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Haematological Responses in *Tilapia Guineensis* Exposed to Butachlor in the Laboratory

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Abstract: *Tilapia guineensis treated to butachlor in the laboratory had its haematological characteristics evaluated. The fish were exposed to the chemical at five different doses over a period of fifteen days: 0.00 (control), 0.05, 0.10, 0.15, and 0.20 mg/L. Temperature, pH, dissolved oxygen, nitrite, and ammonia were the five water quality parameters assessed during the exposure. After the experiment, fish blood samples were taken, and conventional laboratory techniques were used to evaluate the samples for haematological parameters. The study's conclusions showed that as the chemical's concentration rose, so did the levels for ammonia and nitrite. In contrast, the dissolved oxygen values decreased. Both pH and temperature were within the same range. Moreover, among the most important toxic manifestations are significant decreases (P<0.05) in hemoglobin (Hb), packed cell volume (PCV), red blood cell count (RBC), and platelet concentrations; dose-dependent changes were also noted in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Fish exposed to butachlor in aquatic habitats can have their health quickly assessed by looking at these parameters' change.*

Keywords: *Aquatic pollution, Haematology, Tilapia guineensis, butachlor*

1. INTRODUCTION

A growing amount of pollutants end up in the aquatic environment as a result of the discharge of garbage from homes, businesses, farms, and cities. Pollutants affect organisms at all levels, including humans and ecosystems, and have a complex combination of effects on organ function, reproductive status, species survival, population increase, and biodiversity [1]. Agricultural, mining, and industrial discharges of pollutants and hazards have an impact on aquatic habitats. Aquatic life could be negatively impacted by this. Fish and other aquatic species are negatively impacted by biocides, one of these contaminants [2]. Many of these chemicals enter aquatic ecosystems through direct application, spray-drift, run-off, leaching, factory discharge, and sewage, among other routes. All of these have negative health effects on a range of aquatic non-target organisms, especially fish [3, 4]. In addition, fish are commonly used in bio monitoring and can be used to evaluate compounds that may be harmful to humans because they react to toxic agents similarly to larger vertebrates [5]. Exposure to chemicals can alter haematological parameters, which have been demonstrated to be a good measure of physiological changes and overall health in fish [6]. Fish exposure to sublethal amounts of toxins can alter blood parameters such as hemoglobin levels and red blood cell (RBC) counts, according to Gabriel et al. [7]. Compounds, such as biocide, have been used in several studies to assess the harmful effects of different compounds on fish [8]. Fish are useful markers for assessing the toxicity of chemicals. Fish under chemical stress may have different hematological components depending on a variety of biological and non-biological factors, such as the spawning season, sex, and target species, in addition to the type of xenobiotic, exposure duration, and concentration [10]. As blood is a pathophysiological mirror of the entire body, blood parameters are essential for assessing the structural and functional health of fish exposed to toxins [11]. Changes in the blood profile, which show modifications in the organism's metabolism and biochemical processes brought on by the effects of various pollutants, make it possible to investigate the mechanisms behind the effects of these pollutants [12].

Similar to warm-blooded species, changes in fish blood parameters resulting from injury to their tissues or organs can be used to determine and validate the damage or malfunction of the latter (their organs or tissues). In fish, on the other hand, these parameters are more directly related to the response of the entire organism, that is, to the effect on fish survival, growth, and reproduction [13]. It is evident that different species have different fish physiology and biochemical responses to xenobiotics, despite the fact that these systems have not been thoroughly investigated. Because fish have such direct physical and chemical interaction with their environment, they are particularly sensitive to physical and chemical changes that may be reflected in the components of their blood [14, 15].

Fish exposed to chemical pollution may see an increase or decrease in their haematological parameters. Blood tissue is a precise representation of the anatomical and molecular changes occurring within the body. Comprehensive information on fish metabolism and physiological status across a range of age and environmental groups can thus be obtained. Early diagnosis is also feasible when haematological data, especially blood parameters, are analyzed. Thus, in a lab context, this study assessed the haematological responses of Tilapia guineensis subjected to butachlor.

