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Research Article

THE IMPACT OF COPPER SULPHATE ON THE BIOCHEMICAL PARAMETERS IN *CHANNA PUNCTATUS*

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ABSTRACT

The physiological, biochemical and haematological changes in *Channa punctatus* after exposure to sublethal concentrations of the copper sulphate of the heavy metal have been investigated. The toxicity of the 2 ppm copper sulphate with respect to behaviour, and haematological paramaters of the fish *Channa punctatus* has been studied. Red blood cell (RBC) count and haemoglobin (Hb) content were decreased 0.9% with the increasing concentrations of the copper sulphate. However, the white blood cell (WBC) count was increased 1% with increasing concentrations of the copper sulphate. A dissimilar relationship was established with respect to RBC and WBC. The constant increase in the differential count clearly indicates that the heavy metal stress certainly stimulate the white blood cells to produce more at all times of exposure. Biochemical changes of protein, lipid and carbohydrate were recorded. Also, such fish when consumed as food leads to the deposition of the heavy metal in the soft tissues of the human body leading to exposure to a health effects.

Keywords: Copper sulphate, Toxicity, Behaviour, Haematological paramaters, *Channa punctatus*.

INTRODUCTION

Water harbours all types of biotic and abiotic components including metals essential for life processes. The effects of heavy metals and trace elements in water have been well known since the episodes of the Minimata and Nigata bay incidents and with the existence of itaital disease caused due to mercury pollution in Japan. Because of these disasters the study of toxicity of heavy metal to the aquatic life is very much required. Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. Heavy metals are dangerous because they tend to bioaccumulation. As trace elements, some heavy metals like copper, zinc are essential to maintain the metabolism of the human body. Copper has been used to control fungal diseases in plants. High concentrations of this heavy metal were detected in some aquatic ecosystems, collecting runoff water and it is also highly concentrated in ground water (Gerbe, 1996). There are also, anthropogenic sources of environmental contamination by copper including mining, smelting, foundries, municipal waste incinerators, burning of coal for power generation and a variety of copper based products in building and construction. Cadmium (Cd) is a well-known heavy metal toxicant with a specific gravity 8.65 times greater than water. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. The target organs for Cd toxicity have been identified as liver, placenta, kidneys, lungs, brain and bones (Ramamurthy, *et al.,* 2008).

Copper is an essential trace metal in small concentrations for several fish metabolic functions. Essentiality of copper arises from its specific incorporation into a variety of enzymes, which play important roles in physiological processes (e.g. enzymes involved in cellular respiration, free radical defense, neurotransmitter function, connective tissue biosyntheses & other functions), as well as, into some structural proteins. Although the crucial role of copper in several enzymatic processes (Baker, 1969), this heavy metal can exert adverse toxicological effects, when present in high concentrations in water (Pelgrom *et al.,* 1995). In fact, it is potentially toxic when the internal available concentration exceeds the capacity of physiological detoxification processes. Copper sulphate is one of the most widely used algicides for the control of phytoplankton in lakes, reservoirs and ponds; it is also used for aquatic weed control (Karan *et al.,* 1998). Fish are

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widely used to evaluate the health of aquatic ecosystems and physiological changes serve as biomarkers of environmental pollution. The present study aims to document the toxicity of copper sulphate is on biochemical and haematological parameter of *Channa punctatus.*

MATERIALS AND METHODS

For the experimental purpose, the healthy live *Channa punctatus* were collected from the local fish farm at Orathanadu. The collected fish were brought to the laboratory without injury in order to avoid dermal infection and they were made to acclimate themselves to the conditions prevailing in the lab for about a week. *Channa punctatus* were fed with groundnut oil cake, coconut oil cake, rice bran; they were given feeds for 7 to 15 h. The food found unused by them was cleared periodically from that tank, during the acclimation period. The chlorine free water was changed daily. The feeding was stopped one day prior to the experiment.

In a preliminary study the toxic and sublethal levels of 0.5 to 5 ppm copper sulphate were found out for 96 h exposure. The fish were observed for behavioural changes and mortality at 96 hours and 21 days of exposure were recorded and tabulated employing each of the two test media. The 4 ppm concentration at which 100 percent mortality was observed within 96 hours was considered as the lethal concentration (96 h LC_{100}) and the 0.5 ppm concentration at which 100 percent survival was observed by the end of 96 hours was considered as the sublethal concentration (96 h LC_0). The LC_{50}, the lethal concentration, which kills 50% of individuals at 96 h exposure, was found out and the data obtained were tabulated. For these present study heavy metals copper was taken and this was prepared at different concentrations.

