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Full Length Research Article

TOXICITY EFFECT OF HAEVY METAL COPPER SULPHATE ON HAEMATOLOGICAL ALTERATIONS IN FISH CATLA CATLA

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Heavy metal copper is common pollutants of freshwater ecosystems where they induce adverse effects on the aquatic biota. Fish, *Catla catla* is an important carp species in Tamil Nadu region having good nutritional values. Fishes living in close association with may accumulate heavy metals. In the present observation, the toxic effects of the heavy metal copper LC_{50} 1.8 mg/L on the total RBC, WBC and Hb in the fish, *Catla catla* were estimated. The rate of oxygen consumption were observed decrease in fish *Catla catla* exposed to low 10% and high 30% sub lethal concentrations of copper sulphate for a period of 24, 48, 72 and 96 hrs. The sublethal concentrations of copper culphate on (Low 10% and high 30% sublethal concentrations) showed a decreasing trend in the RBC and Hb compared to controls and the WBC analysis revealed a significant increased compared to control for a period of 7, 14 and 21 days exposures. The results indicated the toxic nature of the heavy metal copper.

Key words: Fish Catla catla, Copper sulphate, Haematology, Parameters.

INTRODUCTION

Environmental poisoning by heavy metals has increased in recent years due to extensive use of heavy metals in agriculture, and chemical and industrial processes, posing a serious threat to living organisms. The discharges of heavy metals by industries pose a serious water problem due to the toxic properties of these metals and their adverse effects on aquatic life. According to the survey conducted by Central Inland Fisheries Research Institute (CIFRI, 1981),these heavy metals are well known pollutants which are often encountered in many rivers of India, and there is every possibility of deterioration of water quality and hence including man and various organisms are presenting a potential threat for survival. The increasing industrial activities and the use of $CuSo_4$ as a fungicide in agricultural practices as well as in the control of algae and pathogens in fish culture ponds have increased the copper concentrations in aquatic systems. Copper sulphate is a fungicide used to control bacterial and fungal disease of fruit, vegetable, nut and field crops (Hayes, 1982). Copper and its compounds have been used by man since prehistoric times. Copper is a trace element that is essential in small amounts, but can be toxic in large quantities. There are several sources of copper emission into the atmosphere. Copper reaches the aquatic environment through wet or dry deposition, mining activities, land runoff and industrial, domestic and agricultural waste disposals (Bertine and Goldberg, 1997). Aquatic pollution undoubtedly has direct effects on fish health and survival. Heavy metals are regarded as serious pollutants of the aquatic environment because of their persistence and tendency to be concentrated in aquatic organisms (Veena *et al.,* 1997).

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Most heavy metals released into the environment find their way into the aquatic phase as a direct input by various anthropogenic processes, atmospheric deposition and erosion due to rainwater (Kalay *et al*., 1999). Trace metals are considered to be one of the main causes of pollution in aquatic ecosystems at present, having the highest environmental stress index, often in excess of the recommended threshold limit (Meguid *et al*., 2002). The heavy metal contamination of aquatic system has attracted the attention of several investigators both in the developed and developing countries of the world. Many industrial and agricultural processes have contributed to the contamination of fresh water systems thereby causing adverse effects on aquatic life (Dautremepuits *et al.,* 2004). Copper is a trace element which is essential to the function of specific proteins enzymes. However, at high concentrations, it may be toxic to organisms. Fishes are being at the higher level of the food chain accumulate large quantities of these sediments and the accumulation depends on the intake and the elimination from the body (Karadede *et al.,* 2004). Heavy metals are recognized as a strong biotoxicants, because of their persistent nature and cumulative action to the aquatic flora and fauna (Sharma and Agrawal, 2005). Copper is an essential heavy metal. It plays an important role in various biological processes including oxidative phosphorylation, gene regulation and free radical homeostasis as essential cofactor. However, when it was concentration exceedes metabolic requirements, it becomes harmful and play a major role among pollutants (Singer *et al.,* 2005). Copper has been widely used in the past as an algaecide in fish-bearing water, at concentrations which would be toxic if the metal was present in the toxic ionized form. However most, if not all of this inactive copper will ultimately enter sediment sinks where it may have limited bioavailability for organisms living there (Figuero *et al*., 2006). Among various heavy metals, copper, chromium and iron are the most important pollutants originating from industrial effluents and agricultural wastes in aquatic environment, causing significant damage to aquatic organisms, resulting in imbalance of the ecosystem. Aquatic organisms are characterized by the uptake and retention of heavy metals and the rate of accumulation are affected by chemical form of metal (Aanand *et al*., 2010 and Boyd, 2010). Hematological indices greatly used to assess the toxic effect of environmental pollutants (Kavitha *et al*., 2010). The blood parameters have been used as sensitive indicator of stress in fish exposed to different water pollutants and toxicants, such as metals, biocides, pesticides, chemical industrial effluents, etc. These metallic ions are the probable major cause of the physiological abnormalities in fish. A fall in RBC count, Hb% and PCV%, in the fish, *Channa punctatus* upon treatment with both copper and chromium was noticed along with acute anaemia (Singh, 1995).

