

*Full Length Research Paper*

# Behavioural and hematological effects of zinc on African Catfish, *Clarias gariepinus*

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Laboratory investigations were carried out on toxic stress and changes in *Clarias gariepinus*' blood cells after a 96-h exposure to zinc. The renewal technique was followed using different concentrations in the range of 30 mg/l to 50 mg/l after a preliminary screening exercise. During the treatment, the color of the experimental fish became progressively darker with increasing concentrations and mortality was directly proportional to the concentrations of the tests solution unlike those in the control. The 96-h median lethal concentration (96-h LC<sub>50</sub>) was 36.7 mg Zn/l using the logarithmic method. The data obtained showed that the exposure duration evidently influenced the value of median acutely lethal concentration (LC<sub>50</sub>). The erythrocyte, leucocytes, hematocrit, platelets and hemoglobin count of healthy control indicated a mean value of  $3.2 \pm 1.2$  cells  $\times 10^7$   $\mu$ l,  $8.5 \pm 3.6 \times 10^3$  mm<sup>3</sup>,  $40 \pm 4\%$ ,  $2.04 \pm 1.10$  cells  $\times 10^5$   $\mu$ l and  $2.06 \pm 1.03 \times 10^2$  g/dl respectively. Apart from platelets, other parameters displayed significant reduction with increased concentration of toxicant in the experimental treatment. The results indicate that Zn is toxic at high concentration producing dose-responsive increases in mortality and abnormalities in behavior. The fish species is therefore recommended as good bioindicator for the risk assessment of aquatic environment due to zinc.

**Key words:** Zinc, acute toxicity, LC<sub>50</sub>, behavioral changes, hematology, aquatic toxicity, *Clarias gariepinus*.

## INTRODUCTION

Zinc belongs to a class of microelements which are essential for proper functioning of the body (Hilmy et al., 1987; Kotze et al., 1999). Elevated levels of zinc in aquatic systems can be due to liquid effluent discharge, atmosphere deposition, the leaching of domestic sewage and metal bearing minerals insecticides and galvanizing processes (DWAF, 1996; Nussey, 1998). The toxicity of zinc to aquatic life was intensively investigated during the previous decades and a considerable amount of experimental data was compiled and reviewed (US EPA, 1980; Tuurala and Soivio, 1982; Somasundaram et al., 1984; Larson and Hyland, 1987; Bagdonas and Vosylienė, 2006).

In all these, zinc exerts adverse effect in fish accruing structural damage, which affects the growth, develop-

ment and survival of fish. Sub-lethal levels of zinc have been known to adversely affect hatchability, survival and haematological parameters of fish (Cardeihac et al., 1981; Clarkson, 1998; Dickman and Leung, 1998). For instance, exposure to sub-lethal concentrations (300 mg/l) of zinc sulphate for 20 days resulted in drastic changes in the male testis. Metals that accumulate in adult fish were reported being possibly transferred to the eggs during oögenesis, which in turn induced harmful effects onto the embryonic development (Sehgal and Saxena, 1986).

The studies carried out on various fishes have shown that heavy metals including zinc may alter the physiological activities and the biochemical parameters both in tissues and in blood (Tort and Torres, 1988; Canli et al., 1998; Svoboda, 2001; Basa and Rani, 2003; Witeska, 2003). Acute lethality of zinc salts to teleosts, have been reported (Crespo and Balasch, 1980). In addition, Zn can also cause adverse chronic effects at concentrations less than acutely lethal levels with continuous Zn exposure

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(Chapman, 1978). Although the mode of toxic action of zinc is uncertain, acutely toxic concentrations can kill fish by destroying gill tissue (Skidmore, 1964). Acute exposures can also cause an impaired brachial calcium influx leading to hypocalcemia (Hoogstrand et al., 1994).

Hematological alterations have therefore allowed for a relatively rapid evaluation of the chronic toxicities of a compound. Based on available literature, most reported data on Zn were based on the tissue metal concentration. This study therefore evaluated some toxic stress and hematological profile of *Clarias gariepinus*, after a 96-h exposure to zinc. The fish *C. gariepinus* is a hardy fish and highly valued in Nigeria. It would therefore be of interest to investigate the impact of zinc on the behavior and blood cells; which would then give an indication of the toxic nature of zinc.

