

Alterations in Blood Composition of *Tilapia guineensis* Treated with Atrazine in the Laboratory

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ABSTRACT : Alterations in the blood composition of *Tilapia guineensis* exposed to atrazine was carried out. A total of 60 *Tilapia guineensis* (mean length 12.66cm±1.23SD and mean weight 107.99g±1.45SD) was exposed (5 fish per tank) to 0.00ml/L (control) 0.025, 0.050, and 0.075mg/L of Atrazine in triplicates. The experiment was conducted in the laboratory under a static renewal condition for 10 days to determine the effect of the exposure on the haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), white blood cell (WBC), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), differential counts (neutrophils, monocytes, and lymphocytes) and thrombocytes. Exposure of *Tilapia guineensis* to this chemical, resulted in a concentration dependent reduction in the values of Hb, RBC, PCV, MCH, MCHC, lymphocytes and thrombocytes. However, there was a significant increase in the values of WBC, MCV, neutrophils and monocytes as the concentration of the chemical increased. These alterations were more pronounced in the fish exposed to 0.050, and 0.075 mg/L of the chemical.

Keywords: Blood, Pollutants, Toxicity, Chemical Tilapia

I. INTRODUCTION

Aquatic pollution is recognized in many parts of the world worldwide as a potential threat to both human and other animal populations which interact with the aquatic environments [1]. In recent year's explosive increase in human population and industrial development have been the major causes of aquatic contamination around the world [2]. Discharges of pesticides into rivers through run-offs may cause deleterious effects to the health of aquatic organisms, which will have deleterious effects on aquatic biota [3].

Haematological parameters are important in diagnosing the structural and functional status of fish exposed to toxicants. They are considered to be reliable approach in the assessment of toxicity of different chemicals [4] either singly or in combination on fish health [5]. Changes in haematological parameters depend on the extent of impact of contaminants concentration, the duration of exposure besides fish species, their age and health status [6,7]. Alteration in white blood cells may be regarded as a prognostic tool as well as early warning signal of the disturbance in homeostatic defense abilities of fish [8]. Haematological indices are therefore, ready tools used by fish biologists and researchers in many parts of the world in diagnosing stress. This is so because fish are closely associated with aquatic environment and the blood becomes an indicator of slightest change within the body of fish, well before there is any visible sign of disease [9].

Tilapia species is one of the mostly popular fresh water fish consumed in several countries. They are fish that are well adapted to enclosed water. Therefore, *Tilapia guineensis* was chosen in this experiment. *Tilapia guineensis* is an euryhaline species that is commonly found along the coast of West Africa. The species is usually found in creeks, lagoons, and adjoining rivers [10]. Since most pesticides are discharged continuously into the aquatic medium through run-offs, it becomes necessary to assess the possible effects that pesticides may have on a non-target fish such as *T. guineensis*. The present study was carried out to assess the effect of exposure of *T. guineensis* to atrazine a pesticide on some of its blood parameters under laboratory conditions.

II. EXPERIMENTAL WORK

Experimental Location and Source of Experimental Fish

The experiment was carried out at the laboratory at African Regional Aquaculture Centre, Buguma, Rivers State. A total of sixty (60) *T. guineensis*. (mean length 12.66cm±1.23SD and mean weight 107.99g±1.45SD) were collected from the ponds in the Center during low tide. They were transferred to the laboratory for acclimation process.

Acclimation and Feeding Of Fish

The experimental fish were acclimated in two 200L capacity circular plastic tanks containing 100L de-chlorinated water, for 7 days to experimental conditions at room temperature. Netted materials with central slits was tied to the tops of the tanks to prevent escape of fish. Water renewal was done every two days. The fish were fed with ARAC feed (35% CP).

Experimental Design and Procedure

The experimental design was a completely randomized design (CRD) with five treatments levels and a control with each level having three replicates. Five (10) *T. guineensis*. fish were introduced individually into 12, aquaria tanks of 1.5m x 1m x 0.5m dimension, containing 0.000 (control), 0.025, 0.050, and 0.075 mg/L of atrazine. Each treatment and control had three replicates and lasted for 10 days.

