

# Acute Toxicity and Blood Profile of Adult *Clarias gariepinus* Exposed to Lead Nitrate

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## Abstract

Changes in *Clarias gariepinus*' blood cells were investigated after 96-h of exposure to lead. Ninety (90) *Clarias gariepinus* with average weight of 262.2g and average length of 30.8 were divided into 5 groups (A-E) at six (6) fish per group and in triplicates after being acclimatized for 14 days. They were then exposed to various concentrations (0, 25, 50, 100 and 200 mg/l) of lead nitrate for 96 hours. The 96-hour LC 50 was calculated to be 72.92; 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 of treatments. Other blood parameters did not vary significantly in comparison to the control group, but it is worth noting that the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) 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 in haemoglobin synthesis, stress related release of RBCs from the spleen and hypoxia, induced by exposure to lead. This study therefore gives an insight into toxic effect of acute exposure to lead on *Clarias gariepinus*.

## INTRODUCTION

Environmental stressors and their associated risks have always been an inherent part of society. Aquatic ecosystems are especially sensitive to exposure to toxic contaminants. Pollutants either individually or in combination may have sub-lethal effects at the cellular, organ and individual level. 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 values of one or more of the haematological parameters (Van Vuren, 1986). 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 (Golovina, 1996; Luskova, 1997).

Like in warm-blooded animals, changes in the blood parameters of fish, which occur because of injuries of the latter organs or tissues, can be used to determine and confirm the dysfunction or injuries of the latter (organs or tissue). However in the fish, these parameters are more

related to the response of the whole organism, i.e. to the effect on fish survival, reproduction and growth. It should be noted that although the mechanisms of fish physiology and biochemical reaction to xenobiotics has not been investigated enough, it is obvious that species differences of these mechanisms exist.

Fish live in very intimate contact with their environment, and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components (Wilson and Taylor, 1993). In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels. Blood tissue truly reflects physical and chemical changes occurring in organism; therefore, detailed information can be obtained on general metabolism and physiological status of fish in different groups of age and habitat. Early diagnosis is also possible when evaluating haematological data, particularly blood parameters (Folmar, 1993; Golovina, 1996; Luskova, 1997). Furthermore, it should be noted that haematological indices are of different sensitivity to various environmental factors and chemicals (Lebedeva et al, 1998; Vosylienė, 1999a, b).

Previous haematological study of nutritional effects (Rehulka, 2000), infectious diseases (Rehulka, 2002a) and

pollutants (Rehulka, 2002b) brought knowledge that erythrocytes are a major and reliable indicator of various sources of stress (Rainza-Paiva et al, 2000; O'neal and Weirich, 2001). The main goal in toxicity testing according to Van der Merwe et al. (1993) is to predict, in combination with other environmental factors, with known accuracy, a concentration of a specific toxicant that will not harm an entire system. This study therefore assessed the acute toxicity, haematological and immunological profile of *Clarias gariepinus* exposed to lead.

### MATERIALS AND METHODS

#### FISH SAMPLING

Ninety apparently normal adult *Clarias gariepinus* of both sexes weighing between 200-300 g (262.2 g) and total length of between 28-33 cm (mean = 30.8 cm) were purchased from Zartech farms in Ibadan. Ibadan in Oyo state, Nigeria (Figure 1) is the largest city in West Africa and the second largest in Africa, with land size covering an area of 240km<sup>2</sup>. The city is located on geographic grid reference longitude 3o 5E, latitude 7o 20N (Filani, 1994).

#### Figure 1

Figure 1: A Map Of Nigeria, Showing The Location Of Ibadan City



The fish were considered as normal on the basis of their external appearance and absence of symptoms of diseases. Fishes were transported in a container filled with pond water and then acclimatized under laboratory conditions for two weeks (14 days) prior to the commencement of the experiment. During the acclimatization period, the fish were fed 4% of their body weight with commercial feed pellets (40% crude protein, 4.22% fat, 5.88% crude fiber, 10.30% ash and 10.03% moisture) once daily and the water was renewed every other day. The mortality throughout the period of acclimatization was less than 10%.

### LABORATORY EXPERIMENT

Stock solution of lead nitrate [ $\text{Pb}(\text{NO}_3)_2$ ] was made by dissolving 50 g of lead nitrate in 1 liter of well-water (Water  $T^\circ\text{C} = 27.0 \pm 0.1$ ,  $\text{pH} = 7.26 \pm 1.1$ ). A 96-hour daily static renewal acute toxicity was conducted following the methods described by Sprague (1971).