## MATERIALS AND METHODS

### Sample collection and acclimatization

Healthy specimens of African catfish *C. gariepinus* were obtained from a fish farm in Ondo State, Nigeria. The choice of *C. gariepinus* was informed by its ability to withstand stress and its high commercial value in Nigeria. In the laboratory, fishes were kept in large plastic bowls of 120L capacity containing 60L of clean tap water. The fishes were acclimatized for 6 weeks to the laboratory conditions, during which time they were provided with artificial feed (grower's mash) and ground shrimps obtained locally. The size of the fish varied from 18.1 – 22.7 cm in standard length and 50.6 - 97.4 g in weight. Fish of both sexes were used without discrimination. The fish were inspected for disease conditions and general fitness. Water was changed every other day.

### Determination of water physico – chemical parameters

The water quality parameters of the diluting water used in the tests were determined by standard methods (APHA, 1998). The mean temperature, pH, alkalinity, hardness and dissolved oxygen of the water used were 27.4°C, 6.51, 193.3 mg l<sup>-1</sup> (as HCO<sub>3</sub><sup>-</sup>), 227.5 mg l<sup>-1</sup> as CaCO<sub>3</sub> and 6.56 mg O<sub>2</sub> l<sup>-1</sup> respectively.

### Screening exercise / toxicants introduction

Ten fingerlings were kept per bowl. There were five different treatment groups and each had three replicates. The fish were fed three times daily. Feeding was discontinued while aeration continued during the 96-h test period. Toxicant stock solution was prepared by dissolving necessary amounts of the zinc sulphate in distilled water; the final concentration was recalculated according to the amount of heavy metal ion. Different concentrations required were prepared after a range – finding test using a screening procedure with concentrations of 10 – 50 mg l<sup>-1</sup>. Five sets of ten fish each were subjected to serial dilutions of the stock solution of Zn (from 30 – 50 mg l<sup>-1</sup>) in triplicates. Two sets of control (each consisting 10 fishes) which contains no toxicants were set up. The test was performed by the semistic (renewal) bioassay method in which the exposure medium was exchanged every 24-h to maintain toxicant strength and level of dissolved oxygen as well as minimizing the ammonia excretion levels during this experiment. Initially, the fish were observed at 1-h intervals for the first 6-h after

which they were observed at 2-h intervals. Dead fish were immediately removed from the experimental set-up. After the 96-h expiration of the experiment, blood was collected from the remaining fish to assess the effect of acute exposure to zinc sulphate on haematological parameters. During the experimental period, no death was recorded in the control set-up.

### Fish blood collection

Blood samples were collected from both the control and experimental fish that survived the 96-h toxicant exposure period. The blood samples were taken by puncturing posterior caudal vein using ethylenediaminetetraacetate (EDTA) as anticoagulant (Schmitt et al., 2007). 2 ml of blood was decanted in heparinized bottles for determination of blood parameters. The microhaematocrit method of Snieszko (1960) was used to determine the haematocrit (PVC). Haemoglobin (Hb) concentration was measured with Hb test kit using the cyanmethaemoglobin method (Larsen and Snieszko, 1961). Red blood cell (RBC) and white blood cell (WBC) counts were counted under light microscope with an improved Neubauer haemocytometer (Mgbenka et al., 2003; Shah and Altindag, 2004, 2005). The derived haematological indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae as described by Jain (1986): MCV was calculated in femtoliters = PCV/RBC x 10; MCH was calculated in picograms = Hb/RBC x 10 and MCHC = (Hb in 100mg blood / Hct) x 100. Experimental data and those of control were statistically analyzed by means of analysis of variance (ANOVA) and Duncan's New Multiple-Range Test. Standard deviation (SD) and Pearson correlation coefficient were calculated. Significance was set at P = 0.05. All analysis was performed using SPSS software (version 13.0).

## RESULTS

The present study shows the toxicity of Zn metal in the form of zinc sulphate. The trend in mortality (Figure 1) indicated that the survival time for the 96-h renewal bioassay increased as the concentration of the toxicant decreased. After the 96-h exposure, there were 15, 45, 70, 90 and 95% mortality at the 30, 35, 40, 45 and 50 mg l<sup>-1</sup> concentrations of Zn respectively. Control survival was 100% in the test. Based on logarithmic analysis, the regression equation for the Zn (96-h exposure) is  $Y = 375.66x - 535.8$  which yields an LC<sub>50</sub> of 36.7 mg/l. On time basis, within 24, 48, 72 and 96 h exposures 1, 13, 38 and 63% mortality were recorded respectively across the various concentrations. The color of the experimental fish became progressively darker with increasing concentrations and mortality was directly proportional to the concentrations of the tests solution unlike those in the control. The hematological changes observed are presented in Table 1. In general, hemoglobin concentration displayed significant at P < 0.05 tested concentration compared with the control values (Table 1). The data implicated concentration of metal in water as a major factor that can cause reversible and irreversible changes in the homeostatic system of fish.

