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Haematological Profile of Oreochromis Niloticus (Burchell, 1822) Exposed to Cadmium Chloride

M. S. Isiyaku[1*] and R. Mohammed[2]

[1]*Department of Fisheries and Aquaculture, Bayero University, Kano, Nigeria.*
[2]*Department of Aquaculture and Fisheries Management, Nassarawa State University, Keffi, Nigeria.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Changes in *Oreochromis niloticus* blood cells were investigated after 96-h of exposure to cadmium chloride. One hundred and eighty (180) *Oreochromis niloticus* with average weight of 26.07±1.23g and mean length of 17.50 ± 0.50cm were divided into 5 groups (T1-T5) at ten (10) fish per group and in triplicates after being acclimatized for 14 days. They were then exposed to various concentrations (0, 28, 30, 32, 34 and 36 mg/l) of cadmium chloride. The packed cell volume (PCV) of the treatments decreased significantly relative to that of the control, while their platelet counts increased compared with the control. There was also a reduction in the RBC (2.70, 2.51, 2.24, 1.98, 1.64 and 1.22) of treatments. Other blood parameters did not vary significantly in comparison with the control group, but it is worth noting that, the mean corpuscular volume (MCV) (70.78, 76.09, 83.71, 90.55, 105.49 and 128.68fL), mean corpuscular haemoglobin (MCH) (24.66, 24.49, 27.37, 29.83, 33.14 and 42.18pg) and mean corpuscular haemoglobinconcentration (MCHC) (34.83, 32.19, 32.71, 32.95, 34.41 and 34.78%) increased considerably in all treatments compared to the control. These alterations have been attributed to direct or feedback responses of structural damage to RBC membranes resulting in haemolysis and impairment inhaemoglobin synthesis, stress related release of RBCs from the spleen and hypoxia, which was induced by exposure to cadmium chloride. This study therefore gives an insight into toxic effect of cadmium chloride on fish.

**Corresponding author: Email: imsani.faq@buk.edu.ng;*

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1. INTRODUCTION

The count of red blood cells is quite a stable index and the fish body tries to maintain this count within the limits of certain physiological standards using various physiological mechanisms of compensation. Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the valuesof one or more of the haematological parameters [1]. Blood cell responses are important indicators of changes in the internal and/or external environment of animals. In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels. Their changes depend on fish species, age, the cycle of the sexual maturity of spawners and diseases [2].

Fish live in very intimate contact with their environment and are therefore very susceptible to many physical and chemical changes which may be detected in their blood components [3]. Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health status of fish [4]. In fish, exposure to chemical compounds can induce either increase or decrease in the levels of haematological indices. Since blood tissues clearly reflect physical and chemical changes occurring in organisms, detailed information can be obtained on general metabolism and physiological status of fish in different groups of age and habitat. The study of physiological and haematological characteristics of cultured fish species is an important tool in the development of aquaculture system, particularly with respect to its use in distinguishing healthy from diseased or stressed fish [5]. Early diagnosis is also possible, when evaluating haematological data, particularly blood parameters [2].

However, there is a need to understand the physiological concept of fish health in relation to blood and the quality of dietary protein fed. Any changes in the value of a component of a blood sample, when compared to the normal values, could be used to interpret the metabolic state and health status of animal [6,7]. Low haematological indices are indications of anaemic conditions [8]. In fish, exposure to chemical pollutants can induce either increases or decreases in the levels of haematological indices. Such changes depend on fish species, age, cycle of sexual maturity of spawners and disease [2]. Previous haematological studies on the effect of nutrition [9], infectious diseases and pollutants [10] revealed that erythrocytes are the major and reliable indicators of various sources of stress [5].

