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Haematological Responses of *Tilapia guineensis* Treated with Industrial Effluents

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Abstract A total of 180 *Tilapia guineensis* (mean length 20.62cm±2.1SD and mean weight 150.28g ±3.14SD) were exposed (10 fish per tank) to 0.00mlL^{-1} (control) 0.1, 0.2, 0.3, 0.4, 0.5mL^{-1} industrial effluents in triplicates in plastic tanks under a static renewal condition for 15 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), thrombocytes and blood glucose. Exposure of *T. guineensis* 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 gradual increase in the values of WBC, MCV, neutrophils, monocytes and blood glucose as the concentration of the effluent increased. These alterations were more pronounced in the fish exposed to 0.30, 0.20 and 0.50m/L^{-1} of the effluents. Results from this study suggest that brief exposure of *T. guinensis* to industrial effluents could cause some level of stress as manifested by changes in the haematological parameters of fish under consideration.

Keywords: *haematology, effluents, tilapia, acute toxicity, aquatic population*

1. Introduction

Rapid growth and expansion of industries in recent years has resulted in the substantial increase in effluents which, are normally discharged into open land or aquatic environment causing a number of environmental problems [1.2]. Indiscriminate discharge of these industrial wastes has aggravated the problem of aquatic pollution and contamination thereby causing alterations in the national condition of aquatic medium and consequently results in changes in the internal mechanism as well as morphological imbalance of aquatic organism [3,4]

The use of haematological studies in fish for assessment of impacts of toxicants in environmental research has increased tremendously in recent years [5]. Gabriel *et al.* [6] noted that among the cellular, biochemical, and physiological systems of multicellular animals that can be maintained in ecotoxicology, the use of heamatology has some uniquely attractive features. Likely more than other biological processes, haematology is directly concerned with the internal mechanism of an individual, in relation to its survival or essential factor of ecology [7]. Detrimental changes in blood parameters as a result of contaminants are of great ecotoxicology concern because they have the potential to influence the individual organisms by affecting the susceptibility to disease [8].

In addition, analysis of blood parameters will reveal conditions within the body of the fish long before there is any outward manifestation of disease or effects of unfavourable environmental factors [9]. Also blood parameters can be used to provide an indication of exposure to chemical toxicants and contaminants and predict the toxic effects of pollutants in the aquatic medium [10].

Tilapia guineensis is an eurylabire species that is commonly found along the coast of West Africa. The species is usually found in creeks, lagoons, and adjoining rivers [11]. Since most industrial effluents are discharged continuously into these aquatic medium, it becomes necessary to assess the possible effects that effluent 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 industrial effluents on some of its blood parameters under laboratory conditions.

2. Materials and Methods

One hundred and eighty adult of *T. guineensis* (mean total length 20.62cm±2.21SD and mean weight 150.16g ±3.14SD) were collected from African Regional Aquaculture Centre, Buguma Rivers State, Nigeria and acclimated to laboratory conditions for seven days in two large plastic tanks (6x3x2cm). During this period the fishes were fed with formulated diet containing 35% crude protein once a day. Also the water in the tanks was renewed daily during acclimation..

The fish were exposed to effluents collected from a vegetable oil company located in Port Harcourt, Rivers State Nigeria. The desired effluents concentration (0.10, 0.20, 0.30, 0.40, 0.50m/L^{-1}) and a control (0.00m/L^{-1}) with no toxicants were prepared according to the method described by Yaji and Auta [12]. Each toxicant was replicated three times.

The measured effluents were introduced into 40L of plastic tanks filled up to 30L of brackish water. The mixture was allowed to stand for 30 minutes before introducing the test fishes. A total of 180 fish were stocked in 18 tanks, (3 per concentration) to give a loading rate of 10 fish per tank and the fish were exposed for a period of 15 days. The water in the experimental tank was renewed every two days based on the initial different concentrations.

Water quality parameters in the experimental tanks during the trial were determined, temperature was measured with mercury in glass thermometers, pH with pH meter (Model 3013, Jenway, China), salinity was determined with hand held refractometer (Atago products Model H.191, Japan) while electrical conductivity, nitrate, dissolved oxygen and biological oxygen demand were done according to APHA [13].

Blood samples were collected at the end of 13 days exposure, from the fish (three fish per tank) from caudal region using heparinized 5ml syringes with 21 gauge needle. After collection the blood samples were transferred into Ethylene Diamine tetra Acetic Acid (EDTA) bottles determined using standard laboratory methods as described for laboratory analysis. The haematological parameters were by Blaxhall and Daisley [14], Packed Cell Volume and red blood cell (RBC) were estimated by the method of Rusia and Sood [15] using haemocytometer Haemoglobin content of the blood was determined by cyanmethiamoglobin method, white blood cell (WBC) was evaluated using improved Neubauer counter as described by Yaji and Auta [12]. The differential counts such as neutrophils, monocytes and lymphocytes were determined on blood film stained with Grumwald-Giemsa stain [16]. Thrombocytes and red blood indices such as mean corpsuclar volume (MCV), mean corpuscular haemoglobin (MCH) and means corspucular haemoglobin concentration (MCHC) were calculated using standard formula [17]. Plasma glucose was estimated by Ezymatic cadorimetric method [18].