2. MATERIALS AND METHODS

2.1. Experimental Location and Fish

The study was carried out at the African Regional Aquaculture Center, a division of the Nigerian Institute for Oceanography and Marine Research, in Buguma, Rivers State, Nigeria. Ponds produced 180 *T. guineensis* during low tide, of which 90 were juveniles (mean length: 12.68 cm; mean weight: 70.98 cm; mean weight: 6.98 g) and 90 adults (mean length: 18.32 cm; mean weight: 130.66 cm; mean weight: 21.45 g). Six 50-liter open plastic containers were used to transport the fish to the lab, where they acclimated for seven days.

2.2. Preparation of Test Solutions and Exposure of Fish

Butachlor was purchased for this experiment from a supermarket in Port Harcourt, Nigeria. *T. guineensis* were exposed to the chemical in triplicates at doses of 0.50, 1.50, 2.50, and 2.50 mg/L, as well as 0.00 mg/L as the control. Five random fish were arranged in each test tank. The fifteen-day test was carried out. Fresh water was pumped into the tanks every day. Commercial feed was supplied to the fish twice daily at 3% body weight.

2.3. Haematological Analysis

A small hand net was used to remove each fish one at a time, and they were then arranged belly up on a table. With the help of a 2 mL plastic syringe, blood samples of approximately 5 mL were taken from the caudal peduncle, and 2 mL of the blood were distributed into an anticoagulant called EDTA for hematological investigations. Blood samples that had been obtained and kept were taken to the lab for examination. The packed cell volume (PVC), hemoglobin (HB), red blood cell count (RBC), and platelets red blood cells indices: MCH, MCHC, and MCV were all measured in the blood using an automated analyzer

2.4. Evaluation of Water Quality Parameters

Water temperature was recorded using mercury-filled glass thermometers, pH was measured using a pH meter (Model 3013, Jenway, China), during the investigation. Using the technique outlined by APHA (2005), the values of dissolved oxygen, nitrite, and ammonia were assessed.

2.5. Data Analysis

With the help of SPSS statistics program 22.0 for Windows, the data was compiled and examined. To identify the significant differences in measured variables between the control and experimental groups, a one-way analysis of variance (ANOVA) was used. Tuckey's multiple comparison tests were used to distinguish between the treatments that differed significantly from one another.

3. RESULTS

The results of physico-chemical parameters in the experimental tanks during the exposure period are presented in Table 1. The values of temperature and pH were within the same range in all concentrations of sodium bromide. While ammonia, and nitrite increased significantly. However, the dissolved oxygen reduced with increasing concentrations of the chemical. The effects of the chemical on the haematological of the juvenile and adult sizes of T. guineensis are shown in Tables 2 and 3 respectively. The result showed a significant reductions (P<0.05) in hemoglobin (Hb), packed cell volume (PCV), red blood cell count (RBC), and platelet concentrations and dose-dependent alterations were observed in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) of the exposed fish.

Table 1. *Physicochemical Parameters of Water in Tanks of T.guineensis exposed to Chronic Concentrations of Sodium Bromide (Mean ±SD)*

Concentrations (mg/L)	Physico- Chemical Parameters of Water				
	Temperature (^{0}C)	pH	DO (mg/l)	Nitrite(mg/l)	Ammonia(mg/l)
0.00	29.11±0.67^a	6.78±0.88^a	6.81±0.55^a	0.00±0.00^a	0.02±0.01^a
0.05	29.90±0.77^a	6.71±0.99^a	6.36±0.44^a	0.03±0.00^b	0.21±0.07^b
0.10	29.01±0.54^a	6.72±0.91^a	5.66±0.55^b	0.04±0.00^b	0.31±0.01^c
0.15	29.04±0.43^a	6.72±0.77^a	5.66±0.88^b	0.06±0.00^c	0.34±0.01^c
0.20	29.27±0.88^a	6.61±0.88^a	4.66±0.44^c	0.06±0.00^c	0.37±0.03^c

