Haematological Profile of *Clarias gariepinus* (Burchell, 1822) Exposed to Lead

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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. 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, which was induced by exposure to lead. This study therefore gives an insight into toxic effect of lead on fish.

Key words: Blood indices, African catfish, lead, acute toxicity

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 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; Vosylienë, 1999a; 1999b). Previous haematological study of nutritional effects (Rehulka, 2000), infectious diseases (Rehulka, 2002a) and pollutants (Rehulka, 2002b) brought knowledge that erythrocytes are the major and reliable indicators of various sources of stress (Rainza-Paiva *et al.*, 2000; O'neal and Weirich, 2001). This study therefore assessed the haematological 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. Another set of ninety juvenile fish weighing between 80-90 g (mean = 84.2 g) and a total length of between 15 – 22 cm (mean =19.3 cm) were also purchased from Zartech fish farm 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 240 km^2. The city is located on geographic grid reference longitude 3º5' E, latitude 7º20' N (Filani, 1994). The fish were considered as normal on the basis of their external appearance and absence of symptoms of diseases. The two sets of fish were transported separately in a container filled with pond water. The fish were 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 fibre, 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 [Pb $(No_3)_2$] was made by dissolving 50 g of lead nitrate in 1 litre of well-water (Water TºC = 27.0±0.1, pH = 7.26±1.1). Two sets of experiments (adult and juvenile respectively) in triplicates. A 96-hour daily static renewal acute toxicity was conducted following the methods described by Sprague (1971). Fish in each set 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, 2.5, 5.0, 10 and 20 mg/l, respectively) for the juveniles and (0.0, 25, 50, 100 and 200 mg/l) respectively for the adult fish. Fish allotted to group A served as the control for the two sets of experiment conducted (juvenile and adult *Clarias gariepinus*).

Fish were observed at 2-hour intervals for the first 2 hours after which they were observed at 6-hour intervals. 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 haematological 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 = PCV/RBC x 10, MCH was calculated in picograms = Hb/RBC x 10 and MCHC = (Hb in 100mg blood / Hct) x 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.

Figure 1. A map of Nigeria, showing the location Of Ibadan City (Arrow).

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.

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

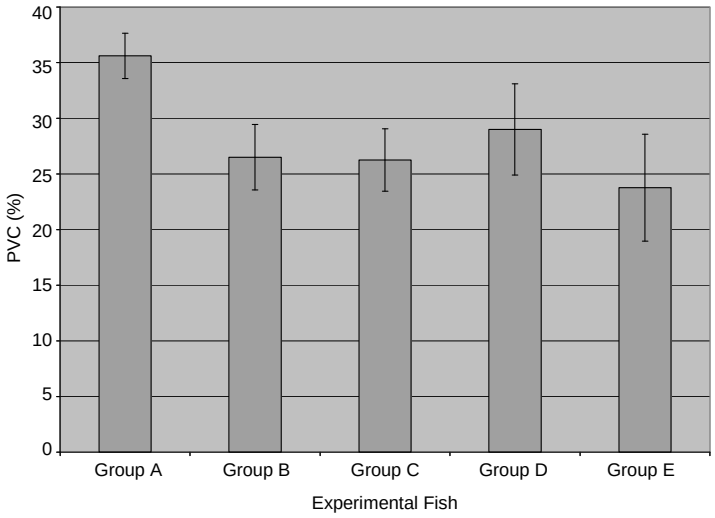

Figure 2. Packed cell volume (pcv %) of adult fish post 96-hour exposure to lead nitrate.