The data were analyzed by one-way analysis of variance ANOVA a Duncan multiple Range Test for variable at 0.05 probability was used for comparism of the treatment [19].

Table 1. Physicochemical Parameters in exposed tanks during the experimental period (Mean ±SD)

Parameter	Concentration of industrial effluents (M/L^{-1})					
	0.00	0.10	0.20	0.30	0.40	0.5
Temperature (^{0}c)	2916 ±0.41^a	29.21 ±0.51^a	29.66 ±1.21^a	29.71 ±1.11^a	24.12 ±1.41^a	29.33 ±1.35^a
pH	6.61 ±0.12^a	6.62 ±0.31^a	6.62 ±0.31^a	6.24 ±0.71^a	6.17 ±0.12^a	6.00 ±0.31^a
Dissolved Oxygen (Mg/L^{-1})	6.91 ±0.31^a	6.80 ±0.12^a	6.54 ±0.24^a	5.50 ±0.33^b	5.20 ±0.14^b	5.11 ±0.25
Salinity (o/oo)	13.29 ±1.21^a	12.31 ±1.31^a	13.30 ±1.22^a	13.29 ±1.30^a	13.30 ±1.27^a	13.30 ±1.28^a
Electrical conductivity (µs/cm)	100.77 ±6.21^a	108.66 ±6.31^a	110.71 ±7.21^a	115.66 ±6.22^b	131.12 ±6.23^c	135.66 ±7.25^c
Nitrate (Mg/L^{-1})	0.65 ±0.001^a	0.06 ±0.01^a	0.09 ±0.01^a	0.09 ±0.00^a	0.09 ±0.01^a	0.09 ±0.01^a
Biological oxygen demand (mgL^{-1})	0.40 ±0.10^a	0.67 ±0.01^a	1.21 ±0.14^a	2.66 ±0.11^{ab}	3.21 ±0.14^c	4.58 ±0.80^c

Table 2. Haematological parameters of *T. guineensis* exposed to different concentration of industrial effluents for 15 days (Mean ±SD)

Parameter	Concentration of industrial effluents (M/L^{-1})					
	0.00	0.10	0.20	0.30	0.40	0.5
Hb (gdL^{-1})	6.41 ±0.18^a	5.21 ±0.12^b	4.20 ±0.16^c	3.16 ±0.48^d	2.17 ±0.19^e	2.00 ±0.28
RBC (x10^aL^{-1})	4.22 ±0.21	3.41 ±0.13	2.91 ±0.14	2.21 ±0.22	2.01 ±0.10	1.6 ±0.91
PCV (%)	20.16 ±2.11^d	18.34 ±2.11^c	16.41 ±2.33^b	14.22 ±2.78^{ab}	12.61 ±1.98^a	11.88 ±1.12^a
WBC (x10^{12})	20.64 ±1.91^a	23.66 ±3.41^b	25.74 ±2.14^{ab}	28.61 ±2.04^c	31.16 ±2.14^d	34.21 ±2.14^{cd}
MCH (pg)	15.38 ±3.21^c	13.28 ±2.86^c	14.43 ±3.01^b	14.29 ±2.71^b	12.28 ±2.11^a	12.1g ±2.61^a
MCHC(%)	31.79 ±2.71^e	28.40 ±3.01^d	25.59 ±2.11^c	22.22 ±1.79^{ab}	19.58 ±1.66^b	16.83 ±1.36^a
MCV (PI)	47,77 ±2.65^a	53.78 ±3.11^b	56.39 ±4.12^b	64.34 ±3.11^{ab}	65.73 ±4.21^{ab}	70.71 ±3.71^c
NEUT (%)	2.12 ±0.11^a	2.42 ±0.12^a	2.98 ±6.21^a	3.01 ±0.11^{ab}	4.00 ±0.91^d	4.21 ±0.18^b
MONO (%)	28.81 ±1.21^a	30.56 ±1.21^b	32.01 ±1.66^b	34.10 ±2.11^c	36.14 ±1.91^d	40.21 ±2.16^c
LYMPH (%)	68.07 ±2.11^a	67.00 ±2.11^a	65.11 ±3.22^{ab}	63.03 ±2.78^b	60.08 ±2.71^c	56.14 ±2.24^d
THRO (%)	98.64 ±4.16^d	87.14 ±3.12^c	71.12 ±3.14^b	61.12 ±2.11^{ab}	51.21 ±2.66^b	39.14 ±3.12^a
GLU (mg/L)	3.75 ±0.26^a	4.89 ±0.29^b	5.99 ±0.31^{ab}	6.91 ±0.41^c	7.91 ±1.11^d	9.91 ±1.12^e