Means within the same column with different superscripts are significantly different (P<0.05)

Table 2. *Haematological Parameters in Juvenile of T. guineensis exposed to chronic concentrations of Butachlor (Mean ±SD)*

Conc. (mg/l)	PCV (%)	HB (g/dl)	RBC (Cells x 10^{12})	Platelets (Cells x 10^{12})	MCV (fl)	MCH (pg)	MCHC (g/dl)
0.00	37.66±8.34^b	12.99±0.77^b	7.99±0.66^b	290.01±12.77^b	76.88±5.77^b	25.88±3.99^a	34.02±6.44^a
0.05	33.04±1.55^b	11.55±2.01^b	6.44±1.77^b	281.08±15.01^b	72.77±7.99^b	22.02±1.11^a	33.02±7.05^a
0.10	31.88±6.02^b	10.91±2.62^b	5.98±1.66^b	260.33±61.99^b	70.33±2.18^b	23.44±3.03^a	32.22±9.66^a
0.15	26.66±2.55^a	9.88±1.99^a	4.61±1.99^a	250.02±11.44^a	68.99±7.12^a	23.21±4.03^a	34.70±9.02^a
0.20	23.88±4.99^a	7.04±1.07^a	3.62±1.09^a	248.44±12.77^a	65.02±7.12^a	23.33±4.44^a	34.66±9.55^a

Means within the same column with different superscripts are significantly different (P<0.05)

Key: PCV – Packed Cell Volume, HB – Haemoglobin, RBC – Red Blood Cell, MCV – Mean Corpuscular Haemoglobin, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular Haemoglobin Concentrations.

Table 3. *Haematological Parameters in Adult of T. guineensis exposed to chronic concentrations of Butachlor (Mean ±SD)*

Conc. (mg/l)	PCV (%)	HB (g/dl)	RBC (Cells x 10^{12})	Platelets (Cells x 10^{12})	MCV (fl)	MCH (pg)	MCHC (g/dl)
0.00	39.88±8.77^b	13.92±0.09^b	9.41±0.88^b	349.07±12.77^b	72.12±6.66^b	25.30±9.11^a	33.44±9.04^a
0.05	35.77±9.11^b	12.06±2.09^b	7.88±1.99^b	309.00±15.66^b	69.90±9.31^a	23.80±9.01^a	34.07±9.11^a
0.10	34.16±6.44^b	10.92±2.57^b	5.12±1.77^a	290.55±12.54^a	67.88±9.03^a	22.88±9.77^a	33.97±9.88^a
0.15	29.12±9.33^a	9.44±1.88^a	4.92±1.07^a	280.79±52.41^a	67.60±9.12^a	21.66±9.22^a	32.77±9.02^a
0.20	27.80±9.10^a	8.77±1.88^b	4.05±1.44^a	250.55±12.02^a	60.12±9.01^a	20.66±7.33^b	32.03±9.17^a

Means within the same column with different superscripts are significantly different (P<0.05)

Key: PCV – Packed Cell Volume, HB – Haemoglobin, RBC – Red Blood Cell, MCV – Mean Corpuscular Haemoglobin, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular Haemoglobin Concentrations.

4. DISCUSSION

The physical and chemistry aspects natural features such as temperature, pH, dissolved oxygen, nitrite, and ammonia are indicators of the health of aquatic life [16]. Measurements of dissolved oxygen, ammonia, and nitrite in the research were observed to deviate significantly from the control. Gabriel et al [17] argues that the temperature and pH values for aquacultural practices are within the recommended ranges. Although ammonia is a vital source of nutrients, fish can perish in water that contains excessive amounts of it. The median average value was high for an aquatic body. Fish metabolism, which uses food to produce the energy, minerals, and proteins required for survival and growth, naturally produces ammonia [18]. This could be the cause of the rise in ammonia's value. When contrasted with other physicochemical parameters, nitrates frequently have fewer direct effects on aquatic life. Nevertheless, aquatic life may find it difficult to survive when nitrite levels in the water are too high. For 96 hours, the experiment's nitrate levels varied from 0.03 to 0.06 ppm. This is consistent with research by Nte and Akinrotimi [19], who discovered that higher concentrations and longer exposure times of nitrite result in increased toxicity to aquatic invertebrates.