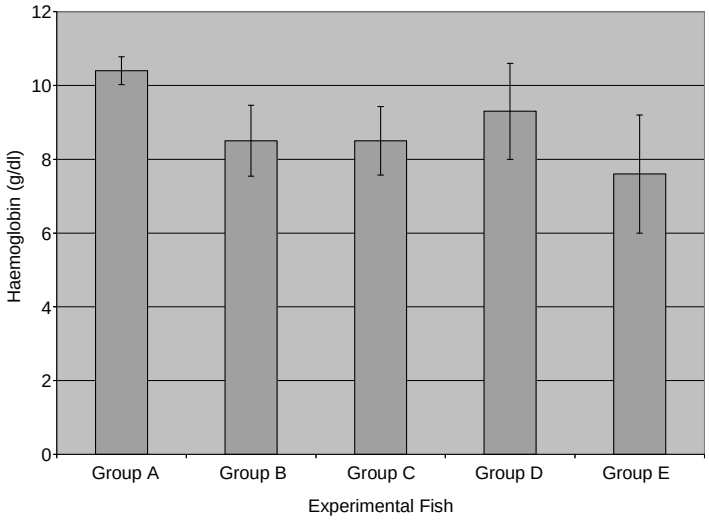

Figure 3. Haemoglobin concentration (g/dl) of adult fish post 96-hour exposure to lead nitrate.

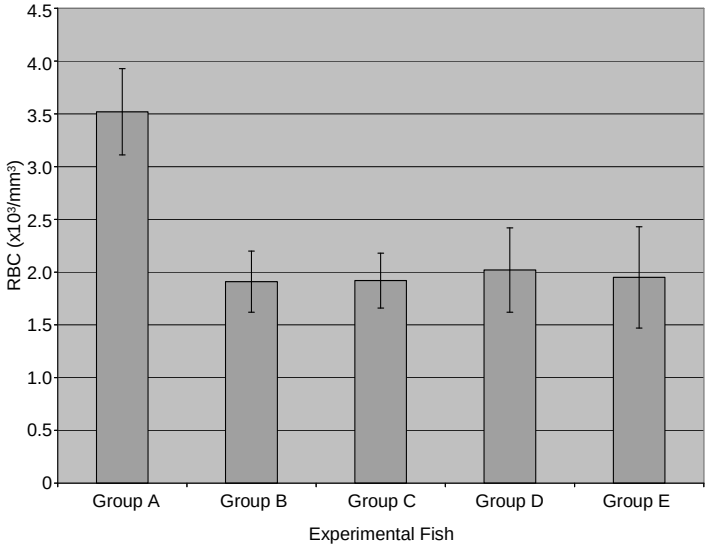

Figure 4. Red blood cell count (x 10^5/mm^3) of adult fish post 96-hour exposure to lead nitrate.

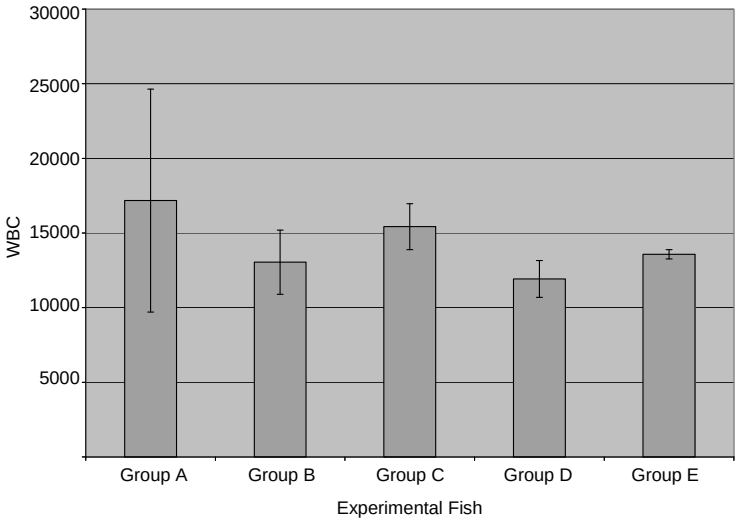

Figure 5. White blood cells (x 10^5/mm^3) of adult fish post 96-hour exposure to lead nitrate.