Evaluation of Water Quality Parameters

The physico-chemical parameters were evaluated in the experimental tanks on daily basis. Ammonia, pH, Conductivity, Dissolved oxygen (DO) and Total Dissolved Solids (TDS), were assessed using the methods described by APHA (1998). The water pH was measured using pH meter, and while Temperature was evaluated by using mercury in glass thermometer.

Blood Sampling and Analysis

Blood sampling was conducted at the expiration of 10 days. Blood samples were collected from a total of 30 *T. guineensis* with heparinized plastic syringe, fitted with 21 gauge hypodermic needle and preserved in disodium salt of ethylene-diamine tetraacetic acid (EDTA) bottles for analysis. Standard blood analytical methods described by Houston [11] were adopted for this study. The cyano-haemoglobin method was used to determine haemoglobin (Hb) using diagnostic kits from Sigma diagnostics USA, and packed cell volume (PCV) was determined by the microhaematocrit method. Red blood cell (RBC), leucocrit (LCT) and thrombocyte count were determined with the improved Neubauer haemocytometer. White blood cells (WBC) was determined with the improved Neubauer counter, while differential counts such as neutrophils, lymphocytes and monocytes were determined on blood film stained with May-Grunwald-Giemsa stain. The values of haematological indices were calculated Hrubec [12]:

Mean Cell Volume (MCV)

Values of MCV were calculated using the formula:

$$\text{MCV} = \frac{\text{PCV} \times 10}{\text{RBC}}$$

Mean Cell Haemoglobin (MCH)

Values of MCH were calculated using the formula:

$$\text{MCH} = \frac{\text{Hb}(\text{g/dl}) \times 10}{\text{RBC}}$$

Mean Cell Haemoglobin Concentration (MCHC)

Values of MCHC were calculated using the formula:

$$\text{MCHC} = \frac{\text{Hb}(\text{g/dl}) \times 100}{\text{PCV}(\%)}$$

Statistical Analysis

Data obtained from the experiments were collated and subjected to ANOVA using Statistical Package for the social Sciences, (SPSS) version 22, differences among means were separated by Turkey's Comparative test at 0.05%.

III.RESULTS AND DISCUSSION

The results of water quality variables in the experimental tanks during the exposure period are shown in Table 1. The result indicated an increase in the values of Ammonia from 0.01 in the control to 0.11 mg/L at 0.075mg/l of the chemical. Conductivity also increased from 123.04 to 199.09 μ S/cm. However, reductions were recorded in the values of dissolved oxygen and total dissolved solids, while the values for Temperature and pH were within the same range in all concentrations of exposure. The result of hematological parameters in *T.guineensis* exposed to Atrazine, for 10 days is presented in Table 2. The result indicated a consistent reduction relative to the control in the values of haemoglobin (Hb), red blood cell (RBC), packed cell volume (PCV), lymphocytes, platelets and mean corpuscular volume (MCV). While the values of WBC increased from 18.00 to 32.91, Neutrophils from 38.40 – 52.58, monocytes from 4.47 – 8.04.

Table 1: Water Quality Variables (Mean \pm S.D) in the Experimental Tanks during the Experimental Period.

Parameters	Concentrations of Atrazine (mg/L)			
	0.000	0.025	0.050	0.075
Ammonia (mg/l)	0.01 \pm 0.00 ^a	0.02 \pm 0.01 ^a	0.09 \pm 0.01 ^b	0.11 \pm 0.02 ^b
Temperature ($^{\circ}$ C)	28.94 \pm 2.11 ^a	29.01 \pm 0.23 ^a	29.02 \pm 0.22 ^a	29.11 \pm 0.99 ^a
pH	7.51 \pm 0.33 ^a	7.65 \pm 0.54 ^a	7.71 \pm 0.33 ^a	7.44 \pm 0.22 ^a
Conductivity(S/m)	123.04 \pm 9.88 ^a	137.33 \pm 13.88 ^a	167.49 \pm 10.11 ^b	199.09 \pm 10.86 ^b
DO (mg/l)	5.99 \pm 0.62 ^c	4.22 \pm 0.51 ^b	3.89 \pm 0.52 ^b	3.28 \pm 0.32 ^b
TDS (mg/l)	1.04 \pm 0.01 ^a	0.09 \pm 0.01 ^a	0.08 \pm 0.01 ^b	0.07 \pm 0.01 ^b

Means with different superscript in the column are significantly different (P<0.05).