Fish were randomly allotted at six (6) fish per group (A, B, C D and E) based on the concentrations of lead nitrate they were exposed to (0.0, 25, 50, 100 and 200 mg/l) respectively. The experiment was set up in triplicates. Fish allotted to group A served as the control. Fish were observed at 2 hours intervals for the first 2 hours after which they were observed at 6 hours interval. Dead fish were immediately removed from the experimental set-up. After the expiration of the experiment, blood was collected from the remaining fish to assess the effect of acute exposure to lead nitrate on blood parameters. Fish were anaesthetized in 8 litres of well-water containing 0.2 g of benzocaine, which had been dissolved in 5 ml acetone.

Blood was drawn from the posterior caudal vein according to Schmitt et al (1999) and 2 ml was decanted in heparinized bottles for red blood cell count (RBCC), haematocrit (PCV), haemoglobin (Hb) and white blood cell count (WBCC). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were derived from the RBC, PCV and Hb as described by Jain, 1986. MCV was calculated in femtoliters =  $\text{PCV}/\text{RBC} \times 10$ , MCH was calculated in picograms =  $\text{Hb}/\text{RBC} \times 10$  and  $\text{MCHC} = (\text{Hb in } 100\text{mg blood} / \text{Hct}) \times 100$ .

### STATISTICAL ANALYSIS

Results are presented as mean with standard error of mean (SEM). The results were also analysed using student's t-test. The level of significance was  $p < 0.05$  at 95% confidence limit.

### RESULTS AND DISCUSSION

The toxic effects of heavy metal on fish are multidirectional and manifested by numerous changes in the physiological and chemical processes of their body systems (Dimitrova et al 1994). Sublethal toxicity of lead to fish produces haematological and neurological effects (Hodson et al 1984). The PCV, Hb, RBC, WBC, Platelet count, Lymphocyte count, Neutrophil count and derived erythrocyte indices (MCV, MCH and MCHC) of the fish exposed to lead nitrate are presented in Figures 2-11.

**Figure 2**

Table 1: 96 Hour Lc 50 Determination In Adult Fish Based On Arithmetic Method Of Karber

Concentration (ppm)	Concentration Difference	Number alive	Number dead	Mean death	Mean death Dose diff
0	-	6	0	-	0
25	25	4	2	1	25.0
50	25	3	3	2.5	62.5
100	50	2	4	3.5	175.0
200	100	0	6	5	500.0
					762.5

$$LC\ 50 = LC\ 100 - \frac{\sum \text{mean death} \times \text{conc. diff}}{\text{Number of organism per group}}$$