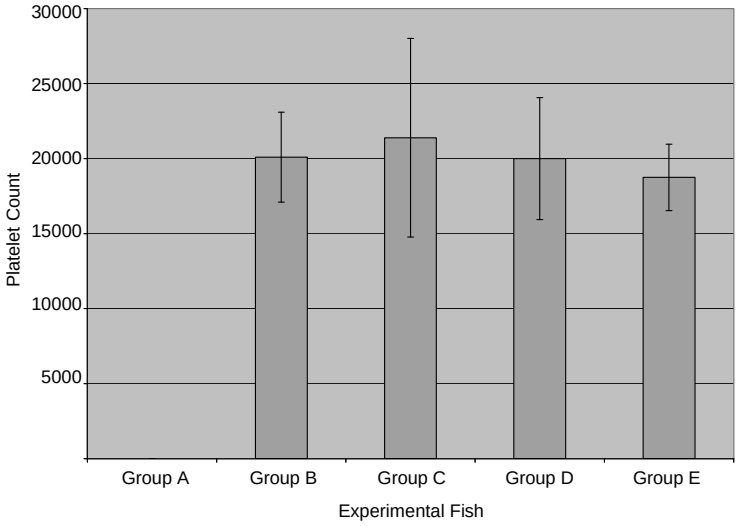

Figure 6. Platelets count of adult fish post 96 – hour exposure to lead nitrate.

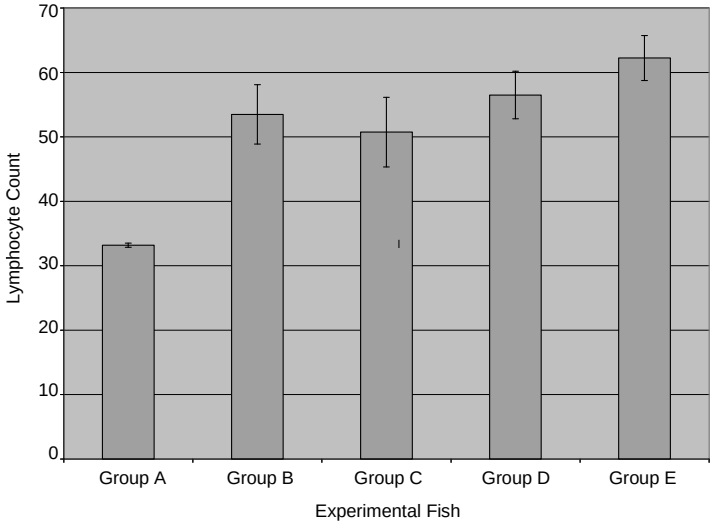

Figure 7. Lymphocyte count (%) of adult fish post 96 – hour exposure to lead nitrate.

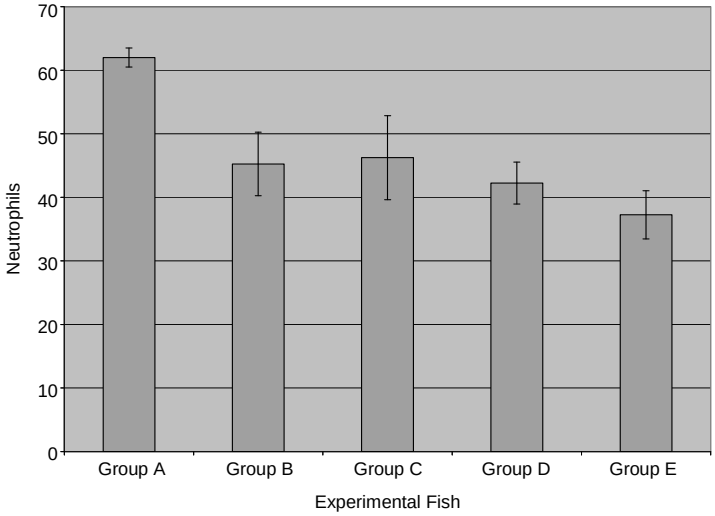

Figure 8. Neutrophil count (%) of adult fish post 96 - hour exposure to lead nitrate.

