Haematological Changes in Black Jaw Tilapia (*Sarotherodon melanotheron*) Exposed to Atrazin and Metalochlor in the Laboratory

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ABSTRACT : A total of 120 Sarotherodon melanotheron (mean length 11.44cm±1.10SD and mean weight 54.26g±1.18SD) were exposed (10 fish per tank) to 0.00mL^{-1} (control) 0.01, 0.02, and 0.03mL^{-1} of Atrazine and metalochlor (combined) 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 S.melanotheron to these toxicants, caused a concentration dependent significant (P < 0.05) 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 0.02, and 0.03 mL^{-1} of the chemical. Results from this study suggest that exposure of this specie to this pesticide could cause some level of stress as indicated by changes in the haematological variables of fish under consideration.

Keywords: Haematology, Toxicity, Pesticides, Aquatic environment

I.INTRODUCTION

Aquatic pollution is recognized globally as a menace to both human and other animal populations which interact with the aquatic environments [1]. Increase in human population and industrial development have been the major causes of contamination in the coastal areas around the world in recent years [2]. Discharges of effluents from industries and wastes from homes into rivers may cause deleterious effects on the aquatic biota [3]. Contaminants in aquatic environments can reach man through the food chain, due to consumption of contaminated fish [4].

In assessment of health status of fish both in the wild and culture medium, haematological parameters are currently considered as essential indices to ascertain the general well being of fish [5]. Changes in haematological variables are commonly use when clinical diagnosis of fish physiology is applied to determine the effects of external stressors such as contaminants on the systems of the fish. It has been illustrated that the use of haematological variables as indicators of stress, chemical exposures as well as incidence of pesticide applications can provide information on the physiological response of fish to a changing external environment [6]. This is because fish has a close association with their immediate environment external environment [7], and any alterations in the system of the fish will be revealed in the blood before any visible external manifestations. Therefore, hematological techniques are the most common method to determine the sub-lethal effects of the toxicants in the system of the fish [8-9].

Tilapia species is one of the most popular fish consumed in several countries. Therefore, specie such *Sarotherodon melanotheron* as chosen in this experiment is an euryhaline species that is commonly found along the coast of West Africa. The specie is usually found in creeks, lagoons, and adjoining rivers and they are one the common fish found in these water bodies [10]. Since most toxicants and wastes from industries, homes and farms, find their way through direct discharge and run offs into these aquatic medium, it becomes necessary to assess the possible effects of atrazin and metaochlor a pesticide on fish such as *S. melanotheron*. Hence, the present study was carried out to assess the effect of exposure of *S. melanotheron* to 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. One Hundred and twenty (120) adult sizes of *Sarotherodon melanotheron* fish (mean length 7.04±2.01cm and mean weight 54.02±1.01g) were harvested 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. 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 three treatments levels and a control with each level having three replicates. Ten (10) *Sarotherodon melanotheron* fish were introduced individually into 12, aquaria tanks of 1.5m x 1m x 0.5m dimension, containing 0.00 (control), 0.01, 0.02, 0.003 and 0.04 mg/L of Delta Force **(**metalochlor /atrazine). The experiment lasted for 10 days. The solution for each concentration was renewed daily, with freshly prepared solution of Delta Force **(**metalochloclor/atrazine).

Evaluation of Water Quality Parameters

The physico-chemical parameters were measured in the experimental tanks on daily basis. Ammonia, Conductivity, Dissolved oxygen (DO) and Total Dissolved Solids (TDS), were determined using the methods described by APHA [11]. 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 taken at the expiration of 10 days. Blood samples were collected from a total of 30 *S. melanotheron* with heparinized plastic syringe, fitted with 21 gauge hypodermic needle and preserved in disodium salt of ethylene-diamine tetraacetic acid (EDTA) bottles for analysis. The methods described by Blaxhall and Daisley [12], and Wedemeyer *et al*. [13] were used 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 according to [14]. 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 [15]. The values of haematological indices were calculated with the method of Brown [16]:

Mean Cell Volume (MCV)

Values of MCV were calculated using the formula:

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

Mean Cell Haemoglobin (MCH)

Values of MCH were calculated using the formula:

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

Mean Cell Haemoglobin Concentration (MCHC)

Values of MCHC were calculated using the formula:

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

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 byTurkeys 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 0.02±0.13 in the control to 0.61±0.05 at 0.03mg/l of the chemical. Conductivity also increased from 120.87±10.23µS/cm to 187.28±11.22 µS/cm. However, reductions were recorded in the values of dissolved oxygen, while the values for temperature, total dissolved solids and pH were within the same range in all concentrations of exposure. The result of hematological parameters in *C. gariepinus* juvenile males exposed to Atrazine/ metalochlor, for 10 days is presented in Table 4.2. 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 20.00±1.02 to 29.10±1.02, Neutrophils 39.00±1.03[a] – 51.18±1.03, monocytes 4.48±0.19 to 7.28±0.17.