Using haematological markers, fish exposed to different types of aquatic pollution and poisons have been used as a sensitive indicator of stress. When sublethal toxin concentrations are present in the aquatic environment, aquatic animals may not always perish. But over time, the bioaccumulation of these toxins may provide major health hazards to higher trophic levels, including people, as well as aquatic organisms like fish. Contaminants can induce a range of physiological dysfunctions in fish that impact haematological parameters due to the interaction between blood and water. Fish health and the amount of oxygen in their environment can be correlated thanks to vital indicators of fish oxygen transport capacity such as packed cell volume (PCV), hemoglobin (Hb), and red blood cell counts (RBC) [20].

With increasing concentration and exposure duration to sodium bromide, T. guineensis showed a large decrease in dissolved oxygen level as well as a significant reduction in PCV, Hb, and RBCs. These findings suggest that erythropenia and hemolysis are brought on by a disruption in osmoregulation across the gill epithelium. Other potential causes include the disruption of iron production or the toxic substance's inhibitory influence on the hemoglobin-producing enzyme system as a result of chemical exposure. Furthermore, a reduction in the PCV values, Hb concentration, and RBC count appeared to suggest erythrocyte hemolysis and/or irreversible impairment of renal function. Adamu and Audu [21] speculate that gill damage and/or poor osmoregulation, which can result in hemodilution and anemia, could be the reason of the significant decline in PCV. The drop in RBCs could be due to a decrease in erythropoietic activity. Most vertebrates, including fish, have erythropoietic activity regulated by erythropoietin, which is produced in the kidney [22]. Erythropoietin facilitates erythropoiesis by promoting the development of hemopoietic stem cells into erythroblasts, which generate red blood cells.

Furthermore, erythropoietin promotes the production of pyridoxal phosphate by growing red blood cells (RBCs) ([23]. Either a slower rate of synthesis or a higher rate of Hb oxidation could be the reason for the decrease in Hb content [24]. Similar to this finding, Akirotimi et al. [25]) observed a decrease in the quantity of red blood cells, hemoglobin, and PCV levels in fish exposed to cypermethrin and connected it to the pesticide's detrimental effects on cell death and/or reduction in cell size. Fish subjected to malathion exhibited decreased RBC counts, Hb concentrations, and PCV levels, per Zaki et al. [26]. Comparable results were noted for juvenile C. gariepinus treated separately with lambda-cyhalothrin, deltamethrin, and cypermethrin [27, 28]. The fish homeostatic system can undergo reversible alterations due to the more sensitive erythrocyte indices, which include mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), variations in these indicators are intimately correlated with variations in PCV levels, Hb concentration, and RBC count. The results showed that MCHC had considerably grown. Both the MCH and MCV showed slight differences from the control. This pattern is in line with a study by Adeyemo [29], who found that lead exposure in experimental fish led to increases in MCV, MCH, and MCHC.

5. Conclusion And Recommendations

In conclusion, butachlor negatively impacted T. guineensis's haematological parameters by decreasing the volume of packed and red blood cells. Even at very low concentrations, the exposed fish's metabolic pathways experienced these abnormal alterations, indicating the chemical's level of toxicity to living organisms. The test fish's increased breathing rate, gasping for air, restlessness, and loss of balance were stress-related behaviors brought on by the toxicant's effect on the physicochemical properties of the water. Therefore, efforts must to be directed toward making sure that initiatives that facilitate the release of this toxin into water bodies are discontinued.

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