Table 2: Haematological Parameters in *Sarotherodon melanotheron* exposed to Atrazine and Metalochlor for 10 days (Mean \pm S.D).

Parameters	Concentrations of Atrazine/Metalochlor (mg/L)			
	0.000	0.025	0.050	0.075
Hb (g/dl)	15.00 \pm 0.49 ^d	13.90 \pm 0.82 ^c	10.09 \pm 0.88 ^b	8.12 \pm 0.76 ^a
RBC (Cells x 10 ¹²)	9.77 \pm 0.98 ^d	7.980 \pm 0.55 ^c	6.92 \pm 0.89 ^b	4.34 \pm 0.11 ^a
PCV (%)	35.00 \pm 1.43 ^c	33.00 \pm 1.07 ^c	30.00 \pm 1.05 ^b	27.00 \pm 1.65 ^b
WBC (Cells x 10 ⁹)	18.00 \pm 1.11 ^a	22.00 \pm 1.54 ^b	28.00 \pm 1.98 ^b	32.19 \pm 1.98 ^c
Neutrophils (%)	38.40 \pm 1.01 ^a	41.80 \pm 1.43 ^a	44.87 \pm 2.78 ^d	52.58 \pm 1.65 ^b
Monocytes (%)	4.47 \pm 0.18 ^a	6.79 \pm 0.65 ^a	7.46 \pm 1.32 ^b	8.04 \pm 0.18 ^b
Lymphocytes (%)	56.07 \pm 6.07 ^c	52.20 \pm 4.07 ^c	47.11 \pm 3.87 ^b	40.34 \pm 2.09 ^a
Platelets (%)	475.98 \pm 2.54 ^c	450.89 \pm 2.35 ^b	430.58 \pm 0.88 ^b	397.54 \pm 0.87 ^a
MCV (f l)	45.47 \pm 3.54 ^a	51.88 \pm 2.71 ^b	58.42 \pm 3.12 ^b	66.99 \pm 4.87 ^c
MCH (pg)	18.59 \pm 2.76 ^a	19.03 \pm 1.98 ^a	21.98 \pm 2.65 ^b	23.98 \pm 1.09 ^b
MCHC (%)	40.65 \pm 1.87 ^c	38.97 \pm 2.18 ^b	35.97 \pm 3.66 ^b	32.78 \pm 1.54 ^a

Means with different superscript in the column are significantly different (P<0.05).

The major haematological responses in this work include decreases in red blood cell counts, packed cell volume and haemoglobin concentration. Sublethal concentrations of contaminants in the aquatic system do not necessarily result in outright mortality of aquatic organisms but could have significant effects, which can result in several physiological dysfunctions in fish [13]. Changes in haematological values occur in relation to physiological stress, disease and toxic environmental conditions [14, 15, 16]. Haemoglobin is the oxygen-carrying component in the blood of fish and its concentration can be used as a good indicator of anaemia [17]. The decreased haemoglobin in the experimental fish exposed to paraquat could thus be an indication that anaemic condition occurred in the fish during exposure to this chemical. Decreased haemoglobin following exposure to pesticide usually results in haemodilution. This haemodilution has been regarded as a mechanism that reduces the concentration of the contaminant in the circulatory system of the fish [18,19,20].