The metal entering into fish system are slowly eliminated (James and Sampath, 1996; James *et al*., 1996), hence the blood parameters get affected on account of metal toxicity. Fish blood is susceptible to contamination-induced stress and its interchanging caused by theFish blood is susceptible to contamination-induced stress and its interchanging caused by the hematological and immunological parameters can be utilized as toxicity indices of xenobiotics (Sancho *et al*., 2000). Hematological indices and blood parameters could be practical to the assessment of the effects of contaminants on fish Oliveira Ribeiro (Oliveira Ribeiro *et al*., 2006). Although the produce of silver carp is expanding annually, the processing of this fish is restricted. In the present investigation, tested variations of haematological changes in carp (*Catla catla*) were exposed to $CuSO_4$.

MATERIALS AND METHODS

Fish, *Catla* was collected from Chidambaram area and were brought to the laboratory in large plastic troughs and acclimatized for one week. Healthy, fish having equal size (length 10 to 15 cm) and weight (100 to 200 g) were used for experimentation. Stock solution of copper sulphate was prepared by dissolving appropriate amount of salt in distilled water. The physico-chemical characteristic of test water have analyzed regularly during the test periods following the standard method describe by APHA (1998). Batches of 10 healthy fishes were exposed to different concentrations of heavy metal copper to calculate the medium lethal concentration LC_{50} value (1.8 mg/L) using probit analysis Finney method (1971). The fishes (Four groups) were exposed to the two sublethal concentrations ($1/10^{th}$ and $1/30^{th}$ mg/L) of copper for 5, 10 and 15 days respectively. Another group was maintained as control. Fish was collected and gently wiped with a dry cloth to remove water. Caudal peduncle was cut with a sharp blade and the blood was collected in a watch glass containing EDTA, an anticoagulant (6% Ethylene diamine tetra acetic acid). The blood was mixed well with the EDTA solution by using a needle and this sample was used for determining the Red Blood Corpuscle Count (RBC), White Blood Corpuscle Count (WBC) and Haemoglobin count (HB).

RESULTS

Heavy metal caused 50% mortality of fish *Catla catla* at 96 hours was 1.8 mg/L. The LC_{50} values of copper sulphate heavy metal for 24, 48, 72 and 96 hours were 1.5, 1.6, 1.7 and 1.8 mg/L respectively.

Oxygen consumption: Oxygen consumption of fish *Catla catla* exposed of sub lethal concentrations of copper is presented in Table 1 and Fig. 1. The rate of oxygen consumption were observed decrease in fish *Catla catla* exposed to low 10% and high 30% sub lethal concentrations of copper sulphate for a period of 24, 48, 72 and 96 hrs. The rate of oxygen consumption in control fish *Catla catla* were 0.94, 0.97 and 0.83 ml/O_2/ g/hr at 24, 48, 72 and 96 hrs respectively (Table 1). The fish exposed to sub lethal concentrations of copper sulphate shown in the oxygen consumption at the rate of 0.56, 0.50 and 0.44 ml/O_2/ g/hr at low sublethal concentration of 24, 48, 72 and 96 hrs and 0.44, 0.41 and 0.38 at high sublethal concentration of 24, 48, 72 and 96 hrs respectively. In the present study the oxygen consumption was gradually decreasing with increasing exposure periods (Table 1 and Fig. 1).

Haematology: The toxic effects of copper sulphate on the haematological parameters of *Catla catla* such as number of red blood corpuscles (RBC), white blood corpuscles (WBC) and haemoglobin content (Hb) were analyzed. The observations were made at the end of exposure periods (7, 14 and 21 days) to calculate the percentage of increase and decrease of different haematological parameters.

