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Effect of heavy metal on biochemical and hematological parameters in *Cyprinus carpio* and its use as a bioindicators of pollution stress

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Abstract: *Study was undertaken to evaluate hematological and biochemical changes resulting from the exposure of a common carp* Cyprinus carpio *to sublethal concentrations (0.05 mg/l) of zinc in water for a period of 10, 20 and 30 days. Four groups of ten fishes were subjected to serial dilutions of the stock solution of zinc of 0 (control), 0.05 mg/l in four large plastic pools of 60 liters capacity by the semi static (renewal) method. At the end of 10, 20 and 30 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, and total plasma protein and plasma glucose concentration). The derived haematological indices of sublethal concentrations (0.05 mg/l) of zinc caused a dose dependent decrease in haemoglobin values, coupled with a decrease in haematocrit values and red blood cell counts are which indication of anemia of the normal 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 was also lower in the exposed fish as compared to the control. In conclusion, the changes observed indicate that haematological parameters can be used as an indicator of zinc related stress in fish on being exposed to high levels of zinc.*

Key Words: Zinc, Haematology, Glucose, Protein, *Cyprinus carpio*

Introduction

Heavy metals in the environment have long biological half-lives and therefore a major threat to aquatic organisms, particularly fishes are found to be more effective fishes. In high concentrations heavy metals will kill aquatic organisms; at sub-acute concentrations they are gradually intensified in various aquatic organisms as they reach higher tropic levels of the food chain. Health hazards caused by heavy metals have become a great concern only when they affected humans via the food chain as in intimate diseases in Japan. The use of fish as an indicator organism for assessing the quality of the aquatic environment seems to be logically justifiable. Certain physiological parameters may be used for descriptive purposes as 'early warning systems'. Therefore an attempt has been made to study the influence of heavy metal zinc sulphate on non-target organisms such as the extensively cultivated species of fish *Cyprinus carpio* var. communis. Among the various toxic pollutants, heavy metals are particularly severe in their action due to tendency of bio-magnification in the food chain. The global heavy metal pollution of water is a major environmental problem. With the advent of agricultural and industrial revolution, most of the water sources are becoming contaminated (Khare and Singh, 2002). Industrial discharges containing toxic and hazardous substances,

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including heavy metals contribute tremendously to the pollution of aquatic ecosystem (Gbem *et al.*, 2001). Zinc is an essential element which acts as a structural component and having specific properties is indispensable for life. The danger of zinc is aggravated by its almost indefinite persistence in the environment because it cannot be destroyed biologically but can only be transformed from oxidation state or organic complex to another. Zn is a potential toxicant to fish, which causes disturbances of acid-base and ion regulation, disruption of gill tissue and hypoxia (Hogstrand *et al.*, 1994). Bioaccumulation of metals reflects the amount ingested by the organism, the way in which the metals are distributed among the different tissues and the extent to which the metal is retained in each tissue type. Accumulation of zinc has attained a serious dimension causing a pathogenic stage like Alzheimer's disease. Zinc in certain concentration is desirable for the growth of freshwater animals but it's over accumulation is hazardous to exposed organisms as well as to those who consume them directly or indirectly through food chain. The pattern of metal accumulation in fish tissue can be utilized as effective indicator of environmental contamination (Sultana and Rao, 1998). Fish exposed to high concentration of trace metals in water may take up substantial quantities of these metals. When exposure to high Zn level occurs than the liver's capacity is exceeded to remove the excess level of Zn, the more toxic type of Zn (Zn^{2+}) may be transported through blood stream to other organs. Zinc can be accumulated via the gills and/or the digestive track; however the role of water as source of Zn uptake is not fully elucidated (Spry *et al.*, 1988). The aim of this study was to evaluate the plasma protein, plasma glucose and hematological effects of zinc sulphate in *Cyprinus carpio*, exposed to sublethal dose at different time interval.

Material and Methods

Healthy specimens of *Cyprinus carpio* were obtained from a local fish farm and were transported in containers to the laboratory. In the laboratory, fish were kept in large plastic pool containing 60 L of clean tap water and acclimatized for 15 days to the laboratory conditions, during which time they were provided with artificial feed tubifex 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 sulphate was prepared by dissolving 43.97 g, equivalent to 1 g of zinc in 1000 ml distilled water at concentration of 1000 mg/l. 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 in three large plastic pools of 60 liters capacity. The test was performed by the semi static (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. The water quality parameters of the diluting water used in the tests was determined by standard methods as presented in Table 1. The exposure period lasted 15 days, after which blood samples were taken from the control and experimental fish. The samples of blood 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). Red and white blood cell counts were counted under light microscope with an improved Neubauer haemocytometer. Total plasma protein and

plasma glucose concentration were determined by Lowry *et al* (1951) and Oser (1965). 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 and Discussion

The observed Physico – chemical characteristics of water used for testing were as follows; temperature 20.8 ± 1.5^{0}C; dissolved oxygen 7.8 ± 1.6 mg/l, hardness 135.60 ± 5.20 mg/l as $CaCO_3$; Chloride 7.50 ± 0.85 mg/l; electrical conductivity 782 ± 42.50 µmhs/cm; pH 7.7 ± 0.20.