The experiments were carried out in such a manner that the *Channa punctatus* were subjected to 0.5 to 5 ppm different sublethal concentrations in order to assess the effect of copper on selected biochemical and haematological parameters. Before the actual starting of the experiments, the test fishes were divided into 3 groups of the same weight one control and two experimental. They were selected from the stock tank and transferred into the test chamber with test solution of various concentrations of copper viz., 0.1 and 0.2 ppm. Each group consisted of 10 fish as per aquarium. These groups were actually subjected to both short (96 h) and long term (21 days) exposure periods. Both long term and short tem exposure were given to all the groups involving the 0.1 & 0.2 ppm concentrations of copper sulfate. A control third group (copper free water 0 ppm) was also maintained in the above said manner. After the exposure period was over, fish was taken out and sacrificed for the analysis of selected haematological parameters viz., RBC, WBC, Hb, MCV, MCHC, and MCH with respect to short term and long term exposure periods of fish to the different concentrations of copper. After 21st day analysis of biochemical and enzymes was estimated in muscle of fish. The estimation of glucose (Dubois *et al.*, 1956), protein (Lowry *et al.*, 1951), Alanine transaminase and Aspartate transaminase (Mohun and Cook, 1957) and lipase (Oser, 1965) were estimated.

RESULTS

Survival Capacity: The study recorded that 96 h LC_{50} value of copper was 3.0-ppm. Percentage of mortality of *Channa punctatus* is varied with effect of copper concentrations and exposure time (Table 1). The mortality of fish was increased with increasing concentrations of copper sulfate. No mortality was observed up to 1-ppm.

Haematological parameters: With respect to haematology, the study illustrative of the fact that there was a gradual decrease in the haematological parameters such as RBC number, Hb content and gradual increase in WBC number, such was actually caused by the use of the heavy metal. The copper sulphate shows significant concentration dependent. The reduction of red blood cells was noticed at lower concentration and also at short term (Acute) exposures i.e. in 0.1 and 0.2-ppm for 96 h. The same individual were treated at a higher a concentration of 0.1 and 0.2-ppm even though there was a short term exposure results a considerable number of decreased RBC. At the same times for a long period, the maximum reduction was observed.

It is evident that the normal blood parameter values of RBC count was 2.80 X 10^6/cmm, WBC 9640 X 10^3/cmm, Hb 10.40 g/100 ml; PCV 30.40%; MCV 108.57 ulX10^9; MCH 37.14 P.gm and MCHC 34.21%. The RBC counts found in the 0.1-ppm copper treated fish after an exposure of 96 h was 2.70 X 10^6/cmm. Later when the fish was introduced at high concentration at 0.2 ppm, its count was 2.60X10^6/cmm with a decrease of 7.14%. When the fish was introduced of choronic exposure period at higher concentration of 0.2 ppm the RBC count was 2.55 x 10^6 cmm with a decrease 8.92%. Like wise, the same results are also true for haemoglobin contents. For a prolonged exposure period of different concentrations of copper medium, the Hb content was decreased in *Channa punctatus* by 6.7% at low concentration and by 9.6% at higher concentration. During the experiments, white blood cell count (WBC) increased more and more at prolonged exposure period. The consistent increase in the WBC indicates that the heavy metal stress certainly stimulates the production of more WBC's at all time of longer exposures.

There are variations in the values of MCV, MCH and MCHC when the fish are exposed to 0.1 and 0.2ppm of copper for 96 h and 21 days (Tables 2 and 3). It has been suggested that enumeration of different cell ratio count provides a useful diagnostic procedure to assess physiological stress in the fish as well as the water quality. There are variations in the values of enzymes and biochemical content when the fish are exposed to 0.1 and 0.2ppm of copper for 96 h and 21 days. The biochemical and enzymes results presented here have clearly demonstrated that the elevated metal ion concentrations for copper substances (Table 4).

Table 1. Effect of different concentration of copper sulphate on mortality (%) of *Channa punctatus* as a function of different exposure time.