Table 1. The rate of oxygen consumption changes of fish Catla catla at different sublethal concentrations of copper sulphate (ml/O_2/ g/hr)

Experimental Group	Exposure Period (days)			
Treatment	24 hrs	48 hrs	72 hrs	96 hrs
Control	0.80 ± 0.04	0.82 ± 0.11	0.81 ± 0.11	0.81 ± 0.09
Low SLC	0.75 ± 0.06	0.68 ± 0.13	0.61 ± 0.11	0.56 ± 0.14
High SLC	0.52 ± 0.11	0.47 ± 0.06	0.43 ± 0.08	0.37 ± 0.08

(Values are mean ± SD).

Table 2. The haematological parameters of fish Catla catla under two sublethal concentrations of copper sulphate

Days	Exposure	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Hb (g/100ml)
7 days	Control	2.88 ± 0.38	15.92 ± 0.58	6.36 ± 0.68
	Low SLC	2.79 ± 0.45	16.47 ± 0.78	6.26 ± 0.38
	High SLC	2.5 ± 0.37	17.75 ± 0.63	6.02 ± 0.55
14days	Control	2.89 ± 0.4	15.93 ± 0.4	6.34 ± 0.75
	Low SLC	2.22 ± 0.27	18.18 ± 0.63	5.78 ± 0.44
	High SLC	2.12 ± 0.33	18.34 ± 0.30	5.5 ± 0.65
21 days	Control	2.86 ± 0.38	15.82 ± 0.61	6.33 ± 0.40
	Low SLC	1.84 ± 0.34	18.47 ± 0.53	5.07 ± 0.47
	High SLC	1.68 ± 0.45	18.68 ± 0.39	4.97 ± 0.35

Values are mean ± SD – or + indicate present decrease or increase over control

Red Blood Corpuscles (RBC): The sublethal concentrations of copper culphate on (Low 10% and high 30% sublethal concentrations) showed a decreasing trend in the RBC counts compared to controls (Table 2 and Fig. 2). The mean values of RBC in control fish were estimated to be 2.88 ± 0.38, 2.89 ± 0.4 and 2.86 ± 0.38 (10^6/mm^3). The control fish, the maximum (2.89 ± 0.4) was recorded for 14 days. In the sublethal concentration (LSLC) values were recorded to be 2.79 ± 0.45, 2.22 ± 0.27 and 1.84 ± 0.34 and the sublethal concentration (HSLC) values were noted 2.5 ± 0.37, 2.12+0.33 and 1.68 ± 0.45 after exposure of 7, 14 and 21 days respectively.

White Blood Cells (WBC): The changes of haematological parameter like, WBC in the fish kidney, both in control as well as sublethal concentrations of copper sulphate exposed to (Low and high sub lethal concentrations) for 7, 14 and 21 days

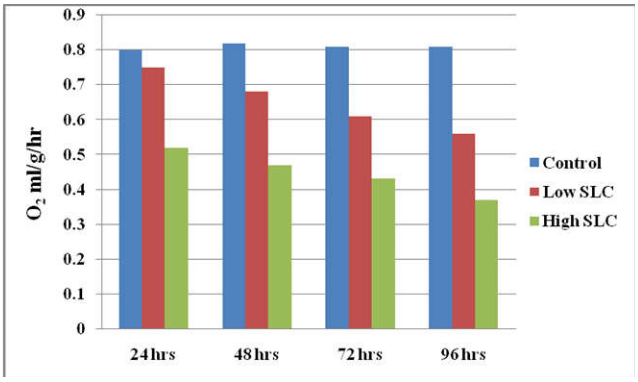

Fig 1. The rate of oxygen consumption changes of fish *Catla catla* at different sub lethal concentrations of copper sulphate (ml/O_2/ g/hr)

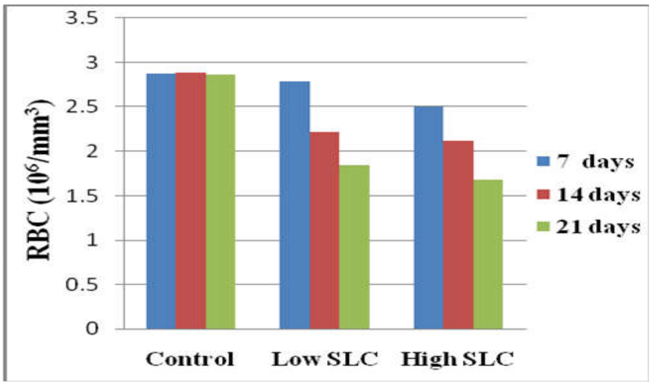

Fig. 2. The total RBC of *Catla catla* under sublethal concentrations of copper sulphate.