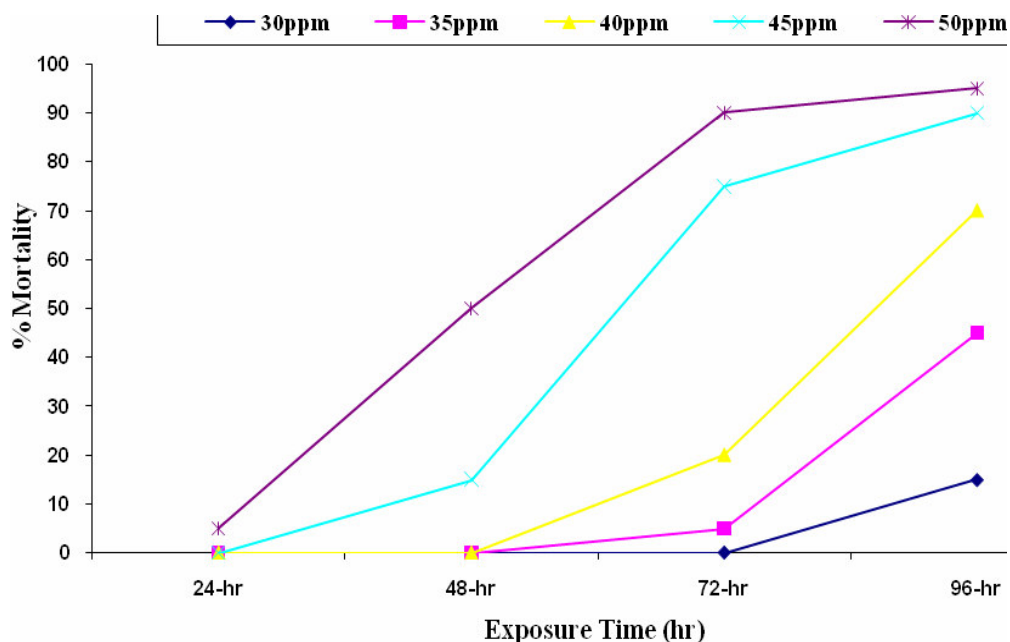


Figure 1. Trend in fish mortality with duration of exposure to zinc sulphate.

Table 1. Blood Parameters of *C. gariepinus*, for various concentrations of Zinc.

Blood Parameters	30 (mg/l)	35 (mg/l)	40 (mg/l)	45 (mg/l)	50 (mg/l)	Control (mg/l)
Haematocrit (%V)	23 ± 2 <sup>c</sup>	20 ± 3 <sup>c</sup>	25 ± 5 <sup>c</sup>	19 ± 3 <sup>c</sup>	14 ± 3 <sup>d</sup>	40 ± 4 <sup>a</sup>
WBC (x 10 <sup>3</sup> mm <sup>3</sup> )	7.6 ± 2.3 <sup>a</sup>	6.7 ± 1.1 <sup>ab</sup>	7.0 ± 3.5 <sup>a</sup>	6.5 ± 2.7 <sup>ab</sup>	5.6 ± 2.2 <sup>c</sup>	8.5 ± 3.6 <sup>a</sup>
RBC (x 10 <sup>7</sup> /μL)	0.9 ± 0.4 <sup>c</sup>	0.32 ± 2.1 <sup>d</sup>	0.42 ± 2.3 <sup>d</sup>	0.36 ± 1.5 <sup>d</sup>	0.17 ± 0.9 <sup>e</sup>	3.2 ± 1.2 <sup>a</sup>
Hb (x 10 <sup>2</sup> g/dl)	1.72 ± 1.05 <sup>b</sup>	1.35 ± 1.24 <sup>b</sup>	1.60 ± 1.12 <sup>b</sup>	0.98 ± 0.41 <sup>c</sup>	0.61 ± 0.32 <sup>c</sup>	2.06 ± 1.03 <sup>a</sup>
PLT (10 <sup>5</sup> /μL)	1.40 ± 0.52 <sup>b</sup>	1.50 ± 0.22 <sup>b</sup>	3.06 ± 1.21 <sup>d</sup>	2.60 ± 1.23 <sup>b</sup>	2.06 ± 1.02 <sup>a</sup>	2.04 ± 1.10 <sup>a</sup>
MCV (x 10 <sup>-6</sup> Fl)	2.56	6.25	8.33	8.06	8.24	1.56
MCH (x 10 <sup>-6</sup> Pg)	1.91	4.22	3.81	2.72	3.59	6.44
MCHC (Pg)	747.8	675.1	640.0	515.7	435.7	412.0

Means in the same horizontal column followed by different superscript are significantly different ( $\alpha = 0.05$ ) according to Duncan's New Multiple Range Test.

