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Full Length Research Paper

Sublethal haematological effects of zinc on the freshwater fish, *Heteroclarias* sp. (Osteichthyes: Clariidae)

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Laboratory study was undertaken to evaluate some haematological changes resulting from the exposure of a freshwater fish, *Heteroclarias* sp. 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, 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.

Key words: Zinc, haematology, anaemia, glucose, protein, *Heteroclarias,* Nigeria.

INTRODUCTION

Zinc, an essential element, is one of the most common heavy metal pollutants. The sources of zinc and other heavy metals in natural waters may be from geological rock weathering or from human activities such as industrial and domestic wastes water discharges and animals where it forms constituent functions in maintaining cytoplasmic integrity (Wheatherley et al., 1980). However at high concentrations, zinc exerts adverse effect in fish accruing structural damage, which affects the growth, development and survival of fish (Tuurala and Soivio, 1982). Zinc accumulates in the gills of fish and this indicates a depressive effect on tissue respiration leading to death by hypoxia (Crespso et al., 1979). Zinc pollution also induces changes in ventilatory and heart physiology (Hughes and Tort, 1985). Sublethal levels of zinc have been known to adversely affect hatchability, survival and haematological parameters of fish (Cardeihac et al., 1981). Annune et al. (1994b) reported that zinc could cause sub-acute effects that change fish behaviours. Such observed behaviours include lack of balance since most fins are motionless in the affected fish, agitated swimming, air gulping, periods of quiescence and death.

Apart from zinc, other toxicants have been known to adversely affect fish haematology. Gobacher and Skaya (1977) observed some chronic effects of organophosphate insecticide on fish haematology. McKim et al. (1970) observed in the haematological parameters of the

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blood of the brook trout (*Salvelinus fontalis*) after a short term and long term exposure to copper. The measurement of specific and biochemical changes in *S. fontalis* exposed for short periods to sublethal environmental stressors, which have provided a sensitive method for predicting the effects of chronic exposure on survival, reproduction and growth. Haematological alterations have therefore allowed for a relatively rapid evaluation of the chronic toxicities of a compound.

Haematological changes apart from those due to heavy metals have also been reported. Bouck and Ball (1966) reported that alterations in fish blood were observed due to the influence of capture. Bouck and Ball (1966) also investigated and reported the influence of capture methods on rainbow trout (*Salmo gardnerii*) as well as a perturbation of blood parameters. Distinct differences in behaviour were noted in the captured fish which led to extensive mortality of some groups.

It is, however known that the toxicity of heavy metals including zinc to fish is sometimes reduced in hard water. Jones (1983) demonstrated that *Gasterosteus aculeralus* survived longer in water containing zinc and calcium than when no calcium was present. Lloyd (1960) demonstrated that zinc was toxic to rainbow trout (*S. gardnerii*) in water of hardness 12 g $CaCO_3$ L^{-1} compared with water of 320 mg $CaCO_3$ L^{-1}. Eisler and Gardner (1973) reported that concentrations of cadmium (10 mg Cd^{2+}) not easily toxic to *Fundulus heteroclutus* for 24 h (in sea water) exerted a negative effect on the survival of fish exposed to a test solution containing sublethal concentrations of copper and zinc.

Clarias sp. is a widely distributed fish in Asia and Africa. In these areas, the fish is extremely popular on account of its tasty flesh, its unparalleled hardness, its rapid growth and high market price. It inhabits tropical swamps, lakes and rivers, some of which are subject to seasonal drying. Furthermore, *Clarias* fishery constitutes one of the major fisheries in many river basins in Africa. Awachie and Ezenwaji (1998) estimated that *Clarias* fishery contributes about 17% of over 6,000 tonnes of annual fish production from all fisheries sectors.

In Nigeria, *Clarias* sp. is an indigenous fish occurring in freshwater throughout the country. It is suspected that apart from tilapia, *Clarias* is the most abundant cultivated fish species in Nigeria. The common species found are *Clarias gariepinus*, *Clarias angullaris* and *Clarias buthupogon*. *Clarias* sp. are generally strong fishes. They possess accessory air breathing organs that enable them to tolerate adverse aquatic conditions where other cultivated fish species cannot survive (Olatunde, 1983). Several investigations have been carried out on various toxicants on *Clarias* sp. These include the sublethal effects of concentrations of water extracts of akee apple on *C. gariepinus* (Onusiriuka and Ufodike, 1998). Toxicity of cassava leaf extracts was observed to adversely affect the African catfish, *C. angullaris* (Aguigwo, 1998).

The sublethal effects of various heavy metals on the haematology of *Clarias* sp. are recently being studied. The haematological characteristics of African mudfish, *C. buthupogon* have been reported (Kori-Siakpere and Egor, 1999). We have previously reported the chronic sublethal haematological effects of copper in fresh water teleost, *Clarias isheriensis* and some alterations in haematological parameters in *C. isheriensis* exposed to sublethal concentrations of water borne lead (Kori-Siakpere (1991, 1995). Annume and Ahume (1998) observed sublethal haematological changes in mudfish, *C. gariepinus* when exposed to copper and lead.