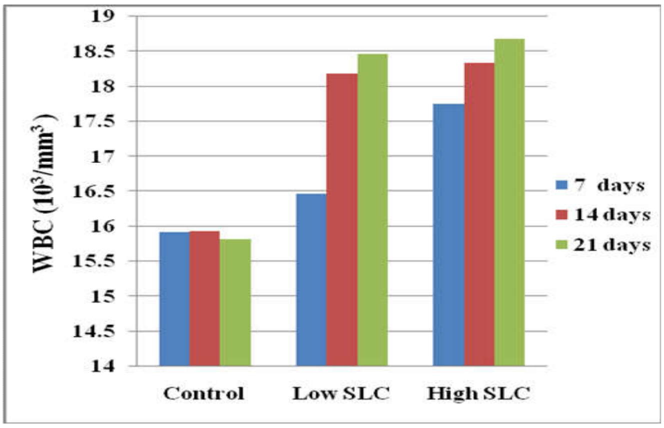

Fig. 3. Total WBC of *Catla catla* under sublethal concentrations of copper sulphate

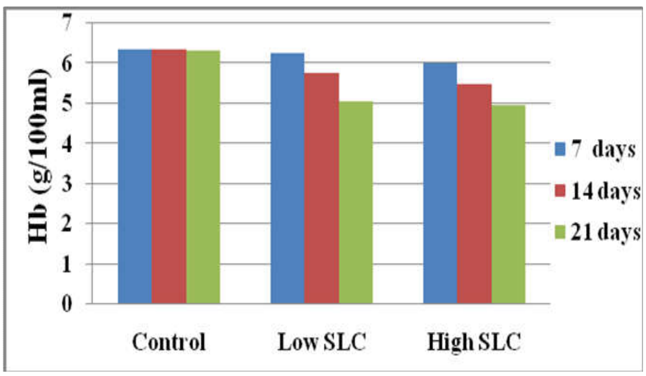

Fig. 4. Total Hb of fish *Catla catla* under sublethal concentrations of copper sulphate

(Table 2 and Fig 3). The WBC analysis revealed a significant increase compared to control fish. The White Blood Cells (WBC) count from 15.92 ± 0.58, 15.93 ± 0.4 and 15.82 ± 0.61 ($10^3/mm^3$) in control fish. The sublethal concentrations (LSLC) values were recorded from 16.67 ± 0.78, 18.18 ± 0.63, and 18.47 ± 0.53 and the sublethal concentrations (HSLC) values were recorded from 17.75 ± 0.63, 18.34 ± 0.30, 18.68 ± 0.39 ($10^3/mm^3$) after exposure of 7, 14 and 21 days respectively.

Haemoglobin (Hb): The changes of haematological parameter like haemoglobin in the fish *Catla catla*, both in control as well as sublethal concentrations of copper sulphate exposed to (Low and high sub lethal concentrations) for 7, 14 and 21 days (Table 2 and Fig. 4). The haemoglobin analysis revealed a significant reduction compared to control fish. The haemoglobin count from 6.36 ± 0.68, 6.34 ± 0.75 and 6.33 ± 0.40 g/100ml in control fish. The maximum value of control fish (6.36 ± 0.68) was recorded for 107 days. Sublethal concentration (Low SLC) values were recorded from 6.26 ± 0.38, 5.78 ± 0.44, and 5.07 ± 0.47 and high sub sublethal concentration values were recorded from 6.02 ± 0.55, 5.5 ± 0.65 and 4.97 ± 0.35 g/100ml after exposure of 7, 14 and 21 days respectively (Table 2 and Fig. 4).