## DISCUSSION

Zinc is known to be an essential element of plants and animals. Within the aquatic environment, at high concentrations, it exerts adverse effects by accruing structural damage, which affects the growth, development and survival of fish (Turala and Soivio, 1982). The LC<sub>50</sub> of 36.7 mg/L as obtained in this study is on the high side. This is of great advantage because levels of Zn that may probably enter the environment in the form of pollutant may not be so high to this level. Thus, the fish population may not be affected significantly. However, increased mortality may result due to bioaccumulation resulting from continuous release of Zn-containing wastes in the water ways. In the present study, some behavioral re-

sponses were observed after the introduction of the toxicants (Zn).

The behavior and mortality rate were found to depend on both duration of exposures. In addition, this mortality profile due to 96-h Zn exposure indicated concentration dependent relationship. This implies of *C. gariepinus* is a good bioindicator of ambient Zn levels in solution. Some behavioral responses include rapid and erratic swimming, darting up and down with occasional jumpy movement. Others include loss of balance and general weakness with majority of the fish gathering near the bottom while their reactions to light and sound were delayed. Similar behavioral pattern has been reported with other metals such as Cd on *Tilapia mossambica* (Ghatak and Konar, 1990), Pb on Brook trout, *Salvalinus fontinalis* (Hole

combe et al., 1976) and Pb on *Oreochromis mossambicus* (James et al., 1996).

Prior to mortality, there was uncoordinated movement with reduced activity evidenced by vertical positioning. Others include curling of spine and vertical movement of the fish which may probably be due to loss of equilibrium. All these observations were more pronounced with increasing concentrations of toxicant. Consequently, the percentage and number of survivors decreased with increasing concentrations of toxicants in water (Table 1). The mortality pattern may be attributed to the injuries (which were quite visible during the study) on the skin caused by progressive exposure to toxicants which is capable of weakening the organism's resistance and the immune system.

Between 24 – 72 h and at 30 and 35 mg l<sup>-1</sup> Zn, the body concentration of Zn does not show any change over an increasing range of dissolved Zn exposures until a threshold external dissolved availability is reached due to continuous exposure for 96-h, although new Zn is entering the body in significant amounts at all exposure but an equivalent amount of Zn is excreted to match the rate of Zn uptake. When the rate of Zn uptake from solution exceeds the rate of excretion especially at 72 – 96 h and at higher concentration, Zn then rises above the regulated values.

Much more damage is envisaged within the internal system. Based on the pattern in Figure 1, it is hoped that recovery of fish emerging from lower concentration of Zn is expected to be faster than those placed in higher concentration when transferred into clean water.

Changes in the haemoglobin level become visible at higher concentrations of 45 and 50 mg l<sup>-1</sup> where  $0.98 \pm 0.41 \times 10^2$  g dl<sup>-1</sup> and  $0.61 \pm 0.32 \times 10^2$  g dl<sup>-1</sup> were reported respectively. The differences between the haemoglobin level in the control ( $2.06 \pm 1.03 \times 10^2$  g dl<sup>-1</sup>) and experimental samples were statistically significant ( $P < 0.05$ ) particularly at higher concentrations (that is, 45 mg l<sup>-1</sup> and 50 mg l<sup>-1</sup>). However, between 30 and 40 mg l<sup>-1</sup> Zn, the %Hb levels were not statistically different ( $P < 0.05$ ). A similar significant decrease in hemoglobin due to exposure of *C. gariepinus* to sublethal concentration of Zn has been reported (Annune, 1994).

The mean PVC was  $40 \pm 4\%$  in the control experiment. This value was observed to decrease progressively in the experimental fish as the concentration increases from 30 – 35 mg l<sup>-1</sup>. Though, within the experimental fish, the changes in haematocrit value of the concentration range (30 – 45 mg l<sup>-1</sup>) were not significantly different ( $P < 0.05$ ). However, an unusual increase in PVC was observed at 40.0 mg l<sup>-1</sup>. Soivoi and Nikinmaa (1981) reported that increase in hematocrit is an indication of stress response causing RBC swelling or haemoconcentration due to plasmatic volume reduction (Wilson and Taylor, 1993). At concentration of 50 mg/l, an unprecedented decrease in PVC was obtained.