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

Parameters	Concentrations of Atrazine/Metalochlor (mg/L)			
	0.00	**0.01**	**0.02**	**0.03**
Ammonia (mg/l)	0.02±0.13[a]	0.10±0.45[a]	0.19±0.08[b]	0.61±0.05[b]
Temperature (^oC)	29.03±2.22[a]	29.91±0.43[a]	29.06±0.17[a]	29.04±0.56[a]
P^H	7.58±0.66[a]	7.69±0.34[a]	7.72±0.21[a]	7.31±0.33[a]
Conductivity(S/m)	120.87±10.23[a]	130.07±11.04[a]	165.45±12.42[b]	187.28±11.22[b]
DO (mg/l)	5.88±0.55[c]	4.13±0.49[b]	3.88±0.61[b]	3.07±0.21[b]
TDS (mg/l)	0.01±0.01[a]	0.02±0.01[a]	0.06±0.01[b]	0.06±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 ± S.D).

Parameters	Concentrations of Atrazine/Metalochlor (mg/L)			
	0.00	**0.01**	**0.02**	**0.03**
Hb (gldl)	14.20±0.34[d]	12.20±0.12[c]	11.00±0.31[b]	9.00±0.52[a]
RBC (Cells x 10^{12})	8.10±0.72[d]	6.70±0.21[c]	5.60±0.16[b]	4.40±0.27[b]
PCV (%)	36.00±1.64[c]	34.00±1.81[c]	32.00±1.62[b]	29.00±1.18[b]
WBC (Cells x 10^9)	20.00±1.02[a]	21.00±1.01[a]	25.00±1.01[b]	29.10±1.02[c]
Neutrophils (%)	39.00±1.03[a]	40.80±1.71[a]	45.34±2.25[d]	51.18±1.03[b]
Monocytes (%)	4.48±0.19[a]	5.70±0.17[a]	6.46±1.19[b]	7.28±0.17[b]
Lymphocytes (%)	55.07±6.31[c]	53.80±4.84[c]	48.03±3.97[b]	41.10±2.01[a]
Platelets (%)	451.27±0.1[c]	470.12±0.12[b]	450.11±0.12[b]	401.00±0.14[a]
MCV (f l)	44.44±3.02[a]	50.70±2.33[b]	57.14±3.07[b]	65.90±4.66[c]
MCH (pg)	17.53±2.08[a]	18.20±1.04[a]	19.64±2.44[b]	20.45±1.66[b]
MCHC (%)	39.44±1.06[c]	35.88±2.03[b]	34.38±3.27[b]	31.03±1.11[a]

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

Increased levels of toxicants in the aquatic environment as a result of discharges from industries and domestic wastes can have both lethal and sublethal effects on organisms including fish. Sublethal concentrations of toxicants in aquatic environment do not necessarily result in outright mortality of aquatic organisms but could have deleterious effects, which can result in several physiological alterations and dysfunctions in fish metabolism [17,18]. Changes in haematological variables occur in relation to physiological stress, disease and toxic environmental conditions in relation to chemical exposure [19,20].

The haematological responses of *S. melanotheron* to atrazine/metalochlor in this study include significant decrease in red blood cell counts, PCV and haemoglobin concentration . This results agrees with the findings of Akinrotimi *et al*.[22] in African catfish (*Clarias gariepinus*), exposed to cypermethrin under laboratory conditions. The decreased red blood cell number following exposure to atrazine/metalochlor 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 [23]. Decreases in the red blood cells in fish exposed to contaminants according to Nte et al.[24] could also be as a result of excessive internal bleeding consequent of damaged or impaired kidney

Conversely, haemoglobin is the oxygen-carrying component in the blood of fish and its concentration can be used as a good indicator of anaemic conditions induced by stressors, such as handling and pollutants [25]. The decreased haemoglobin in the experimental fish exposed to pesticide as observed in this study could thus be an indication that anaemic condition occurred in the fish during exposure which was pronounced at the concentration of 0.03mg/l of the chemical. Decreased haemoglobin following chemical exposure usually results in haemodilution. This haemodilution has been regarded as a mechanism that reduces the concentration of the contaminants in the circulatory system of the exposed fish [26]. Furthermore, packed cell volume (PCV), is used to determine the ratio of plasma to corpuscles in the blood as well as the oxygen-carrying capacity of the blood [27]. In *S.melanotheron* exposed to varying concentrations of this chemical, the values of PCV reduced with increasing concentrations of the chemical. This observation is in line with the report of Akinrotimi and Amachree [28] in *Clarias gariepinus* exposed to detergents. This reduction could be attributed to gill damage and/or impaired osmoregulation causing anaemia and haemodilution [28].

The white blood cell counts increased in response to exposure of the chemical. The erythrocytic indices of MCH and MCV were similarly increased in most of the exposure media. While MCHC reduced. The observed trend correspond with that of George *et al*. [29] in *Clarias gariepinus* exposed to different concentrations of atrazine/metalochlor in the laboratory. In fish, the white blood cells respond to various stressors including infections and chemical irritants [30]. Thus increasing numbers of white blood cells are a normal reaction to a chemical such as atrazine/metalochlor in the present study, demonstrating the effect of the immune system under toxic conditions. The decreased number of white blood cells (leucopaenia) may be the result of bioconcentration of the test metal in the kidney and liver [31].

CONCLUSION

In conclusion, the results of this study highlight the stress to which fish are exposed through the uncontrolled discharge of chemicals such as pesticide in the aquatic environment. Results obtained from exposure of fish to the various toxicants, can give a prediction of the fate of fish populations exposed to these conditions in the wild.

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