DISCUSSION

Hematological indices are very important parameters for the evaluation of fish physiological status under metallic stress. The changes in blood indices and their peculiarities depend on the concentrations of heavy metals and duration of exposure of fish to them. Hematological indices are of different sensitivity to various environmental factors and chemicals. The count of erythrocytes is quite a stable index and the fish body tries to maintain this count within the limits of physiological standards, using various physiological mechanisms of compensation, especially under stress. Dethloff *et al.,* (2004) observed significant reduction in the total count of Red blood cells, in Oncorhynchus mykiss 6M exposed to 0.5 mg/L copper sulphate for 120 days. It may be due to the decreased rate of production of red blood cells or increased loss of these cells or impaired erythropoiesis due to a direct effect of heavy metals on haematopoietic centers i.e. kidney and spleen, or accelerated erythroclasia due to altered membrane permeability or increased mechanical fragility and defective iron metabolism or impaired intestinal uptake of iron due to mucosal lesions. The decrease in RBCs count may perhaps be from the inhibition of DNA synthesis in red blood cell production, or impaired intestinal absorption of iron or possible disruption of hematopoiesis and hypoxia, induced by exposure to the selected toxicants (Shah, 2006). Thus, the significant decrease in these hematological parameters is an obvious clue of prevalence of severe anemia induced by exposure of the experimental fish to copper and lead in the water, as reported by Maheswaran *et al.,* (2008). The decrease in population of immature RBCs might be as compensation for the increased degeneration of cells. The morphological changes in RBCs may be taken as a serious indication of heavy metal intoxication in fish. The reduced oxygen carrying capacity in the present study up to the day 21 may be attributed to the fall in RBC count, intense haemolysis, and haemodilution induced by the copper stress. (Aranudova *et al*., 2008).

In the present observation total erythrocytes of *Catla catla* showed a significant decreasing tendency at low and high sublethal concentrations of copper sulphate heavy metal, when compared to control. The changes in WBC count can be manifest with the form of leucocytosis, as lymphopenia and heterophilia are common characteristic responses of the animals under stress (Joshi, 2002). Similarly, Dethloff *et al*., (2004) also reported the increase in the count of lymphocytes and decrease in neutrophils, eosinophils and basophils and no change in monocytes cell in Oncorhynchus mykiss on exposure to 0.5 mg/L copper sulphate for 120 days. The observed reduction in WBC count for toxicant exposed groups agrees with the reports that fish is in stress and the release of cortisol during stress causes a decrease of leukocyte count, which shows the weakening of the immune system (Olanike *et al.,* 2008). In the leukocyte series, WBCs count were found to exhibit a highly significant decrease, compared to control. The significant decrease of neutrophils, thrombocytes and lymphocytes count may be due to increased concentration of toxicants in various tissues of fish (Ololade and Oginni, 2010). In the present observation, the number of leucocytes in *Catla catla* was increased with the increasing concentrations of copper sulphate when compared to control. Copper sulphate exposure of 0.05 mg/L significantly decreased haemoglobin and haemocrit values in Asian catfish (*Saccobranchus fossilis*) Khangarot *et al*., 1988).

According to Dethloff *et al.,* (2004) has been observed that the haemoglobin content decreased significantly after 2 mg/L copper sulphate exposure the time period of 30, 60 and 90 days as compared to the controlled one. In fishes after 5 mg/L copper sulphate exposure for the period of 30, 60 and 90 days, the haemoglobin content have also got decreased significantly as compared to the controlled one. It is inferred that the decrease in haemoglobin percentage in the earlier study may be due to anaemia caused by copper sulphate. Haematology has been widely used for the detection of physiopathological alterations following different stress conditions. In recent years hematological studies are increasing used to determine the systematic relationship to elucidate the physiological adaptation, to assess the health of the fish and to estimate the water pollution (Akhtarunnessa Chowdhury and Masudul Haq, 2012; Enis Yonar *et al*., 2012; Ibrahim *et al*., 2012; Zubair Ahmad, 2012; Abedi *et al.,* 2013). In the present estimation, the haemoglobin content of *Catla catla* in low and high sublethal concentrations showed decreasing trend with a significant reduction of copper, when compared to control.

Conclusion

The study suggested that the physicological and hematological indices of *Catla catla* for low 10% and high 30% sublethal concentrations of copper sulphate. This data verifies that the vicissitudes in physicological and hematological indices may be used as sensitive biomarkers for animal health evaluation, especially in regions that are naturally affected by heavy metals, causing stress in fish on exposure to elevated levels in the water. Exposure of *Catla catla* and other allied fish species to higher concentrations of copper demonstrated a toxic poisoning. The consumption of fish as a diet from such metal polluted areas is directly toxic threat to human blood characteristics. Thus sincere attentions should be devoted to minimize the risk of copper pollution in the ambient environment to save living organism including human population from adverse effects of these pollutants.

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