This final reduction in PVC to 14% as compared with

the 40% in the control is indicative of the immune depression resulting from zinc intoxication. The study implicated a drastic reduction in the total haematocrit which was observed to be dosage-dependent. This is in line with earlier study (Skidmore, 1964). The observed reduction in the haemoglobin and haematocrit values in the fish could also be attributed to the lysing of erythrocytes. Similar reductions have been reported by Samprath et al. (1993) and Musa and Omoregie (1999) when they exposed fish to polluted environment under laboratory conditions. Thus, the significant reduction in these parameters is an indication of severe anaemia.

Similar pattern were observed in the erythrocytes and leucocytes values in the experimental fish; a fluctuated pattern of changes which followed the tendencies exhibited by the haemoglobin. The sudden increase in WBC count from  $6.7 - 7.0 \times 10^3$  mm<sup>3</sup> can indicate hyper-splenism, inflammation and stress (Nordenson, 2004). However, a statistically significant ( $P < 0.05$ ) difference in erythrocyte was observed between all the experimental fish and the control while in leucocytes, significant difference between experimental and control mainly occurred at higher concentrations of toxicants (Table 1). The data in Table 1 also shows that platelets in the control fish falls within those of the experimental fish with higher values ( $2.04 \pm 1.10 \times 10^5$   $\mu$ l) when compared with data from lower concentrations (30 and 35mg/l) but comparatively lower with data from higher concentration (that is, 40 – 50 mg l<sup>-1</sup>).

Hematological indices such as hemoglobin, haematocrit and red blood cell count, have been reported to indicate secondary responses of an organism to irritants (Rogers et al., 2003). Studies on freshwater fish such as Catfish (*Clarias lazera*) and tilapia (*Tilapia zillii*) in a 96-h exposure to 22 and 32 mg l<sup>-1</sup> of Zn respectively have resulted in decreased level of the haemoglobin and haematocrit (Hilmy et al., 1987). Increase in leucocytes in the blood of Carp (*Cyprinus carpio*) and decrease in the blood of dogfish (*Scyliorhinus canicula*) in a 96-h acute toxicity test has been reported (Torres et al., 1984). The reduction in leucocytes of the experimental fish that was observed agrees with the report that the release of epinephrine during stress causes the contraction of spleen and a decrease of leucocytes count, which shows the weakening of the immune system. The potential for chemicals to cause damage to the immune system of considerable public health significance, as alterations in immune function can lead to increased incidence of hypersensitivity disorders, autoimmune and infectious diseases.

The derived haematological indices of MCHC, MCH and MCV displayed a fluctuated pattern. The highest MCHC (747.8 Pg) was recorded in blood samples from the least concentration (30 mg/l). This value decreased with increased concentration of toxicants. Unlike MCHC values, the Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) increased with increased concentrations of toxicant. The significant

change in the MCH may be due to the reduction in cellular blood iron, resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis (Hodson et al., 1978). Significant mean differences ( $P < 0.05$ ) were recorded between WBC/RBC; Hct/Hb and MCH/MCHC. The MCH and MCV content were higher in zinc-exposed fish when compared with the control. The MCV was statistically significant ( $P < 0.05$ ) especially with increased concentration of toxicants. Similarly, significant positive correlation were observed between Hct/RBC ( $P = 0.05$ ,  $r = 0.833$ ) and MCH/Hct ( $P = 0.05$ ,  $r = 0.822$ ). Similarly, positive correlations ( $P = 0.01$ ) was observed between MCH/RBC ( $r = 0.971$ ).

Exposure of *C. gariepinus* to acute toxicity due to Zn revealed significant changes in the hematological variables. Majority of these parameters displayed significant reduction with increased concentrations of toxicant. This reduction is capable of altering the metabolic function of the fish. This indicates that Zn, in all its bioavailable form, when introduced in excess into the river system is capable of affecting aquatic life negatively. It should be noted that although the mechanisms of fish physiological and biochemical reaction to xenobiotics has not been enough investigated, it is obvious that species differences of these mechanisms exists. Further work needs to be done on other species of fish and on the impact of mixed toxicants (that is, mixture of two and more metals) since most wastes introduced into the river system consists of mixtures of these metals.

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