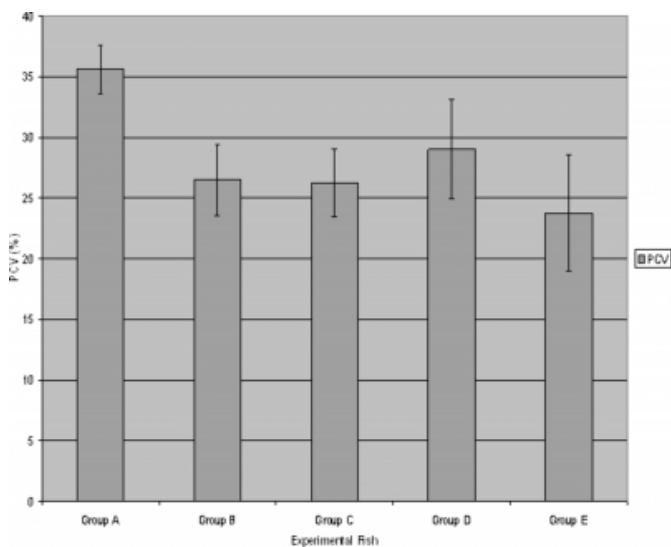
$$= 200 - \frac{762.5}{6}$$

$$= 200 - 127.08$$

$$= 72.92\ \text{ppm}$$

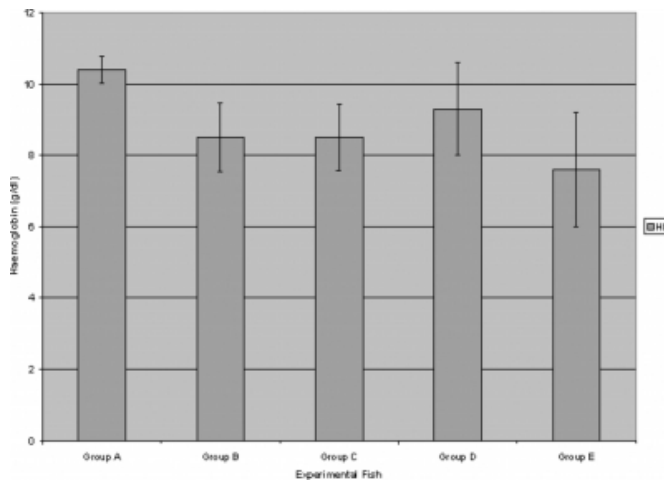
**Figure 3**

Figure 2: Packed Cell Volume (Pcv %) Of Adult Fish Post 96-Hour Exposure To Lead Nitrate



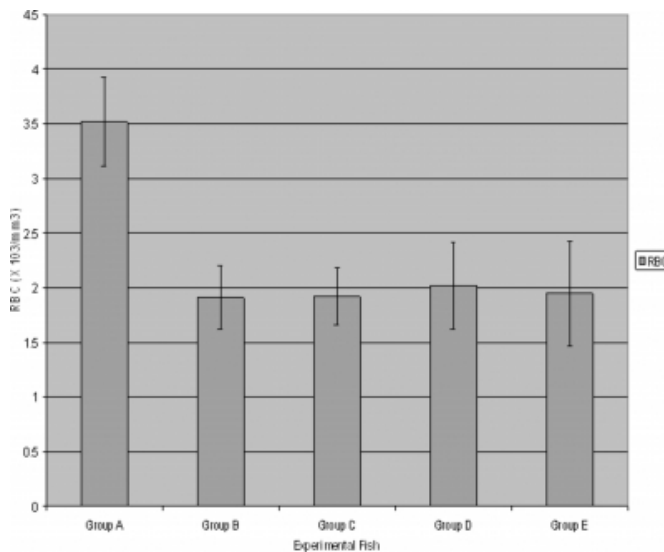
**Figure 4**

Figure 3: Haemoglobin Concentration (G/Dl) Of Adult Fish Post 96-Hour Exposure To Lead Nitrate



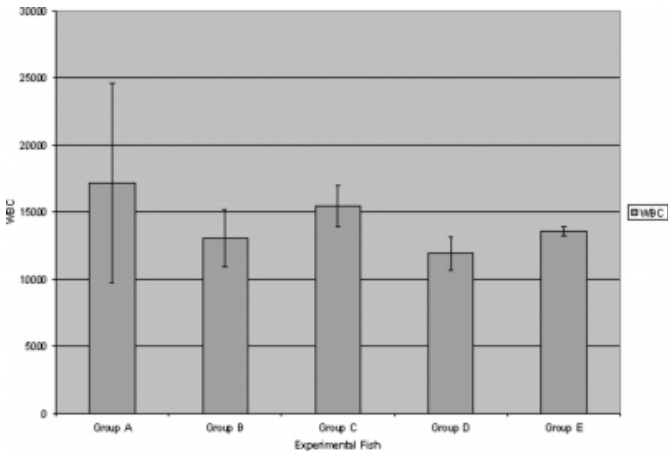
**Figure 5**

Figure 4: Red Blood Cell Count (X 10<sup>5</sup>/Mm<sup>3</sup>) Of Adult Fish Post 96-Hour Exposure To Lead Nitrate