Packed cell volume is used to determine the ratio of plasma to corpuscles in the blood as well as the oxygen-carrying capacity of the blood [21]. The significant decrease in the packed cell volume in this study could be linked to gill damage and/or impaired osmoregulation causing anaemia and haemodilution. Blood cells of teleost are produced from haemopoietic tissues of the kidney and spleen [22]. The red blood cells have the important function of haemoglobin transport which carries oxygen to all tissues in the body [23] (Hibiya, 1982). The decreased red blood cell number following exposure to cadmium could be as a result of haemolysis or destruction of the red blood cells. The cause of the reduction of circulating erythrocytes of stressed fish has been attributed to aggregation of red blood cells in damaged gills [24]. Decreases in the red blood cells could also be as a result of internal bleeding caused by damaged kidney.

The white blood cell counts also increased with change in the composition as seen from the differential white blood cell counts. The erythrocytic indices of MCHC, MCH and MCV similarly increased fish exposed to paraquat at different concentrations exposure media. In fish, the white blood cells respond to various stressors including infections and chemical irritants [25]. Thus increasing numbers of white blood cells are a normal reaction to a chemical such as paraquat as in the present study, demonstrating the effect of the immune system under toxic conditions. The decreased number of lymphocytes may be the result of bioconcentration of the test pesticide in the kidney and liver [26].

CONCLUSION

On the whole, the results of this study highlight the stress to which freshwater fish are exposed through the uncontrolled discharge of heavy metals in the aquatic environment. Results obtained from exposure of fish to the various toxicants, despite specific shortcomings such as experimental error, water hardness, water-pH and capture stress enable us to predict the fate of fish populations exposed to these conditions

REFERENCES

1. Beyer, J., Sandvik, M., Hylland, K., Fjeld, E., Egaas, E., Aas, E., Skaare, J.U. and Goksoyr, A. Contaminant accumulation and biomarker responses in flounder (*platichthys flesus* L.) and Atlantic cod (*Gadus Morhua* L.) exposed by carrying to polluted sediments in Sorfjorden, Norway. *Aquat.Toxicol*(1996): 36: 75-98.
2. Chindah, A. C and Hart, A. I. Occurrence and distribution of epi fauna and in-fauna community in shallow mangrove wet land in the tropical West African Region. *Afri. J. Env. Stud*(2000): (1&2): 76-83.
3. Chindah, A. C. Toxicity of Cypermethrin to *Tilapia guineensis* juveniles. *Journal of Agric. Biotech Env.* (2004): 2 (2):60 - 66.
4. Gabriel, U. U., Anyanwu P. E and Akinrotimi, O. A. Blood characteristics associated with confinement stress in black chin tilapia *Sarotherodon melanotheron*. *J Fish Int* (2007): 2:186-189.
5. Gabriel, U. U., Anyanwu P. E and Akinrotimi, O. A. Haematological profile of Black chinned tilapia *Sarotherodon melanotheron* from Buguma creek, Niger Delta. *Agric. J* (2007): 2 (3): 384-387.
6. Gabriel, U. U., Akinrotimi, O.A and Eseimokumo, F. (Haematological responses of wild Nile tilapia *Oreochromis niloticus* after acclimation to captivity. *Jordans. J Biol. Sci* 4(2011): (4): 225-230
7. Akinrotimi, O. A., Abu O. M. G, Ansa E. J., Edun O. M. and George O. S. Haematological responses of *Tilapia guineensis* to acute stress. *J. Nat Appl. Sci*(2009): 5:338 - 343.
8. Akinrotimi, O. A, Gabriel U. U, Anyanwu P.E. and Anyanwu.A.O. Influence of sex, acclimation methods and period on haematology of *Sarotherodon .melanotheron*. *Res J Biol Sci.*(2007): 2: 348 -352.