Key: PCV – Packed Cell Volume (%), Hb – Haemoglobin (gd/L); RBC- Red Blood Cells (x10^9/L), WBC – White Blood Cell (x10^{IL}): MCH – Mean Corpuscular Haemoglobin (pg); MCHC – Mean Corpuscular Haemoglobin Concentration (%); MCV – Mean Corpuscular Volume (R1); NEUT – Neutrophils (%); MONO – Monocytes (%). LYMP – Lymphocytes (%); THRO – Thrombocytes (%); GLU – Glucose (mg/L)

3. Results

The water quality parameters in the various treatments level (Table 1) indicated that there were no significant differences ($p > 0.005$) in the values of temperature, pH, salinity and nitrate, while significant changes ($p < 0.05$) were observed in dissolved oxygen, electrical conductivity and biological oxygen demand. These changes were recorded from 0.30M/L^{-1} concentrations of the effluents (Table 1). The haematological changes produced by the effects of exposure of *T. guineensis* to different levels of effluent under a static conditions showed a significant different in all the parameters of exposed fish and the control (Table 2).

There was a significant increase ($p < 0.05$) in the values of white blood cells, mean corpuscular volume (MCV), neutrophils, monocytes and glucose compared to the control value. These increases were more noticeable in the fish i exposed to 0.30mlL^{-1} of effluents and above (Table 2). Exposure of *T. guineensis* to effluents caused a concentration dependent reduction in the values of Haemoglobin (Hb), red blood cell (RBC), packed cell volume (PCV), mean corpuscular haemoglobin (MCH),

mean corpuscular haemoglobin concentration (MCHC), lymphocytes and thrombocytes (Table 2).

4. Discussion

Water quality parameters are an indicator of toxicants effects in the aquatic medium. [20]. In the study no changes were observed in the values of temperature, pH, salinity and nitrate between the control and various concentrations of effluent, the result agreed with that of Nte *et al.*, [21] who reported no significant changes in water quality in the experimental water on exposure of *Sarotherondon melanotheron* to industrial effluents. There were changes in the values of dissolved oxygen which decreases as the concentrations of the effluent increases, while electrical conductivity and biological oxygen demand increased considerably with increasing concentrations of the effluents. The reduction in the value of dissolved oxygen is in line with the report of Gabriel *et al.* [6] who reported same in *Clarias gariepinus* exposed to proxone under laboratory conditions. This may be due to stress induced condition by the effluents which results in agitation and aggressive behaviour of the fish and thereby reduces the dissolved oxygen level of the water and consequently increased their biological oxygen demand in the exposure tanks.

Haematological assessment is a pathophysiological reflector of the whole body and therefore, blood parameters are important in diagnosing the structural and functional status of fish exposed to contaminants [22]. Exposure of *T. guineensis* to sublethal levels of industrial effluents which is obtained in the environment, results in concentration dependent decreases in Hb, RBC, PCV, MCH, MCHC, thrombocytes, lymphocytes and plasma glucose. The reduction in RBC, Hb and PCV consequently led to reduction in oxygen carrying capacity of blood, resulting to anaemic condition in the fish. This result corroborates the findings of Ramesh and Saravana [23] in *Cypinus* carpio exposed to chlorpyrifos under laboratory conditions. The anaemic status, may be as a result of inhibition of erythropoeiesis and haemosynthesis consequent to an increase in the rate erythrocytes destruction in haemopoietic organs. A decreased in value of lymphocytes and thrombocytes which are concern with the specialized defense and blood clotting respectively in the body of the fish, is in line with that of Ajani *et al.* [24] in *Clarias gariepinus* exposed to water borne toxicants. This may be as a result of lymphopenia and thrombocytosis which characterized animal undergoing stress consequent of unfavourable environment.

Increase in the various WBC, neutrophilis, and monocytes can be correlated with an increase in antibody production which helps in survival and recovery of fish exposed to toxicants as observed by Tiago *et al.* [25] in *Brycon amazonica* exposed to low concentrations of phenol in the laboratory. In the present study the significant increase in WBC, neutrophils and monocytes counts indicate hypersensitivity of these cells to effluents and the changes may be due to immunological reaction to produce more antibodies to combat the stress induced through exposure to industrial effluents.

Changes in blood glucose has been suggested as useful indicator of stressed condition and probably the easiest and most commonly measured secondary response to stressors in fish [26,27]. The plasma glucose concentration in circulation is dependent upon glucose production and its clearance from circulation. The production of glucose assists the animal to cope with stress. In the present study, the significant increase of plasma glucose level might have resulted from gluconeogenesis to provide energy for the increased metabolic demands imposed by effluent stress.

5. Conclusion

The present investigation revealed that exposure of *T. guineensis* to low concentrations of industrial effluents affects the physiology of the fish, such as reduction in the volume of red blood cells. Therefore, care should be taken in discharging the effluent into water bodies so as to minimize its impact on aquatic organism.

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