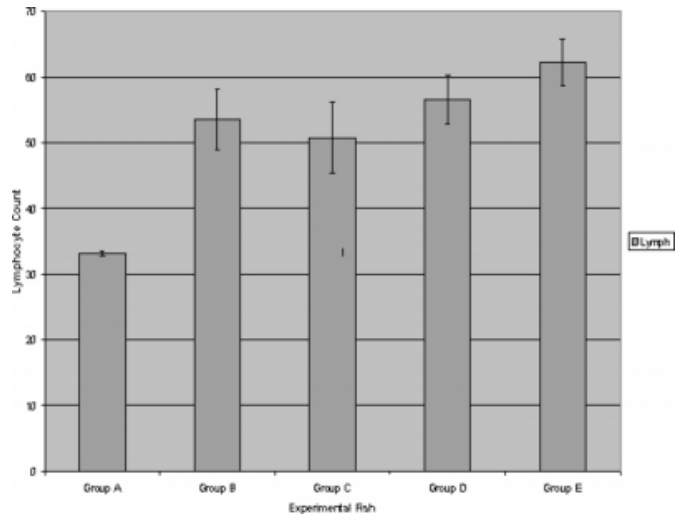
**Figure 6**

Figure 5: White Blood Cells(X 10<sup>5</sup>/Mm<sup>3</sup>) Of Adult Fish Post 96-Hour Exposure To Lead Nitrate



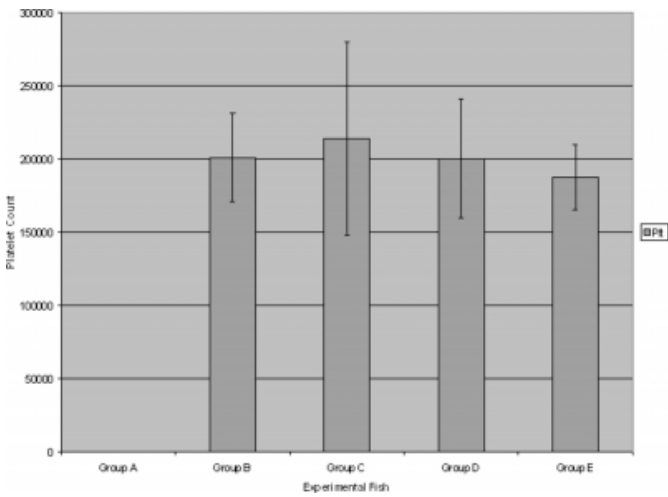
**Figure 8**

Figure 7: Lymphocyte Count (%) Of Adult Fish Post 96 – Hour Exposure To Lead Nitrate



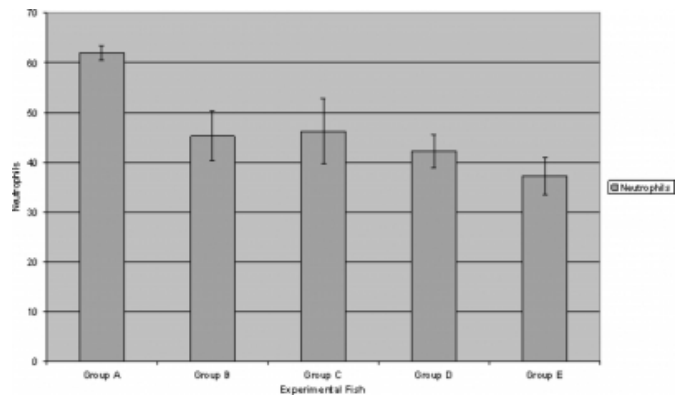
**Figure 7**

Figure 6: Platelets Count Of Adult Fish Post 96 – Hour Exposure To Lead Nitrate



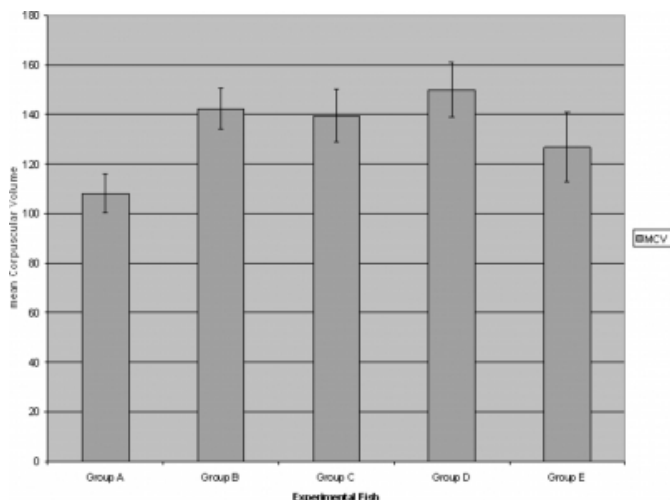
**Figure 9**

Figure 8: Neutrophil Count (%) Of Adult Fish Post 96 - Hour Exposure To Lead Nitrate



