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Toxic effects of zinc sulphate on haematological indices of fresh water catfish *Clarias batrachus* (Linn.)

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Abstract

The aim of the study to evaluate some haematological changes resulting from the exposure of a freshwater fish, *Clarias batrachus* to sublethal concentrations (5.0 and 10.0 mg L-1) of zinc in water for a period of fifteen (15) days. Three groups of ten fish were subjected to serial dilutions of the stock solution of zinc of 0 (control), 5.0 and 10.0 mg L-1 in three large plastic bowls of 60 litres capacity by the semistatic (renewal) method. At the end of the 15 days exposure period, blood samples were taken from the control and experimental fish. Blood was assayed for selected haematological parameters (haematocrit, haemoglobin, red blood cell counts, white blood cell counts, differential white blood cell counts, erythrocyte sedimentation rate, and total plasma protein and plasma glucose concentration). The derived haematological indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated. Sublethal concentrations (5.0 and 10.0 mg L-1) of zinc caused a dose dependent decrease in haemoglobin values, coupled with a decrease in haematocrit values and red blood cell counts are obvious indication of anemia of the norm chronic type. The total white blood cell counts and the differential white blood cell counts were decreased except for the lymphocytes in which there was a slight increase. Plasma level of protein and glucose were also lower in the exposed fish when compared to the control. The haematological indices MCHC, MCH and MCV were also lowered. In conclusion, the changes observed indicate that haematological parameters can be used as an indicator of zinc related stress in fish on exposed to elevated zinc levels.

Keywords: zinc, haematology, anaemia, glucose, protein, *clarias batrachus*

Introduction

The aquatic environment is continuously being contaminated with chemicals from agriculture and urban activities. In many due to a continuous release of metals from industrial and agricultural sources. Frequently, metals are present as mixtures in the environment because of their concomitant release from mining activities or industrial uses. Zinc plays an important role in cellular metabolism acting as co-factors in a number of important enzymes. However, they can become toxic when elevated concentrations are introduced into the environment (Marr *et al.*, 1996; Karan *et al.*, 1998). Studies on the toxicity of metals for fish have been focused on the effects of short term exposures to single metal at relatively high concentrations; rather than investigating the toxic impact of long-term exposures to metal mixtures at environmentally realistic concentrations. Under conditions of acute, high-dose metal exposure, the maintenance of branchial osmoregulation and gas exchange is of prime importance for the survival of the fish; whereas under conditions of sublethal, chronic metal intoxication, the adaptive capacity of internal metal accumulating organs such as the liver may gain importance (McDonald and Wood, 1993; Schlenk *et al.*, 1999; Stubblefield *et al.*, 1999). Several biochemical and physiological responses can occur when a toxicant is absorbed by aquatic organisms which may be a compensatory response or a toxicity mechanism (Begum, 2004). Chronic exposure of fish to ions, Cu, Cd or Zn, has been shown to cause a variety of behavioral, biochemical and physiological changes including loss of appetite, reduced growth, decreased aerobic scope and mortality (McGeer *et al.*, 2000; Sloman *et al.*, 2003).

Hematological parameters are used as an index to detect physiological changes and to assess structural and functional status of health during stress conditions in a number of fish species (Adhikari *et al.*, 2004; Suvetha *et al.*, 2010). Fish blood is sensitive to pollution-induced stress, and changes on the hematological parameters, such as hemoglobin content, hematocrit and number of erythrocytes can be used to monitor stress caused by pollutants such as heavy metals (Romani *et al.*, 2003; Barcellos *et al.*, 2004). Enzymes activity are considered as sensitive biochemical indicators before hazardous effects occur in fish and are important parameters for testing water for the presence of toxicants (El-Demerdash and Elagamy, 1999; Gul *et al.*, 2004). Estimation of enzymes like aspartate and alanine aminotransferase (AST, ALT) and lactate dehydrogenase (LDH) are considered useful biomarkers to determine pollution level during chronic exposure (Asztalos *et al.*, 1990; Basaglia, 2000; Ozman *et al.*, 2006). Therefore, the present study was undertaken to evaluate some haematological effects resulting from the exposure of the freshwater fish, *Clarias batrachus* sp. to sublethal concentrations of zinc in the water.

