in the air breathing catfish *Clarias batrachus* (Linn) exposed to malathion. Environ Polut Ser Ecol Biol 22(2): 149-158.

32. Thakur GK, Pandey PK (1990) BHC (Gammaxene) poisoning effect on leucocytes of an air breathing fish *Clarias batrachus* (Linn). J Env Bio 11(2): 105-110.

33. Agrahari S, Pandey KC, Gopal K (2006) Effect of monocrotophos on erythropoietic activity and hematological parameters of the freshwater fish, *Channa punctatus* (Bloch). Bull Environ Contam Toxicol 76(4): 607-613.

34. Benarjee G, Narayana RB, Srikanth K, Ramu G (2003) Haematological changes in the fresh water fish, *Channa punctatus* due to the effect of Rayon industry effluents. J Chem Pharma Res 5: 178-183.

35. Shrivastava AK, Mirsha J (1985) Lindane induced hematological changes in the cat fish, Heteropneustes fossilis. Wat Acad Sci Lett 8: 391-392.

36. Akinrotimi OA, Agokei EO, Aranyo AA (2012) Changes in haematological parameters of *Tilapia guineensis* exposed to different salinity levels. Journal of Environmental Engineering and Technology 1(2): 4-12.

37. Svobodova M, Luskova V, Drastichova J, Zlabek V (2001) The effect of diazinon on hematological indices of common carp (*Cyprinus carpio L.*). Acta Vet Brno 70: 457-465.

38. Metelyev VV, Grischenki I (1970) Toxicology, chemical aspects, pathogenesis and diagnosis of organophosphate TKHM-3 phosphamide and MNP and carbamate poisoning. Eksp Vid Toxicol Mater Veres Simp 1: 36-38.

39. Sharma RC, Gupta N (1982) Carbon tetrachloride induced haematological alterations in *Clarias batrachus* (L). J Environ Biol 3: 127-131.

40. Shrivastava AK, Mirsha J (1987) Heptachlor induced haematological and biochemical changes in Indian catfish *Heteropneustes fossilis* (Bloch). Acta Hydrobiol 29(4): 489-495.

41. Venkateshwarlu P, Rani VJS, Janaiah C, Prasad MSK (1990) Effects of endosulfan and kelthane on hematology and serum biochemical parameters of the teleost, *C. batrachus*. Ind J Comp Anim Physiol 8: 8-13.