The haematological alteration of *C. carpio* resulting from exposure to various concentration of zinc sulphate in the water for 10, 20 and 30 days showed significant reduction in RBC count and haemoglobin percentage where as WBC count and clotting time of the blood increased significantly as compared to control. The RBC count in control was 3.8 x 10^6/mm^3 whereas in 10 days treated fish it decreased to 3.3 x 10^6/ mm^3 and then showed a gradient increase in 20 days (3.4 10^6/mm^3) and 30 days (3.7 x 10^6/mm^3) exposed fish (Fig 1). The haemoglobin percentage in control was 11.20±0.20, where as, in 10days treated fish it increased to 11.90±0.20, decreased in 20 days to 10.70±0.40 and after 30 days it went to 10.80±0.20 exposed fishes (Fig. 2). The percentage of haematocrit decreased only after 10 and 20 days exposure to varied $ZnSO_4$ treatment in the test fishes. When compared to control (38.4) the haematocrit value of 10 days exposed fish decreased to 37.6 and then gradually increased in 20 days (37.5) and 30 days (38%) exposed fishes (Fig. 3). The clotting time also increased for all the three intervals of experimentation, the clotting time of fish when exposed to $ZnSO_4$ for 30 days showed maximum clotting time of 40 sec when compared to 20 days (38 sec), 10 days (30 sec) and of control (35 sec) (Fig. 4). The treated fish showed significant increase in total WBC count when exposed to $ZnSO_4$ for 10, 20 days. The WBC count in control fish was 2.5 X10^3/ mm^3, when exposed to 10 days, it increased significantly to 3.5 X10^3/mm^3 followed by a small increase after 20 days exposure (3.6 X10^3/ mm3) and then decreased to near the control value after 30 days exposure (2.8 X10^3/mm3) (Fig 5).

The mean value in plasma protein in the *C. carpio* in control 3.14 ± 0.32mg/l and sublethal (0.05 mg/l) concentration were sufficient to produce significant decrease in plasma protein level ($P < 0.05$) of treated fish The plasma protein level showed gradual decrease from 3.14±0.32 in control to 2.8±0.141 after 10 days, 2.7±0.063 after 20 days and 2.5±0.088 after 30 days exposed fish during the experimentation (Fig. 6). However, the plasma glucose concentration increased in treated fish *C. carpio* from 100mg/l (control) to after 10 (230.9±7.090), 20 (269.5±4.75) and 30 (273±3.38) days on the sublethal exposure (0.05 mg/l) concentration of zinc sulphate (Fig.7).

Till date, haematological variables have been used to determine the sublethal concentrations of pollutants than for other purposes. The use of immune system parameters to assess alterations in fishes experiencing exposure to heavy metals and its interaction in defense mechanisms stem from the need to develop healthy management tools to support rapidly growing aquaculture industry (Jones, 1983). The physiological stress resulting from metal poisoning is clearly reflected by blood patterns of the experimented fish. 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