The Nile tilapia *Oreochromis niloticus* is a deep-bodied fish with cycloid scales, silver in colour with olive, grey or black body bars, the Nile tilapia often flushes red during the breeding season [11]. It grows to a maximum length of 62 cm, weighing 3.65 kg (at an estimated 9 years of age) [12] and its average size (total length) is 20 cm [13]. *O. niloticus* is surface-feeding omnivore fish belongs to the family Cichlidae. It is the most widely cultured fish in the tropics [14]. It is commonly available and easy to manipulate both in capture and culture fisheries due to its qualities such as good taste, hardy nature, fast growth, resistance to diseases, ease with which to reproduce in captivity and switching of diet. Apart from being a good candidate for aquaculture, it serves as an important source of high level of animal protein especially in the developing countries where there are high levels of animal protein deficiencies [15,16] (Fagbenro and Adebayo, 2002).

The choice of cadmium chloride in this experiment is because of its lower toxicity compared to the other forms of heavy metals, it is used in Agriculture as fungicide, in Medicine as tropical antiseptic, disinfectant, dental amalgams and in research laboratories as an intermediate in the production of other compounds, thermometers, barometers, batteries, lamps, refining, lubrication oils and pressure-sensing devices. It is therefore, pertinent to determine the tolerance limit of some of these chemicals.

This study therefore assessed the haematological profile of *Oreochromis niloticus* exposed to cadmium chloride.

2. MATERIALS AND METHODS

2.1 Experimental Site

The study was carried out in the Department of Fisheries and Aquaculture, Bayero University Kano, Kano State

2.2 Experimental Fish

Juveniles of *Oreochromis niloticus* of a mean weight of 26.07±1.23g and mean length of 17.50

± 0.50cm were obtained from Rumbin kifi fish farm, Kano, Kano State. The fish were acclimatized for 14 days in the fish hatchery Department of Fisheries and Aquaculture, Bayero University Kano. The fish were fed twice daily at 0800 and 1600 hours at 5% of their body weight. Prior to and during exposure period fish were starved for 24hrs, to reduce faeces and ammonia in the experimental containers that could act as contaminants to the experiment.

2.3 Source of Mercuric Chloride

The cadium Chloride was procured from Emole Nigeria Limited, Sabon Gari, Kano a supplier of Laboratory Chemicals and Equipment.

2.4 Physicochemical Parameters of Test Solution

Water quality parameters such as temperature, pH, conductivity, total dissolved solids (TDS) and dissolved oxygen (DO) concentration were routinely (Water quality was monitored at 4 hours internal) monitored in the tanks using digital multi-parameter checker (HI 98126).

2.5 Experimental Design

A completely randomized design was used for the experiment. A total of one hundred and eighty (180) juvenile of *Oreochromis niloticus* were randomly distributed into the plastic containers (60x40x40) size and 70 litres capacity at a stocking rate of 10 fish. The eighteen (18) tanks were assigned to 5 treatments with (control inclusive). In order to determine the LC_{50}, the *Oreochromis niloticus* were exposed to five different concentrations of Cadmium chloride for 96hrs.

2.6 Haematological Studies of *Oreochromis niloticus* Exposed to sub-lethal Concentrations of Cadmium Chloride

Blood samples were taken by randomly selecting two fish from the various treatments and injecting in a 2mm needle and syringe through the dorsal aorta puncture and placed in ethylene-diamine-tetra-acetic-acid (EDTA) treated bottles to prevent coagulation and analyzed at Animu Kano Teaching Hospital Kano, Kano State for the following: haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) using an automated haemoglobin analyzer (Cobus U 411) model, while Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were determined by calculations.

2.7 Statistical Analysis

All results were collated and analyzed using computerized, Probit analysis. The mean lethal concentration LC50 at selected periods of exposure and an associated 95% confidence interval for each replicate toxicity test were subjected to probit analysis (Finney, 1971) using statistical package (SPSS, version 22) to determine (LC_{50}).

3. RESULTS AND DISCUSSION

The mortality rates of *Oreochromis niloticus* exposed to various acute concentration of cadmium chloride are shown on Table 1.