9. Akinrotimi O. A.; Abu O. M. G; George O.S; Ucdeme, N. B and Aranyo A. A. Haematological characteristics of *Tilapia guineensis* from Buguma creek Niger Delta, Nigeria. League Res. Nig. (2010): 11:177- 182.
10. Bittencourt N.S (JR), Peter E. Gibbs and J O AfO Semir. Histological Study of Post-pollination Events in *Spathodea campanulata* Beauv. (*Bignoniaceae*), a Species with Late-acting Self-incompatibility. (2003) Annals of Botany 91: 827-834, Braz. J. Med. Biol. Res., 34: 719-726.
11. Houston, A. H. Components of the haematological response of fish to environmental changes: a review (In: Environmental physiology of fishes. Ed. M. A. Ali). Nato Advances study Inst. Series. A life science (1980): 35:241-298.
12. Hrubec, T. C., Cardinale, J. L., & Smith, S. A. Haematology and plasma chemistry reference intervals for cultured tilapia *Oreochromis* hybrid. *Vet Clin. Pathol* (2000): 29: 7-12.
13. Arnold, J. E. Haematology of sand bar shark, *Carcharinus plumbeus* standardization of complete blood count techniques for elasmobranchs. *Vetclin. Pathol* (2005): 34 (2): 115-123.
14. Omeregie, E., T. G. Eseyin & P. C. Ofojekwu. Chronic effects of formalin on erythrocyte counts and plasma glucose of Nile tilapia *Oreochromis niloticus*. *Asian Fish sci.* (1994): 7: 1-6.
15. Akinrotimi, O.A., Bekibele D.O., & Orokotan, O.O. Selected haematological values of African catfish (*Clarias gariepinus*) raised in water recirculating system. *International Journal of Recirculating Aquaculture*, 2011, 12: 1-12.
16. Akinrotimi, O.A., & Gabriel, U.U. Haematological profiles of *Clarias gariepinus* broodfish raised in water recirculating aquaculture system. *Advances in Agriculture, Science and Engineering Research*, (2012): 2(2): 97-103.
17. Svobodova, Z., Machova, *JKolarovo*, J., Vykosovo, B. and Piacka, V. The effect of selected negative factors on haematological parameters of common carp *Cyprinus carpio*. *Review Zoology* (1996): 31:112 –116.
18. Akinrotimi, O.A., & Gabriel, U.U.. Haematological profile of Nile Tilapia (*Oreochromis niloticus*) from ARAC Reservoir Aluu, Port Harcourt, Nigeria. *Advances in Students Research*, (2012): 2(1), 31-37.
19. Akinrotimi, O.A., Gabriel, U.U., & Ariweriokuma, S.V. Haematotoxicity of cypermethrin to African catfish *Clarias gariepinus* under laboratory conditions. *Journal of Environmental Engineering and Technology*, (2012): 1(2), 20-25.
20. Akinrotimi, O.A., Agokei, E.O., & Aranyo, A.A. Changes in haematological parameters of *Tilapia guineensis* exposed to different salinity levels. *Journal of Environmental Engineering and Technology*, (2012). 1(2), 4-12.
21. Thannickal, V. J. and Fanburg, B. L. Reactive oxygen species in cell signaling. *Am J Physiol Lung Cell Mol Physiol*, (2000): 279:1005-1028.
22. Akinrotimi, O.A., Opara, J.Y., & Ibemere, I.F. Changes in haematological parameters of *Tilapia guineensis* exposed to different water pH environment. *Innovation in Science and Engineering*, (2012): 2, 9-14.
23. George A.D.I, & Akinrotimi O.A. Influence of Sex on Haematological Response of *Clarias gariepinus* Juveniles Treated with Atrazine and Metalochlor. *Trends Green Chemistry*, 2017, 3: 1-6.
24. George, A.D.I., Akinrotimi O.A. & Nwokoma, U. K. Haematological Changes in African Catfish (*Clarias gariepinus*) Exposed To Atrazine and Metolachlor in the Laboratory. *Journal of Fisheries Science.com*. 2017:11 (3): 48-54
25. Wilhelm Filho, D., Torres, M. A., Tribess, T. B., Pedrosa, R. C. and Soares, C. H. L. Influence of season and pollution on the antioxidant defences of the Cichlid fish, *Geophagus braziliensis*. *Braz. J. Med. Biol. Res.*, (2001): 34: 719-726.
26. Akinrotimi, O. A. & Amachree, D. Changes in haematological parameters of *Tilapia guineensis* exposed to different concentrations of detergent under laboratory conditions. *Journal of Aquatic Sciences*, (2016): 31 (1), 95-103.