S.NO	Concentration of copper (ppm)	Exposure time (h)			
		24	48	72	96
1	0.5	-	-	-	-
2	1.0	-	-	-	-
3	1.5	-	-	-	10
4	2.0	-	-	20	30
5	2.5	-	10	20	40
6	3.0	-	10	30	50
7	3.5	30	40	50	80
8	4.0	40	60	80	100
9	4.5	60	80	90	100
10	5.0	70	90	100	100

Table 2. Effect of sublethal concentration at 0.1ppm of copper sulphate on selected haematological parameters in *Channa punctatus.*

S. No	Blood parameters	Control	Acute 96 h exposure	Chronic 21 days exposure
1	RBC X 10^6/cmm	2.80 ± 0.52	2.70 ± 0.75	2.60 ± 0.59
2	WBC X10^3/cmm	9640 ± 0.12	9790 ± 0.85	9795 ± 0.73
3	Hb g / 100ml	10.40 ± 0.11	10.35 ± 0.76	9.70 ± 0.55
4	PCV %	30.40 ± 0.72	29.50 ± 0.76	28.60 ± 0.85
5	MCV µl X 10^9	108.57 ± 0.12	109.25 ± 0.85	110.00 ± 0.85
6	MCH (Pg)	37.14 ± 0.72	38.33 ± 0.29	37.30 ± 0.11
7	MCHC (%)	34.21 ± 0.75	35.08 ± 0.23	33.91 ± 0.35

Table 3. Effect of sublethal concentration at 0.2 ppm of copper sulphate on selected haematological parameters in *Channa punctatus.*

S. No	Blood parameters	Control	Acute 96 h exposure	Chronic 21 days exposure
1	RBC X 10^6/cmm	2.80 ± 0.52	2.65 ± 0.75	2.55 ± 0.59
2	WBC X 10^3/cmm	9640 ± 0.12	9990 ± 0.85	10110 ± 0.73
3	Hb g / 100ml	10.40 ± 0.11	10.30 ± 0.76	9.40 ± 0.55
4	PCV %	30.40 ± 0.72	29.10 ± 0.76	23.10 ± 0.85
5	MCV µ l X 10^9	108.57 ± 0.12	109.81 ± 0.85	110.19 ± 0.85
6	MCH (P g)	37.14 ± 0.72	38.86 ± 0.29	36.96 ± 0.11
7	MCHC (%)	34.21 ± 0.75	35.39 ± 0.23	33.45 ± 0.35

Table 4. Effect of sublethal concentration at copper sulphate on selected biochemical and enzyme parameters in *Channa punctatus.*

S. No	Parameters	Control	0.1 ppm copper sulphate		0.2 ppm copper sulphate	
			Acute 96 h exposure	Chronic 21 days exposure	Acute 96 h exposure	Chronic 21 days exposure
1	Total protein	24.7	22.3	15.9	16.2	12.8
2	Carbohydrate	10.5	10.2	9.0	9.1	8.2
3	Phosphatase	18.7	16.5	15.8	16.0	14.5
4	Protease	6.8	5.9	5.5	5.4	4.5
5	Lipase	1.08	1.5	1.0	1.2	0.95
6	AST	4.52	3.8	3.2	3.5	2.36
7	ALT	0.44	0.55	0.74	0.68	0.96

DISCUSSION

Heavy metals are present in aquatic environment from mining activities and industries that use these metals in various processes (Lloyd, 1992). Aquatic ecosystems polluted with heavy metals, may therefore threaten human nutrition and health directly. Fish are widely used to evaluate the health of aquatic ecosystems and physiological changes serve as biomarkers of environmental pollution. The survival of aquatic animals depends on not only the biological state of the animals and physico-chemical characteristics of water but also on kind, toxicity, type and time of exposure to the toxicant. In the present study, the mortality increased with an increase in concentration of copper and also the duration of the exposure. Table 1 depicts the percentage mortality for different exposure periods at different concentrations of copper. LC_{50} value of copper for the fish *C. punctatus* was determined.

A capacity of copper sulphate destruction fish and aquatic animals is largely a function of its toxicity, exposure time, does rate and persistence in the environment. Immediately after transfer to the test solution, *C. punctatus* became hypersensitive and showed a rapid of opercular movements accompanied by occasional gulping of air. The higher the concentration, the more pronounced was this behaviour. After several minutes of exposure the individual lost their equilibrium. They were floated with complete cessation of movements and finally dead.