Not much work has been carried out on the effect of zinc on the haematology of *Clarias* sp. However, Annune et al. (1994b) observed several haematological alterations in *C. gariepinus* and *Orechromis niloticus* when they were exposed to the sublethal concentrations of zinc.

Apart from the studies on the blood of *Clarias*, Oluah (1998) studied the effect of sublethal concentrations of copper II ions on the serum transaminase activity in *Clarias albopuntatus* and the effect of mercury and zinc on the plasma alanine aminotransferase activity in freshwater catfish, *C. albopuntatus* (Oluah and Amalu, 1998). Therefore, the present study was undertaken to evaluate some haematological effects resulting from the exposure of the freshwater fish, *Heteroclarias* sp. to sublethal concentrations of zinc in the water.

MATERIALS AND METHODS

Healthy specimens of *Heteroclarias* 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, $ZnSO_4.7H_2O$ 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.

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) was used to determine the haematocrit. Haemoglobin concentration was measured by the cyanmethaemoglobin method (Larsen and Snieszko, 1961) using a commercially available kit (Cromatest Linear Chemicals, Barcelona Spain). Red and white blood cell counts were counted under light microscope wit h an improved Neubauer haemocytometer. Diffe-

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}

rential 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 *Heteroclarias* sp. to various concentrations of zinc in the water for 15 days includes significant decrease in haematocrit and haemoglobin concentration, and non-significant 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 mean corpuscular haemoglobin (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 ($P < 0.05$) only in the 10 mg Zn L^{-1} of water concentration.

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) reported that toxicants and pollutants have significant effects, which can result in several physiological dysfunctions in fish. Dysfunction in the fish induces changes in blood parameters possible as a result of blood water content.

Zinc is known to be an essential element of plants and animals. However at high concentrations, it exerts adverse effects by accruing structural damage, which affects the growth, development and survival of the fish (Tuurala and Soivio, 1982). Zinc affects tissue respiration leading to death by hypoxia. It also induces changes in vein and heart physiology. The exposure of *Heteroclarias* 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), Neumosok and Hughes (1998) 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 *Heteroclarias* may

Table 2. Changes in haematological parameters as a result of exposure of *Heteroclarias* sp. 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 (10^6 mm^{-3})	1.63±0.12	1.41±0.06*	1.24±0.08*
WBCC (10^3 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 (ρg)	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 *Heteroclarias* sp. 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.

be an indication of haemodilution. Tort and Torres (1988) 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), 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 caused by exposure of the experimental fish to zinc in the water. Flos et al. (1987) observed an increase in haematocrit levels in different fish species after zinc treatments. They attributed such an increase in haematocrit values to increase in the size of the erythrocytes as being demonstrated for chromium and zinc treated rainbow trout. Observed depression in haematocrit and haemoglobin values coupled with decreased and deformed erythrocytes are obvious signs of anaemia.

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) 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). 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 *Heteroclarias* sp. may be attributed to the swelling of red blood cells. Flos et al. (1987) 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). 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). 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).

Plasma proteins were found to decrease with Zn exposure in the present study. This could be attributed to renal excretion or impaired protein synthesis or due to liver disorder (Kori-Siakpere, 1995). On the other hand, the observed decrease of plasma protein could also result from the breakdown of protein into amino acids first and possibly into nitrogen and other elementary molecules. Similar reduction in protein has also been reported in *Saccobranchus* fossils following exposure to chlordane (Verma et al., 1979).

A reduction of plasma glucose was observed in this study. It was found to be significant at low concentrations of zinc. Changes in carbohydrate metabolism occur in fish exposed to various sublethal concentrations of pollutants. Blood glucose has been employed as an indicator to environmental stress (Silbergeld, 1974). The increase in blood glucose is usually correlated with the mobilization of glycogen and development of a status of hyperglycaemia. The hyperglycaemia response varies with the nutritional status of the fish (McLeay, 1977). It is not known whether zinc exposure affects glucose reserve directly or indirectly via other internal factors. The most likely source of glucose loss is through the kidneys, which could indicate a suppression of energy dependent glucose retention in kidney tubules. Decreased glucose absorption has been reported in *Pontius conchnius* exposed to mercury nitrate (Gill and Pant, 1981) and in *C. isheriensis* (Sydenham) exposed to water borne lead (Kori-Siakpere, 1995).

The white blood cells in fish respond to various stressors including infections and chemical irritants (Christensen et al., 1978). Thus increasing or decreasing numbers of white blood cells are a normal reaction to a chemical such as zinc in the present study, and cadmium in a previous study (Kori-Siakpere et al., 2006), demonstrating the effect of the immune system under toxic conditions. 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 non-specific response to any environmental stressor (Iwama et al., 1976; Ellis, 1981).

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) when juveniles of mudfish, *Clarias batrachus* where exposed to carbon tetrachloride. Musa and Omoregie (1999) 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) 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.

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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