As the concentration of the toxicant increases, the rate of stress responses observed are the fish becomes restless, weak and settled at the bottom of the bowl and death. This shows that increase in concentration of cadmium chloride resulted in higher mortalities. The highest mortality rate (83.3%) was recorded in T5 (36 mg/L) while the least mortality rate (20.0%) was recorded in T1 (28 mg/L) Omitoyin et al., [17] and Isiyaku et al. [7] report similar trend in exposure of Oreochromis niloticus to mercuric chloride. There was no mortality in the control treatment throughout the 96hrs exposure.

The regression equation for the relationship was calculated to be probit y =34.02x +24.19, log concentration and on R-square value, R^2=0.985. The expression, R^2 value indicates that, mortality rate of fish increased with increase in concentration of mercuric chloride.

3.1 Behavioral Changes of *Oreochromis niloticus* Juveniles in Cadmium Chloride

The results presented in Table 2 summarized the behavioural changes observed in *Oreochromis niloticus* juveniles exposed to different concentrations of cadmium chloride. Abnormal behaviours observed with *O. niloticus* exposed to various concentration of cadmium chloride in this study were excessive gulping for air, erratic swimming behaviour, restlessness, loss of equilibrium, fin and barbell deformation, skin

Table 1. Mortality rates of *Oreochromis niloticus* juveniles exposed to acute concentrations of cadmium chloride for 96hrs

S/No	Treatment (mg/L)	No of fish	Total Mortality	%Mortality	Log conc.	Probit kill
Control	**0.0**	**30**	**0**	**0.0**	**0.00**	**0.00**
T1	28.0	30	6	20.0	1.45	4.16
T2	30.0	30	10	33.3	1.48	4.56
T3	32.0	30	15	50.0	1.51	5.00
T4	34.0	30	21	70.0	1.53	5.52
T5	36.0	30	25	83.3	1.56	5.95

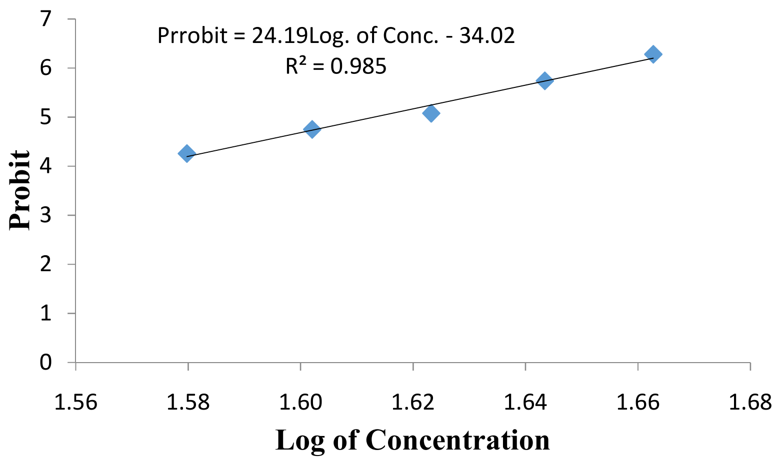

Fig. 1. Linear relationship between mean probit mortality and log concentration of *Oreochromis niloticus* juveniles exposed to acute concentrations of cadmium chloride for 96 hours

Table 2. Behavioral changes of *Oreochromis niloticus* juveniles in cadmium chloride during 96-hour exposure at different concentrations

Fish behaviour	Concentration (mg/L)					
	0.00	**28.0**	**30.0**	**32.0**	**34.0**	**36.0**
Air gulping	-	-	+	++	+++	+++
Barbel deformation	-	-	-	-	-	-
Discoloration	-	-	+	++	++	++
Erratic swimming	-	-	+	++	+++	+++
Scratching on plastic tank	-	-	-	+++	+++	+++
Jumping	-	-	+	++	++	++
Resting at bottom	-	+	++	++	+++	+++
Hanging vertically in water column	-	+	++	++	++	+++
Fin deformation	-	-	+	++	++	++

+ = slightly present
++ = moderately present
+++ = highly present
- = absent

haemorrhage, discolouration and finally death.These behaviour were also reported by Omitoyin et al., [17], Ladipo et al. [18]; Aderolu et al. (2010), Okomoda and Ataguba [19] and Isiyaku et al. [7] to varying toxicants. Bobmanuel et al. [20] stated that behavioural response of fish to toxicants and different reaction time are due to the effect of chemicals, their concentrations, species, size and specific environmental conditions.