Materials and methods

Healthy specimens of *Clarias batrachus* were obtained from a local fish farm at Ughelli town, Delta State, Nigeria and were transported in containers to the laboratory. In the laboratory, fishes were kept in large plastic bowls containing 60 L of clean tap water and acclimatized for 14 days to the laboratory conditions, during which time they were provided with artificial feed (grower's mash from Bendel Feed and Flour Mill, Ewu, Nigeria) and ground shrimps obtained locally to avoid possible effects of starvation on any of the haematological parameters of the fish. The size of the fish varied from 18.6 - 28.2 cm in standard length and 38.5-112.5 g in weight. Fish of both sexes were used without discrimination. Stock solution of the test metal compound zinc tetraoxosulphate IV heptahydrate, ZnSO4.7H2O was prepared by dissolving 43.97 g of Merck grade reagent equivalent to 1 g of zinc in 1000 ml distilled water at concentration of 1000 mg L-1. Three groups of ten fish were subjected to serial dilutions of the stock solution of zinc of 0 (control), 5.0 and 10.0 mg L-1 in three large plastic bowls of 60 litres capacity. The test was performed by the semistatic (renewal) 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 (Kori-Siakpere, 1995). The water quality parameters of the diluting water used in the tests and determined by standard methods are presented in Table 1.

Table 1: Water quality parameters

Parameter	Values
pH	6.54 + 0.38
Temperature	28.3 + 1.3°C
Dissolved oxygen	6.32 + 1.04 mg L^{-1}
Free carbon dioxide	5.25 + 0.07 mg L^{-1}
Alkalinity	32.8 + 1.75 mg L^{-1}
Hardness	124.56 + 11.75 mg L^{-1}
Turbidity	0.305 + 0.07 mg L^{-1}

The exposure period lasted 15 days, after which blood samples were taken from the control and experimental fish. The blood samples were taken by puncturing the caudal vessels, using EDTA (ethylenediaminetetraacetate) as anticoagulant. The microhaematocrit method of Snieszko (1960) [22] was used to determine the haematocrit. Haemoglobin concentration was measured by the cyanmethaemoglobin method (Larsen and Snieszko, 1961) [14] using a commercially available kit (Cromatest Linear Chemicals, Barcelona Spain). Red and white blood cell counts were counted under light microscope with an improved Neubauer haemocytometer. Differential leucocyte counts were made from Leishman/Giemsa stained blood smears. Erythrocyte sedimentation rate was determined with microhaematocrit tubes filled with blood and allowed to stand for 60 min.

The derived haematological indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae. Total plasma protein and plasma glucose concentration were determined using commercial kits (Randox Laboratories Ltd, United Kingdom. The mean values of the various haematological parameters for the control and experimental fish were analysed for statistical significance using the student's t-test. The calculations of statistical significance by the student's t-test at 0.01 and 0.05 levels were made using Microsoft Excel 2000.

Results

The main haematological alteration resulting from exposure of *Clarias batrachus* sp. to various concentrations of zinc in the water for 15 days includes significant decrease in haematocrit and haemoglobin concentration, and nonsignificant decrease in red blood cell counts and erythrocyte sedimentation rates (Table 2). The white blood cell counts also decreased with a change in the composition as seen from the differential white blood cell counts (Table 3). While a decrease in the total plasma protein was recorded in fish exposed to elevated zinc concentrations. Plasma glucose levels decreased in all the concentration of zinc to which the experimental fish were exposed; the drop in plasma glucose was only statistically significant at exposure of 5 mg Zn L-1 of water. The haematological indices of MCHC, MCH and MCV were similarly decreased in most of the exposure media. Results of the haemoglobin and haematocrit values in 10 mg Zn L-1 of water decreased significantly (P <0.01) compared to those of the control. The haemoglobin value also decreased significantly (P <0.05) in the 5 mg Zn L-1 of water exposed fish. Though there was a decrease in red blood cell and erythrocyte sedimentation rate values of fish exposed to both 5 and 10 mg Zn L-1 of water, the decrease was not significant (P >0.05) compared to the control fish. The haematological indices of mean corpuscular volume and mean corpuscular haemoglobin content (MCV and MCHC) respectively were in zinc-exposed fish when compared with the control but were not statistically significant (P >0.05).