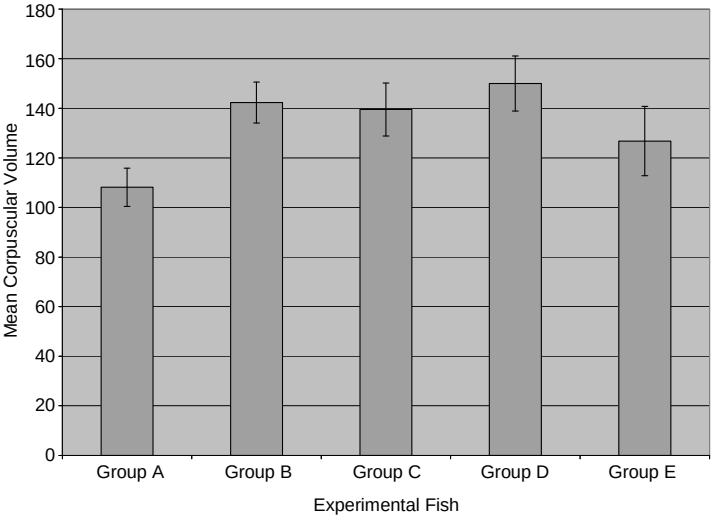

Figure 9. Mean corpurscular volume (fl) of adult fish post 96 – hour exposure to lead nitrate.

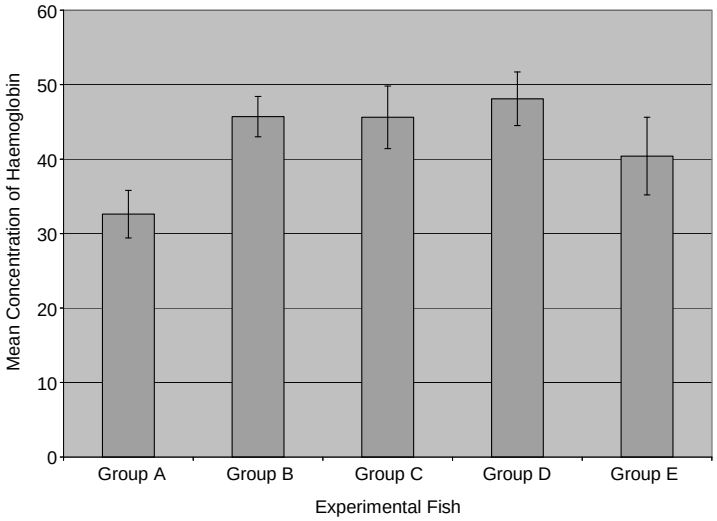

Figure 10. Mean concentration of haemoglobin (pg) of adult fish post 96 – hour exposure to lead nitrate.

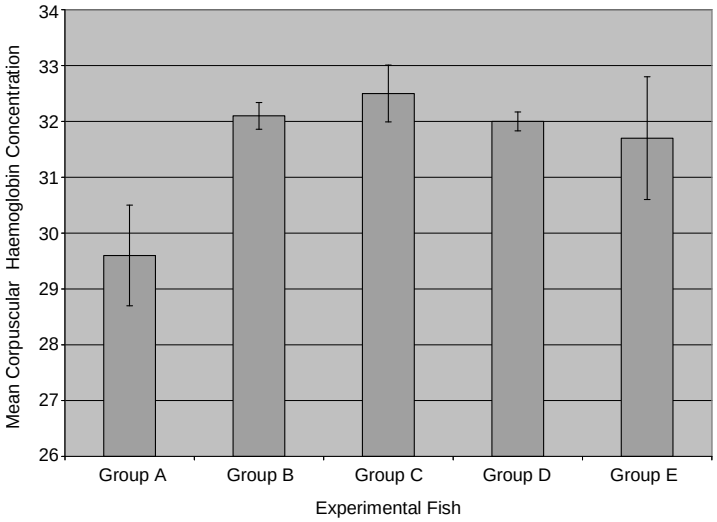

Figure 11. Mean corpuscular heamoglobin concentration (g/dl) of adult fish post 96 – hour exposure to lead nitrate.

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 (Figure 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 (Figure 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 compared to the control (Figures. 9-11). However, the increase in MCV was significant ($p<0.05$) only in group 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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