Changes in Immunology of Guinean Tilapia (*Coptodon guineensis)* Exposed to Glyphosate in the Laboratory

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Abstract

This study assessed immunological alterations in two sizes of Coptodon guineensis exposed to glyphosate at five different concentrations: 0.00 (control), 0.05, 0.10, 0.15, and 0.20 mg/L for a period of 15 days. During the experiment six water quality variables which include temperature, pH, salinity, dissolved oxygen, nitrite, and ammonia were evaluated. After the experiment, blood samples were taken from the fish, which were then examined for immunological profiles using standard laboratory techniques. The results showed that the chemical had a negative impact on the organism based on all the parameters that were assessed. Additionally, the results demonstrated that the values for nitrite and ammonia rose in direct proportion to the chemical's concentration. Meanwhile, there was a sharp decline in the values of dissolved oxygen ($P<0.05$). While, salinity, pH, and temperature were within the same range. The results also indicated a significant ($P<0.05$) reduction in the values of thrombocytes, leucocrit, lymphocytes and monocytes. While, white blood cells, neutrophils and eosinophils were significantly ($p<0.05$) elevated. According to the study, the chemical's impact on the immunological profiles of the fish varies with the concentrations of the chemical. Applying this chemical near an aquatic environment should therefore be done with caution.

Key words: Immune systems, Contaminants; Tilapia, Fish, Aquatic environment

1. INTRODUCTION

Unwanted material additions to water bodies can cause an ecological imbalance by changing the biological, chemical, and physical characteristics of the aquatic system [1]. Aquatic life is put at risk by pesticides and other chemicals, which are significant causes of water contamination [2]. Many of the pollutants can biomagnify and bioaccumulate, which can cause a number of stressors and adverse impacts on aquatic life [3]. Because to pollution, the number of fish and other aquatic species is constantly decreasing. Compared to many other animal species, fish are more vulnerable to stress because of their strong dependence on their environment [4]. Hematologic investigations in fishes have gained increased importance since these parameters were utilized as an efficient and sensitive index to monitor the physiologic and pathologic changes caused by anthropogenic or natural factors such as bacterial or fungal infection or contamination of water resources [5]. Therefore, blood parameters are understood to be useful indicators or instruments for assessing the body's response to various stressors [6]. The biochemical and molecular processes of the life-supporting system are severely hampered by stressors known as toxicants, which accumulate in fish through the food chain or are absorbed through the fish's skin.Pollution usually causes a rapid change in fish immunological characteristics [7].

Fish reactions to various toxicants that reflect the ecological health of the habitat can be effectively monitored using the immunological index, a commonly used method to assess the sub-lethal effects of the pollutant [8]. Therefore, blood parameters, especially toxicological ones, are used as indicators to predict the health and symptoms of organisms, particularly fish [9]. Akinrotimi et al. [10] state that internal factors including sex, age, size, reproductive stage, and health, as well as extrinsic factors like water temperature, nutrition, stress, and seasonal dynamics, have an impact on fish immunological parameters. The changes in blood parameters might be a sign of a fish's physiological response to outside stressors [11]. Blood variables have been shown to be stress indicators and to provide information on fish physiologic responses to changing external conditions [12]. Numerous haematological indicators, such as neutrophils, leucocrit, white blood cells, lymphocytes, monocytes, and thrombocytes, have been used to detect pollutants in the aquatic environment [13].

Fish lymphocytes are responsible for the production of antibodies as a consequence of an immune response since they are believed to be immunocompetent [14]. The immune system depends on white blood cells, which are mainly made up of lymphocytes, monocytes, and granulocytes [15]. When lymphocytes and monocytes are affected, the fish's immune system will be affected. The fish deteriorate as a result, making them more vulnerable to illnesses. It is thought that the negligent application of glyphosate may affect the immunity of the species *C. guineensis*. Many of the innate immune system's components seem to have undergone minimal alteration across evolution. Given the intricacy of the immune system, variations in the immunological response and disease resistance integrity are especially sensitive indicators of toxic damage in mammalian systems [16]. The immune system's vulnerability to a particular poison may therefore vary depending on the species. The relationship between environmental toxicity and fish population disease has long been debated. Therefore, the purpose of this study was to determine the effects of exposure to different amounts of glyphosate on *C. guineensis* immunological profiles.

2. MATERIALS AND METHODS

2.1 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 *C. guineensis*, used for the experiment were sourced from the recruitment ponds in the centre. They were later sorted and grouped into two based on their sizes, juveniles were of the size (mean length 13.55cm±2.55SD and mean weight 77.99g±6.98SD) and adults were of the size (mean length 22.56cm±8.12SD and mean weight 188.45g±34.45SD). The fish were brought to the lab in six open, 50-liter plastic containers, where they acclimated for seven days.