The (MCH) decreased in the 5 and 10 mg Zn L-1 of water treatments but the decrease was only significant (P<0.05) in 10 mg Zn L-1 of water concentration. Total plasma protein values of fish held in both Zn concentrations were decreased. The decrease was only statistically significant (P <0.05) in 10 mg Zn L-1 of water. However, highest decrease in plasma glucose was Observed in the 5 mg Zn L-1 of water concentrations which was statistically significant (P <0.01). In the total white blood cell count, there was a slight decrease in the percentage of neutrophils and eosinophils when compared with the control. However, the eosinophils values in the 5 and 10 mg Zn L-1 of water were statistically significant at (P <0.05) and (P <0.01). A slight statistically insignificant decrease in percentage was recorded in the basophils and thrombocytes, with the decrease being lower in the 5 mg Zn L-1 of water exposed fish. Finally, a sharp increase in percentage was observed in the lymphocytes of fish exposed to Zn. This increase was significant (P <0.05).

Table 2: Changes in haematological parameters as a result of exposure of *Clarias batrachus* to various sublethal concentrations of zinc in the water.

Parameter	Control	5 mg Zn L^{-1} of water	10 mg Zn L^{-1} of water
Haematocrit (%)	38.4±0.94	30.8±0.93	24.8±0.68**
Haemoglobin (gd L^{-1})	15.31±0.81	9.70±0.07*	8.34±0.69**
RBCC (106 mm^{-3})	1.63±0.12	1.41±0.06*	1.24±0.08*
WBCC (103 mm^{-3})	8.42+0.06	8.22±0.09	7.54±0.13**
ESR (mm/h)	21.84±1.28	23.63±1.86	27.82±2.88
MCHC (%)	35.47±5.14	31.97±4.35	34.63±4.03
MCH (μg)	97.28±12.63	75.02±17.14	68.30±5.99*
MCV (μg)	240.18±20.12	232.40±24.04	204.85±20.28
Plasma protein (gd L^{-1})	3.14±0.32	2.95±0.11**	1.89±0.37**
Plasma glucose (mgd L^{-1})	246.51±4.61	147.05±9.90**	198.25±3.42

The values are expressed as the mean ± S.E. (n = 5).* = Significance at 0.05 level; ** = significance at 0.01 level.

Table 3: Changes in differential white blood cell counts as a result of exposure of *Clarias batrachus* to various sublethal concentrations of zinc in the water.

Parameter	Control	5 mg Zn L^{-1} of water	10 mg Zn L^{-1} of water
Lymphocytes	29.41+0.33	36.65+0.03	50.00+0.02*
Basophils	12.95+0.54	12.24+0.02	9.09+0.01
Neutrophils	15.29+0.24	12.43+0.08	9.09+0.02
Eosinophils	15.27+0.32	14.28+0.12**	9.09+0.01**
Thrombocytes	27.06+0.03	24.41+0.34	22.73+0.03

The values are expressed as the mean ± S.E. (n = 5). * = Significance at 0.05 level; ** = significance at 0.01 level.

Discussion

Contamination of aquatic environment by heavy metals whether as a consequence of acute and chronic events constitutes additional source of stress for aquatic organisms. Sublethal concentrations of toxicants in the aquatic environment will not necessarily result in outright mortality of aquatic organisms. Omoregie *et al.* (1990) [19] reported that toxicants and pollutants have significant effects, which can result in several physiological dysfunctions in fish. The exposure of *Clarias batrachus* sp. to sublethal concentrations of zinc caused a significant decrease in haemoglobin and haematocrit of the fish. A similar reduction has been reported by Annune *et al.* (1994b). The fish muscle has been known as the water exchange tissue with blood. Haemoconcentration and haemodilution have been described in previous works.