The haematology of fishes has gained recognition, as an applied science. Haematology tests have become important diagnostic tools in medicine. Recent studies have shown that the haematological parameters may be equally valuable, in indicating the disease or the stress in the fish. The composition of blood of fishes varies with the changing conditions of the environment and responds immediately to any change in water quality because of intimate contact. Out of varied haematological parameters differential red blood cells counts are of immense physiopathological importance. In the present investigation, an attempt has been made to elucidate the effects of copper with different sublethal concentration on certain physiological properties of the blood of *C. punctatus*. *Channa punctatus* exposed to sublethal concentrations of copper resulted in a significant decrease in RBC's count leading to anaemia as a result of inhibition of erythropoiesis, haemosynthesis and increase in the rate of erythrocyte destruction in haemopoietic organs. Natarajan (1981) reported a reduction in Hb content. RBC count and PCV values resulting in hypochronic anaemia due to deficiency of iron and decreased utilization for Hb synthesis.

The anaemic condition recorded in the present study could be due to the destruction of mature RBC or inhibition of erythrocyte production. Such decreases in RBC and anaemic suspense have been observed in *Sarotherodon mossambius* after exposure to lethal concentration of sumithion (Koundinya and Ramamurthi, 1979), *Puntius ticto* treated with herbicide (Chouhan *et al*., 1983) and *H. fossilis* exposed to malathion (Lal *et al*., 1986). It is evident that in the present study the reduction in number of RBC and Hb content and decrease in MCV values might have caused microcytic anemia as suggested in *Glossogobicus giuris* after exposure to sublethal concentration of Malathion. Baskaran (1991) reported that a reduction in the number of RBC and Hb content with an increase in MCV and MCHC values might cause macrocytic anaemia in *Oreochromis mossambicus.* Since the Hb and RBCs are oxygen-carrying devices, the quantitative decrease in their levels might have led to the rearrangement of the oxidative metabolism with a concomitant decrease in the tissues of respiratory potential.

The fish facing asphyxia of undergoing physical exercise and facing hypoxic stress required increased amount of Hb to cope with the decreased oxygen availability. This consequently is partially achieved by immediately releasing mire and more cells, which also bring more erythroblasts in circulation. In the present study also, the RBC and Hb content are more or less the same due to the tolerance to short term exposure, at longer exposure period, depletion or reduction of RBC numbers and Hb content was obtained.

The long- term exposure to copper treated fish reduced the red blood cell count and haemoglobin value. This indicates that he high doses of copper produce anaerobic condition and limits the oxygen carrying capacity and there by decrease the mobility. Most of the blood corpuscles were very thin after long-term exposure and the hypochromic cells naturally contained the decreased concentration of haemoglobin. Total leucocytes count showed an increase in their number in the higher concentrations, but at lower concentration, there is a gradual increase in their number. In the present investigation at higher concentration and longer exposure period produces an increase in total WBC and MCHC count as in *G. hiuris* suggested in *Oreochromis mossambicus* after exposure to malathin and Ekalak Ec-25 respectively. Nair *et al.* (2000) suggested that this leucocytosis was the result of direct stimulation of the immunological defenses due to the presence of toxic substance or may be associated by induced tissue damage. A linear relationship was established with respect to heavy metal copper and total leucocytes. The constant increase in the differential count clearly indicates that the copper stress certainly stimulate the white blood cells to produce more at all times of exposure. It has been suggested that the enumeration of differential cell ratio counts provide of useful diagnostic procedure to assess the physiological stress in the fish.

A significant hyperglycaemia was also recorded after exposure to this wastewater e.g. control fish had a mean plasma glucose of 56.80 mg/100 cm3 while the-treated fish exhibited an increase in the levels of plasma glucose to 65.30 and 81.00 mg/100 cm3, respectively. This means that the fish were subjected to some sort of hypertoxic stress

(Ramamurthy *et al.*, 2008). In the present study, tissue biochemicals were generally influenced by this heavy metal that may be attributed to the relative changes in the mobilization of protein and carbohydrates. Changes in the biochemical concentrations may be a result of increased production of metals, which is a sequestering agent. On the other hand, the elevation of enzymes that runs parallel to a decrease in muscle biochemical content may be on indication of a gluconeogenetic response. This additional source of biochemical may support the fish with the required energy highly demanded to manage with the presence of a potentially harmful substances such as copper sulphate.

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