Water quality parameter is very important in determining fish health and safety within their environments. This is because a good water quality influences fish survival, growth and reproduction. Table 3 Shows physico-chemical parameters of the toxicant used in the acute bioassay test on *Oreochromisniloticus.*

The toxic effects of heavy metal on fish are multidirectional and manifested by changes in the physiological and chemical processes of their body systems [21]. Sublethal toxicity of Cadmium chloride to fish produces haematological and neurological effects [22]. The PCV, Hb, RBC, WBC, Platelet count and derived erythrocyte indices (MCV, MCH and MCHC) of the fish exposed to cadmium chloride are presented in Table 4.

Literature Omitoyin et al., [17], Ladipo et al. [18]; Aderolu et al. (2010), Okomoda and Ataguba [19] and Isiyaku et al. [7] shows that changes in haematological indices of fish caused by heavy metals and their mixtures are different. They are predetermined both by the concentration of heavy metals in the water and time of exposure, and both these factors can cause reversible and irreversible changes in the homeostatic system of fish.It is well known that lead causes early mortality of mature red blood cells and inhibition of haemoglobin formation through inhibition of erythrocyte alpha-amino levulinic acid dehydratase (ALA-D).In the light of the present study, the mean value of PCV was 19.11 % in the control group, which decreased progressively in the treatments groups. A decrease in the erythrocyte count or in the percent of haematocrit indicates the worsening of an organism state and developing anaemia.

Haemoglobin concentrations reflect the supply of an organism with oxygen and the organism itself tries to maintain them as much stable as possible. This study shows that mean haemoglobin in the control was 6.66. A decrease in the concentration of haemoglobin in blood is usually caused by the effect of toxic metals on gills, as well as decrease in oxygen, which also suggests anaemia or confirms toxic impact of cadmium chloride in *Oreochromis niloticus*.

Haematological indices (RBC count, concentration of haemoglobin and haematocrit) have been reported to indicate secondary responses of an organism to irritants [23] reported in their research that mechanism of lead toxicity occurs by ionregulatory disruption. The reduction in WBC count of the treatment groups that was observed agrees with the report that the release of epinephrine during stress causes a decrease ofleucocyte count, which shows the weakening of the immune system. Morsy and Protasowicki, [24] demonstrated cadmium bioaccumulation resulting in reduction of erythrocytes count, haemoglobin contents andhaematocrit in compassion to the control.

In the present investigation, leucocytes showed greater and quite different pattern of change with the effect of cadmium chloride when compared with erythrocyte level of the control fishes. Blood of all experimental groups contained lower concentration of leucocytes than those of the control. Allen, [25] observed increased WBC count in *Oreochromis aureus* after mercury exposure. The decrease in number of WBC observed in the present study may be attributed to the stimulation of immune system in response to tissue damage caused by mercuric chloride. Dhanekar et al. [26] reported the increase in large lymphocytes, reduction in small lymphocyte and thrombocytes populations as also elevation in monocytes, neutrophils and eosinophils cells in *Heteropneustes fossilis, Channa punctatus*and *Mastaemebalus puncalus* on long exposure to least effective concentration of mercuric chloride.

Increase in the MCV and MCH in this study indicate that haemoglobin in the RBC was much lower in the exposed fish than in the control fish as reported by Bhagwart and Bhikajee, [27]. Annune et al. [28] reported increases in MCH and MCHC when *O. niloticus* was exposed to zinc.