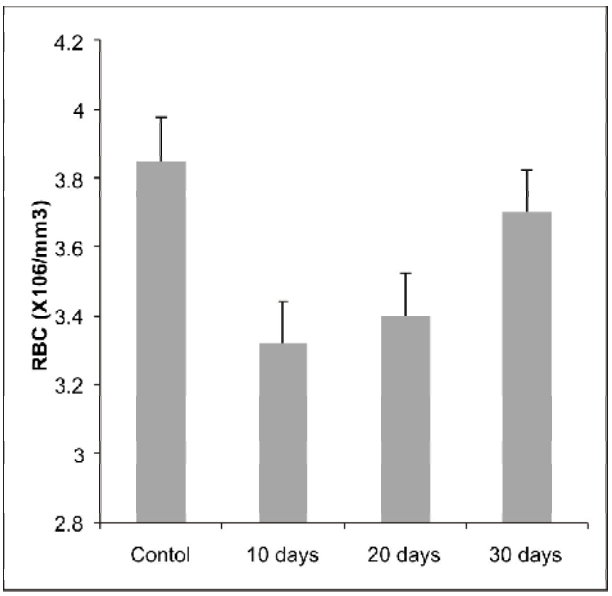

Fig. 1. Level of red blood cell (RBC) for control and exposed fish

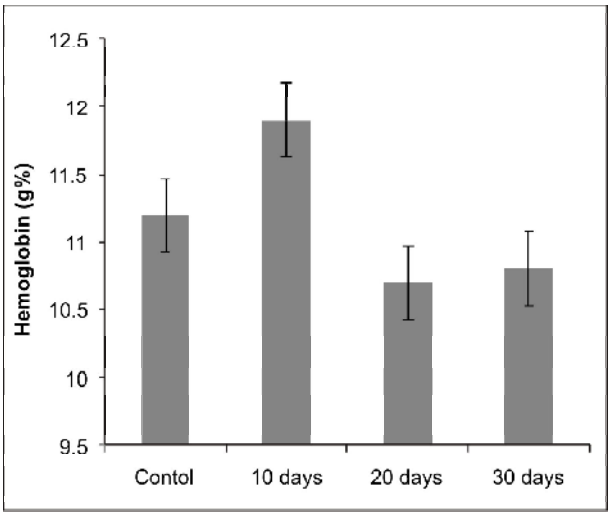

Fig. 2. Level of hemoglobin (%) in the blood of control and exposed fish.

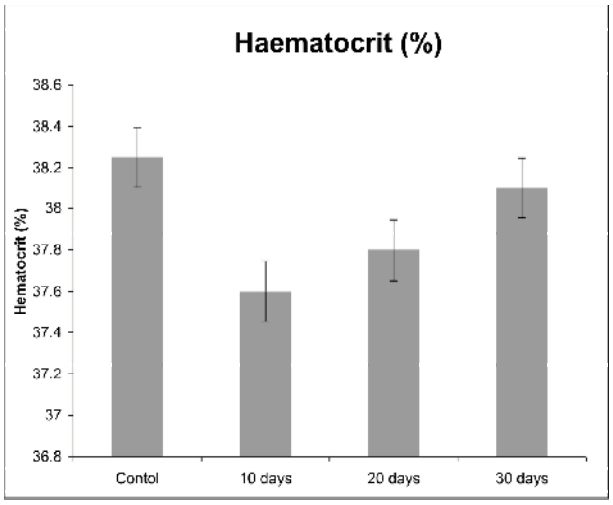

Fig. 3. Level of hematocrit for control and exposed fish

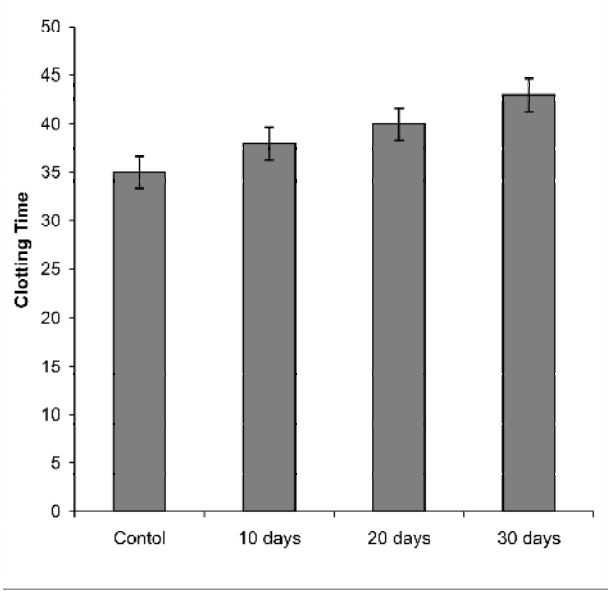

Fig. 4. Level of clotting time for control and exposed fish

haematological parameters. Thus, water quality is one of the major factors, responsible for individual variations in fish hematology, since they live in close association with their environment and are sensitive to slight fluctuation that may occur within their internal milieu. 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 always result in 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 possibly as a result of blood water

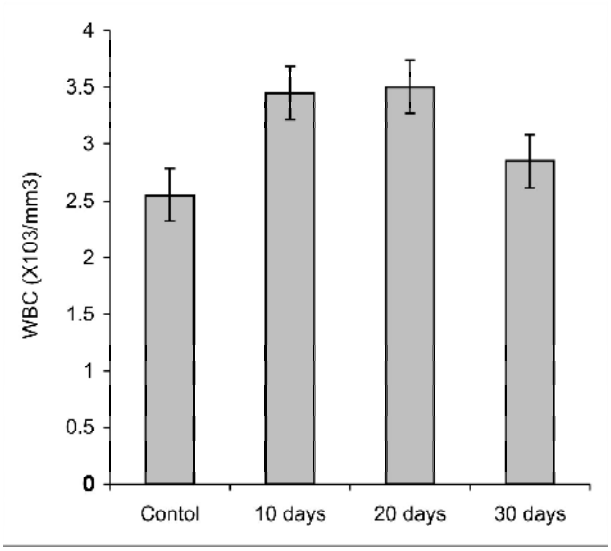

Fig. 5. Level of WBC for control and exposed fish (*) Significant different in relation to control group ($p< 0.05$).