Mishra and Strivastava (1979, 1980) [15, 16], Neumosok and Hughes (1998) [18] observed haemoconcentration after copper exposure and haemodilution following zinc exposure in *Colis fasciatus.* In the present study, the decrease in haematocrit following zinc exposure in *Clarias batrachus* may be an indication of haemodilution. Tort and Torres (1988) [23] reported decrease in haematocrit following 24 h exposure of dogfish, *Scyhorhinus canicula* to cadmium contamination. They attributed this decrease to haemodilution. The observed depiction 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) [20], and Musa and Omoregie (1999) [17] when they exposed fish to polluted environment under laboratory conditions. Thus, the significant reduction in these parameters is an indication of severe anaemia caused by exposure of the experimental fish to zinc in the water. Flos *et al.* (1987) [7] observed an increase in haematocrit levels in different fish species after zinc treatments. There was no significant change in erythrocyte count and erythrocyte sedimentation rate. The red blood cell count of *C. gariepinus* was reported to have increased significantly by Annune *et al.* (1994a) [3] when the fish was subjected to zinc treatment. They attributed the red blood cell elevation to blood cell reserve combined with cell shrinkage as a result of osmotic alterations of blood by the action of the metal (Tort and Torres, 1988) [23]. Annune *et al*. (1994b) also observed a non-significant decrease in red cells for *O. niloticus*. The non-significant decrease in erythrocyte count and erythrocyte sedimentation rate of *Clarias batrachus* sp. may be attributed to the swelling of red blood cells. Flos *et al.* (1987) [7] reported that the swelling of the red blood cells (erythrocytes) may be due to an increase in protein and carbon dioxide in the blood. Sampling procedure could also be as a result of hypoxia or stress that causes these changes. In the values obtained in the haematological indices, no significant change was recorded in the mean corpuscular volume (MCV) and mean corpuscular haemoglobin content (MCHC) but there was significant change in the mean corpuscular haemoglobin (MCH). However, slight fluctuations were recorded in the MCV and MCHC when compared with the control. Spleen contractions after stress have been detected in fish (Abrahamsson and Nilsson, 1975) [1]. Cells released from the spleen. which is an erythropoietic organ would have lowered the MCV values. A similar observation was made for *Cyprinus carpio* after cadmium exposure (Koyama and Ozaki, 1984) [13]. 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) [9]. The decreased number of white blood cells (leucopaenia) may be the result of bioconcentration of the test metal in the kidney and liver (Agrawal and Srivastava, 1980). Decreased number of white blood cells may also be related to an increased level of corticosteroid hormones, whose secretion is a nonspecific response to any environmental stressor (Iwama *et al*., 1976; Ellis, 1981) [12, 6]. In the white blood cell count, a sharp decrease was observed in the percentage neutrophils and esosinophils. The decrease in eosinophils was found to be significant. The reduction in the percentage neutrophils and eosinophils here are in agreement with the findings of Sharma and Gupta (1984) [21] when juveniles of mudfish, *Clarias batrachus* where exposed to carbon tetrachloride. Musa and Omoregie (1999) [17] also reported a decrease in neutrophils of *C. gariepinus* (Burchell) exposed to malachite green. This was attributed to tissue damage. Finally, a slight but statistically significant increase of lymphocytes was recorded in this investigation. This is in agreement with the findings of Samprath *et al.* (1993) [20] when they exposed the Nile tilapia *O. niloticus* to a toxic environment. This they attributed to stimulation of the immune mechanism of the fish to eliminate the effects of the pollutants.

Conclusion

In conclusion, the changes in the haematological parameters indicate that they can be used as indicators of zinc related stress in fish on exposure to elevated zinc levels in the water.

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