2.2 Preparation of Test Solutions and Exposure of Fish

In the present study, glyphosate was used. Glyphosate is a chemical used in herbicides to kill weeds and grasses. It's a broad-spectrum systemic herbicide that works by blocking an enzyme essential for plant growth The pesticide was purchased from a commercial outlet in Port Harcourt, Nigeria. *C.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.

2.3 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 [17]. The leucocytes count was made using improved Neubauer haemocytometer after diluting the blood 1:100 with Shaw's solution.

2.4 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 [18].

2.5 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 $P < 0.05$.

3. RESULTS

The physico-chemical parameter results in the experimental tanks over the course of the exposure period are displayed in Table 1. The salinity, pH, and temperature readings were all within the same range. However, ammonia and nitrite levels significantly increased. Nevertheless, the amount of dissolved oxygen dropped as the chemical concentration rose. Tables 2 and 3 illustrate the immunological profiles of *C. guineensis* juveniles and adults exposed to varying glyphosate concentrations, respectively. The chemical's effects on the total leucocyte and differential white blood cell counts in *C. guineensis* juvenile and adult sizes were demonstrated by the results. According to the findings, the treatment groups' total leucocyte counts were considerably higher ($P < 0.05$) than the control group's. Additionally, there was a significant difference ($P < 0.05$) in the leucocyte counts across the groups.

The results also showed that the peripheral blood of *C. guineensis* had three different kinds of white blood cells: neutrophils, monocytes, and lymphocytes. These were classified as either granulocytes or a granulocytes based on whether or not granules were present in their cytoplasm. Lymphocytes are the most common form of leucocyte found in *C. guineensis* blood. The control group's leucocyte counts were significantly higher ($P<0.05$) than those of the treatment groups. The development of lymphocytosis occurred as exposure duration increased. Monocytes are spherical cells with oval nuclei and clumped chromatin, the second kind of granulocytes seen in blood. Compared to the control group, the exposed fish's monocyte count dropped dramatically (P 0<05). The development of monocytopenia occurred as exposure duration increased. The neutrophils and eosinophils were the granulocytes found in *C. guineensis* blood. Neutrophils and eosinophils were significantly higher in the treated groups (P<0.05) than in the control. The most severe neutropenia was seen in the fish that had been exposed to the toxin.

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

Parameters	Concentrations of Glyphosate (mg/L)				
	0.00	**0.05**	**0.10**	**0.15**	**020**
Temperature (^{0}C)	28.08±1.03[a]	28.71±1.77[a]	28.77±1.77[a]	28.69±1.33[a]	28.99±1.08[a]
pH	6.66±1.03[a]	6.65±1.33[a]	6.43±1.99[a]	6.88±1.98[a]	6.88±1.98[a]
Ammonia (mg/l)	0.16±0.01[a]	0.35±0.01[ab]	0.55±0.18[b]	0.68±0.77[b]	0.98±0.87[c]
DO (mg/l)	6.67±0.07[c]	6.44±0.51[c]	5.51±0.69[5]	4.41±0.88[b]	3.88±0.45[a]
Nitrite (mg/l)	0.02±0.01[a]	0.05±0.01[b]	0.07±0.01[b]	0.09±0.01[b]	0.09±0.01[c]
Salinity (ppt)	11.55±1.21[a]	11.44±3.66[a]	11.46±1.99[a]	11.66±3.88[a]	11.70± 2.11[a]

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

Table 2: Immunological Changes in Juveniles of *C. guineensis* Exposed **to Different Concentrations of Glyphosate (Mean ± SD)**

Parameters	Concentrations of Glyphosate (mg/L)				
	0.00	**0.05**	**010**	**0.15**	**0.20**
WBC (cellsx10^9)	15.68±2.62[a]	18.99±1.08[a]	23.66±2.41[b]	29.22±3.77[b]	34.98±2.02[c]
Leucorit (%)	13.88±1.82[c]	10.66±1.99[b]	9.02±1.09[b]	6.29±1.66[b]	4.98±0.91[a]
Thrombocytes (%)	149.12±9.88[c]	132.88±3.98[c]	115.99±9.03[b]	90.88±5.90[a]	82.99±7.88[a]
Neutrophils (%)	10.49±3.99[a]	13.08±1.88[b]	16.55±1.90[b]	20.66±1.89[a]	37.88±2.88[a]
Lymphocytes (%)	71.88±4.86[c]	67.88±1.92[c]	63.04±1.05[ab]	50.66±1.77[a]	44.99±1.04[a]
Monocytes (%)	17.15±2.23[a]	15.45±1.88[a]	13.06±1.03[b]	11.89±1.90[c]	10.68±1.88[c]
Eosinophils (%)	1.01±0.55[a]	3.02±0.89[a]	6.88±0.99[a]	7.04±1.99[a]	8.08±1.84[a]

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

Table 3: Immunological Changes in Adults of *C. guineensis* Exposed to Different Concentrations of Glyphosate (Mean ± SD)