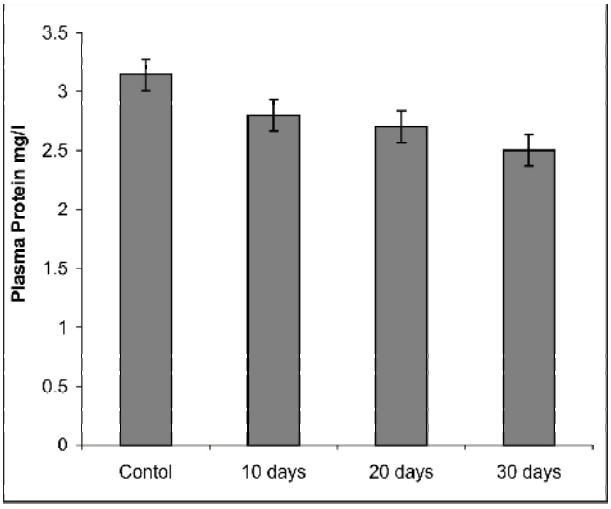

Fig. 6. Level of plasma protein for control and exposed fish (*) Significant different in relation to control group ($p< 0.05$).

content.

Zinc is known to be an essential element for 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). The result of present investigation shows that the $ZnSO_4$ treatment inflicted a drastic reduction in the total RBCs

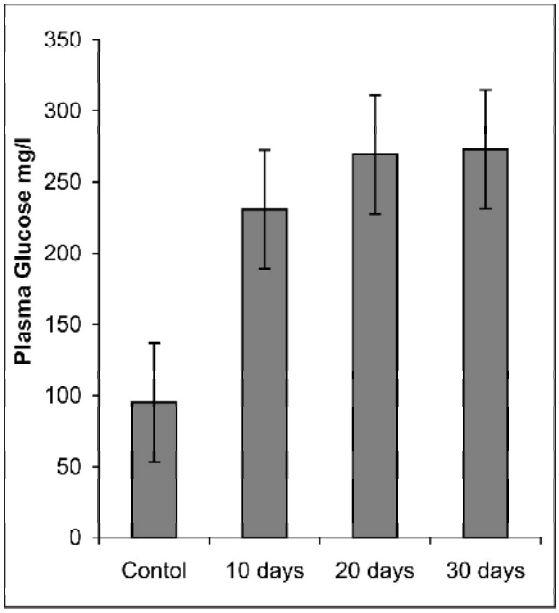

Fig. 7. Level of plasma glucose for control and exposed fish (*) Significant different in relation to control group ($p< 0.05$).

count with the sudden exposure (10 days) to sublethal doses and then as the immune system started to respond to the change the total RBCs count started to increase gradually (20-30 days exposure) compared to control. 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. The fish muscle has been known as the water exchange tissue with blood. Haemoconcentration and haemodilution have been described in previous works of Mishra and Srivastava (1980). In the present study, decrease in haematocrit following zinc exposure in *Heteroclarias* may 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 Musa and Omoregie (1999) when they exposed fish to polluted environment under laboratory conditions. Thus, significant reduction in these parameters is an indication of severe anemia 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 an increase in haematocrit values to increase in the size of the erythrocytes compared to chromium and zinc treated rainbow trout. Observed depression in haematocrit and haemoglobin values coupled with decreased and deformed erythrocytes are signs of anemia. 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 a*l. (1994) 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). They 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) might 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. Spleen contractions after stress have been detected in fish. A similar observation was made for *Cyprinus carpio* after cadmium exposure. 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. 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. 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). An increase of plasma glucose was observed in this study. It was found to be significant at low concentrations of zinc. Changes in carbohydrate metabolism occurs in fish exposed to various sublethal concentrations of pollutants. Blood glucose has been employed as an indicator to environmental stress. The increase in blood glucose is usually correlated with the mobilization of glycogen and development of hyperglycaemia. The hyperglycaemia response varies with the nutritional status of the fish. 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). The white blood cells in fish respond to various stressors including infections and chemical irritants. 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 (unpublished), 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 nonspecific response to any environmental stressor (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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