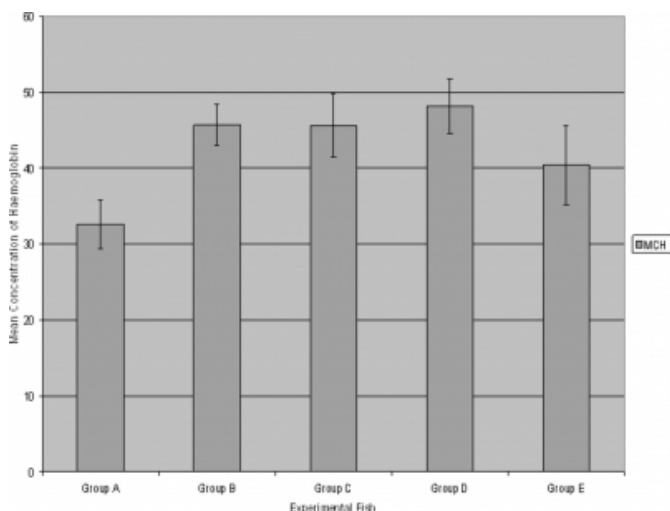
**Figure 10**

Figure 9: Mean Corpuscular Volume (Fl) Of Adult Fish Post 96 – Hour Exposure To Lead Nitrate



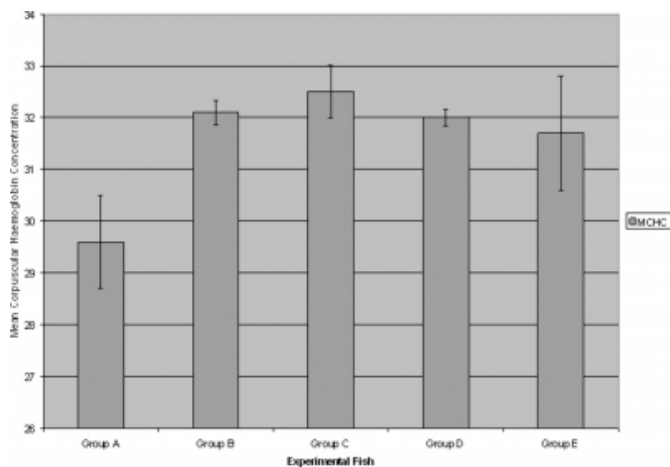
**Figure 11**

Figure 10: mean concentration of haemoglobin (pg) of adult fish post 96 – hour exposure to lead nitrate.



**Figure 12**

Figure 11: mean corpuscular haemoglobin concentration (g/dl) of adult fish post 96 – hour exposure to lead nitrate.



Literature 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). The result is anaemia at high lead exposures or compensating erythropoiesis at lower exposures (Hodson et al., 1984). In the light of the present study, the mean value of PCV was 35.6 in the control group (group A), which decreased progressively (26.5, 26.25, 29, 23.7) in groups B, C, D and E, respectively with groups B, C and E having significantly ( $p = 0.0003, 0.00029, 0.00022$ , respectively) lower PCV compare to the control. 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 10.42, 8.52 in group B, 8.55 in group C, 9.3 in group D and 7.67 in group E. 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 lead in *Clarias gariepinus*.

A non-dose dependent reduction in RBC level of the treatments was observed (Fig. 4). Haematological indices (RBC count, concentration of haemoglobin and haematocrit)

have been reported to indicate secondary responses of an organism to irritants (Rogers et al., 2003) who concluded after their research that mechanism of lead toxicity occurs by ionregulatory disruption. The reduction in WBC count of the treatment groups that was observed (Fig. 5) agrees with the report that the release of epinephrine during stress causes a decrease of leucocyte count, which shows the weakening of the immune system.

The MCV, MCH and MCHC increased considerably in all treatments compare to the control (Figs. 9-11). However, the increase in MCV was significant ( $p < 0.05$ ) only in groups C and E ( $P = 0.033$  and  $0.016$ , respectively), while the increase in MCH and MCHC recorded by the treatments was significant ( $p < 0.05$ ) only in group C ( $p = 0.00026$ ,  $0.00034$ ). This is in agreement with the work of Shah, (2006) following a short-term exposure of tench (*Tinca tinca*) to lead. These alterations were attributed to direct or feedback responses of structural damage to RBC membranes resulting in haemolysis and impairment in haemoglobin synthesis, stress related release of RBCs from the spleen and hypoxia, induced by exposure to lead (Shah, 2006).

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