Table 3. Physico-chemical parameters of the toxicant solution used in the acute bioassay test of *Oreochromis niloticus* juveniles exposed to cadmium chloride

Treatment	pH	TEMP(^{0}C)	TDS(mg/l)	EC(µS)	DO
Control 0.0	7.84±0.00^f	25.35±0.01^a	207.33±0.33^a	409.67±0.67^a	4.55±0.00^f
T1 (28.0)	7.80±0.00^e	25.35±0.01^a	206.33±0.67^b	412.67±1.33^b	4.50±0.00^e
T2 (00.0)	7.75±0.01^d	25.38±0.02^{ab}	211.33±0.33^c	423.33±0.67^c	4.46±0.00^d
T3 (32.0)	7.68±0.00^c	25.36±0.01^{ab}	216.00±0.33^d	433.67±1.15^d	4.43±0.01^c
T4 (34.0)	7.62±0.00^b	25.36±0.01^{ab}	224.33±0.33^f	441.33±0.67^e	4.40±0.00^b
T5 (36.0)	7.57±0.01^a	25.39±0.01^b	230.33±0.33^e	449.67±0.67^f	4.36±0.00^a

Mean in the same column with different superscript differ significantly (P<0.05)

Table 4. Haematological parameters of juveniles of *Oreochromis niloticus* exposed to cadmium chloride

Treatment (mg/L)	0.0	28	30	32	34	36
Parameters						
HCT (%)	19.11 ± 0.01^{d}	19.06 ± 0.01^{d}	18.70 ± 0.30^{d}	17.93 ± 0.03^{c}	17.24 ± 0.01^{b}	15.64 ± 0.04^{a}
HB (g/dL)	6.66 ± 0.03^{e}	6.14 ± 0.02^{d}	6.12 ± 0.01^{d}	5.91 ± 0.02^{c}	5.42 ± 0.02^{b}	5.13 ± 0.03^{a}
WBC($x10^{9}$/L)	2.53 ± 0.03^{a}	2.63 ± 0.02^{b}	3.36 ± 0.04^{c}	4.14 ± 0.02^{d}	6.69 ± 0.02^{e}	7.66 ± 0.01^{f}
RBC($x10^{12}$/L)	2.70 ± 0.02^{f}	2.51 ± 0.01^{e}	2.24 ± 0.04^{d}	1.98 ± 0.03^{c}	1.64 ± 0.04^{b}	1.22 ± 0.01^{a}
PLT($x10^{9}$/L)	15.29 ± 0.04^{a}	16.64 ± 0.04^{b}	18.21 ± 0.01^{c}	20.31 ± 0.01^{d}	23.18 ± 0.03^{e}	26.52 ± 0.02^{f}
MCH (Pg)	24.66 ± 0.28^{a}	24.49 ± 0.01^{a}	27.37 ± 0.40^{b}	29.83 ± 0.53^{c}	33.14 ± 0.80^{d}	42.18 ± 0.03^{e}
MCV (fL)	70.78 ± 0.49^{a}	76.09 ± 0.19^{a}	83.71 ± 2.65^{b}	90.55 ± 1.50^{c}	105.49 ± 2.20^{d}	128.68 ± 0.25^{e}
MCHC (%)	34.83 ± 0.15^{c}	32.19 ± 0.10^{ab}	32.71 ± 0.55^{b}	32.95 ± 0.04^{b}	31.41 ± 0.10^{a}	32.78 ± 0.09^{b}

Mean in the same row with different superscripts differ significantly

4. CONCLUSION

Contaminations of aquatic environment by heavy metals whether as a consequence of acute or chronic events constitute additional stress for aquatic organisms.Haematological indices of fish, caused bycadmium chloride toxicity to *Oreochromis niloticus*can be secondary responses totoxicants, including exposure to lowconcentrations of heavy metals, whichreflect the launch of stress reaction in theaffected fish.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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