Parameters	Concentrations of Glyphosate (mg/L)				
	0.00	**0.05**	**0.10**	**0.15**	**0.20**
WBC (cellsx10^9)	19.89±4.05[a]	26.87±1.99[a]	28.82±6.02[b]	34.88±7.77[b]	41.03±5.01[c]
Leucorit (%)	18.45±1.88[c]	14.99±1.68[b]	12.01±3.41[b]	10.65±1.91[b]	7.12±1.02[a]
Thrombocytes (%)	192.23±9.77[c]	170.66±9.88[c]	152.88±9.88[b]	129.98±5.90[a]	120.03±11.02[a]
Neutrophils (%)	12.88±5.82[a]	17.88±5.85[b]	20.71±4.61[b]	35.49±1.92[a]	53.99±5.67[a]

Lymphocytes (%)	72.88±9.89[c]	67.99±1.82[c]	57.72±3.41[ab]	47.70±6.99[a]	30.99±4.05[a]
Monocytes (%)	15.23±6.90[a]	14.75±1.88[a]	14.23±3.81[b]	8.19±1.02[c]	7.89±0.88[c]
Eosinophils (%)	2.66±0.99[a]	5.15±0.86[a]	8.12±2.02[a]	8.69±1.61[a]	10.33±3.09[a]

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

4. DISCUSSION

Fish exposed to pesticide doses below the lethal threshold are more likely to survive and recover when their WBC count rises, which is associated with the production of more antibodies [19]. Akinrotimi and Amachree [24] conducted similar studies that demonstrated the impact of detergents on *Clarias gariepinus* and showed significant decreases in WBC. Furthermore, Akinrotimi et al. [21] discovered alterations in the immunological indices of fish (*S.melanotheron)* throughout the acclimatization period and techniques. In this study, the immunological index values, specifically those of WBC, thrombocytes, neutrophils, lymphocytes, monocytes, and eosinophils, varied, they said; notably, a sharp drop in levels was noted in females. Similar changes in immunological values were noted by Akinrotimi *et al.* [22] in male and female *Tilapia guineensis* fish exposed to different water pH conditions. These changes were associated with differences in the exposed fish's immunological concentration.Moreover, Akinrotimi *et al.* [23] reported a significant decrease in leucocyte count after exposing Tilapia guineensis to industrial effluents, while Gabriel *et al.* [24] found a significant increase in leucocyte concentration in tilapia, Oreochromis niloticus, after adaptation to captivity [25]. Documented was a significant decrease in lymphocyte count and a marked increase in neutrophils in *Tilapia guineensis* exposed to handling stress; in this study, the number of lymphocytes drastically decreased while neutrophils increased. Neutrophils have also been seen to increase in freshwater fish treated with fenvalerate and malathion [26]. The pulp mill effluent's toxic stress was also linked to a significant rise in neutrophils [27]. In the freshwater fish *Channa punctatus*, monocrotophos increased lymphocyte counts while decreasing neutrophil counts [28].

In addition, the loss of hematopoetic tissue and a decline in the non-specific immune system brought on by high levels of protective toxin may be the causes of the decrease in lymphocyte and rise in neutrophil content. After being exposed to Rayon industry effluents, a study was conducted to evaluate the haematological parameters of *C. punctatus*, the effluents decreased the number of monocytes [29]. The quantity of monocytes in *H. fossilis* dramatically dropped with exposure to lindane [30]. The erythropoetic activity and haematological parameters of C. punctatus were impacted by monocrotophos, which led to a decrease in the monocyte count [31]. Sublethal levels of the synthetic pesticide glyphosate raised the proportion of monocytes in *C.guineeensis* in this investigation. Glyphosate harmful effects on the kidney and spleen (haematopoetic tissues) may be the cause of the current study's decrease in monocyte count. A higher concentration of eosinophils in fish has been linked to chemical exposure [32]. In a similar study, *Cyprinus carpio's* eosinophil count rose as a result of diazinon's toxicity [33]. There were fewer eosinophils in the experimental fish after they were exposed to malathion [34]. While, *C. batrachus* exhibited a decrease in basophils and an increase in eosinophils following a 60-day exposure to carbon tetrachloride [35]. In this investigation, *C.guineensis* treated to sublethal levels of glyphosate showed a decreased blood coagulation time and a decrease in thrombocyte count. After being treated with heptachlor, *Heteropneustes fossilis* equally showed a marked reduction in thrombocyte count [42]. In *C. batrachus*, endosulfan treatment led to a significant rise in thrombocytes in circulation [37]. Defective hemopoiesis and thrombocyte disintegration through capillary bleeding may be the cause of the current study's notable drop in thrombocyte count brought on by carbofuran poisoning [38].

5. CONCLUSION

In summary, the results of this study show how fish are stressed by the uncontrolled release of chemicals, such pesticides, into the aquatic environment. The fate of wild fish populations exposed to pollutants in their natural environments can be predicted using the outcomes of fish exposure to different toxicants. Additionally, using this chemical close to an aquatic medium should be done with caution.

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