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Acute Toxicity and Ethological Responses of Fish, *Clarias batrachus* (Linn.) Exposed to Copper Sulfate

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Abstract: The present study was undertaken to investigate the acute toxicity of copper in freshwater catfish, *Clarias batrachus* (Linn.). The acute toxicity test was performed according to the standard methods by APHA and the values were calculated by probit analysis. The LC₅₀ values at 96 h was found to be 15.0196 mg/l for copper sulfate. Furthermore, the exposed fish showed behavioural changes such as hyperactivity, loss of equilibrium, rapid swimming, disturbed opercular movements, increased surface activity and loss of equilibrium. Mucus deposition all over the body and gills, damaged fin, colour fading and haemorrhage were found as major morphological alterations. The results indicate that copper is toxic at higher concentration producing dose-responsive increases in mortality and abnormalities in behaviour. The fish species is therefore recommended as good bio-indicator for the risk assessment of aquatic environment due to copper.

Keywords: Copper, LC₅₀, Behavioural responses, Morphological changes, *Clarias batrachus*

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Introduction

Contamination of water by heavy metals are hazardous to aquatic life as they are in direct contact with the contaminated medium. The introduction of many relatively toxic heavy metal cations in small amounts into an aquatic environment causes various changes in the internal dynamic of aquatic organism, even at sublethal levels. Non-degradable heavy metals have tendency for bioaccumulation (Ali *et al.*, 2021). Heavy metal copper belongs to a class of microelements which are essential for proper functioning of the body. The sources of copper in natural waters may be from geological rock

weathering or from human activities such as industrial and domestic wastes water discharges. Fishes are in direct contact with the environment and are relatively sensitive to any change in their surroundings.

The effects of heavy metals have been reviewed by various researchers in the field of fish biology. The range of their stressful effects is quite broad and encompasses behavioural modification, alterations in feeding strategies, swimming activity, schooling, predator- prey avoidance, reproduction, learning, abnormal osmoregulation, respiratory stress as well as tissue damage

(Srivastava and Prakash, 2018). Copper (Cu) is an essential trace metal and micronutrient for cellular metabolism in living organisms on account of being a key constituent of metabolic enzymes. However, it can be extremely toxic to intracellular mechanisms in aquatic animals at high concentrations when it exceeds normal levels. Copper sulfate (CuSO₄) is often used as an algicide, fungicides, molluscicides and insecticides. Fish can accumulate copper via diet or ambient exposure (Sfakianakis *et al.*, 2015). Copper-induced histological alterations are found in the gill, kidney hematopoietic tissue, mechanoreceptors, chemoreceptors, and other tissues (Sorensen, 1991).

Acute toxicity is the severe effect suffered by an organism from short term exposure to toxic substances. LC₅₀ is the estimation of the dose/concentration necessary to kill 50% of a large population of the test animals. Experimentally, this is done by administering a chemical at different doses or concentrations to a group of organisms and then observing the resulting mortalities in a set time periods like 24, 48 72 and 96 h. The LC₅₀ values derived from toxicological investigations are highly useful in determining the sublethal concentration of a metal.

Clarias batrachus is widely distributed fish in Asia. It is extremely popular on account of its tasty flesh, its unparalleled hardness, its rapid growth and high fish market price. It inhabits tropical swamps, lakes and rivers. These fishes possess accessory respiratory organ for air breathing that enable them to tolerate adverse aquatic conditions where other cultivated fish species cannot survive. Therefore, the aim of the present study was to investigate the impact of copper sulfate on the morphology and ethology of fresh water catfish, *Clarias batrachus* exposed to acute concentrations of Copper sulfate for 96 h.

Materials and Methods

Healthy specimens of fresh water catfish, *Clarias batrachus* were collected from local fish farm and

were transported in containers to the laboratory. In the laboratory, the fishes were carefully examined for any injury and then kept in 1% solution of KMnO₄ to get rid of dermal infection. Finally they were kept in large plastic jar containing 50 L of clean tap water and acclimatized for 15 days to the laboratory conditions, during which time they were fed on boiled egg yolk and commercial fish food. The fish (both sexes; weighing 50-55 g; measuring 16-21 cm) were selected for experiments. Water was changed on alternate days. Feeding was stopped 48 h prior to the toxicity test, to minimize the contamination from metabolic wastes.

Stock solution of copper sulfate was prepared by dissolving 3.92 g copper sulfate in 1000 ml distilled water at concentration of 1000 mg/l. The final concentration was recalculated according to the amount of heavy metal ion. Acute toxicity tests were conducted to determine the 96h LC₅₀. Initial range finding experiments were performed by using a screening procedure with five different concentrations of copper sulfate (6, 12, 18, 24, and 30 mg/l). Seven sets of ten healthy fishes each were subjected to serial dilutions of the stock solution of CuSO₄ (9, 12, 15, 18, 21, 24, and 27 mg/l). One set of control group (containing ten fishes) which contains no toxicants was set up. Toxicity test have been performed in accordance with the standard methods given by APHA (2005). Mortality of fishes were monitored at regular intervals throughout the test and dead fishes were immediately removed which did not respond even to strong tactile stimuli. Fish mortality data obtained with respect to time and concentration was statistically analyzed for determination of LC₅₀ values. During the experiment morphological and behavioural alternations were also critically observed. During the experimental period, no death was recorded in the control set up.

Results and Discussion

Acute toxicity:

Static bioassay tests, being an important tool to assess the toxic potential of various toxicants and

sensitivity of various organisms at different trophic level in ecosystem has been widely used for evaluation of toxicity (APHA, 2005). 96 h LC₅₀ value revealed by static bioassay tests is given in Table 1. LC₅₀ values of copper sulfate for 96 h was found to be 15.0196 mg/l. Fishes in the control aquarium were observed to be healthy and normal and no mortality was recorded in it. Present findings indicate that the mortality of test fish was dose- and time-dependent reflecting the regular mode of action which may be due to bioaccumulation of copper causing toxicity that lead to fish death. Within the aquatic environment, at high concentrations, copper exerts adverse effects by accruing structural damage, which affects the growth, development and survival of fish (Turala and Soivio, 1982). This is of great advantage because levels of copper that may probably enter the aquatic bodies in the form of pollutant may not be so high to this level. Thus, fish population may not be affected significantly. However, increased mortality may result due to bioaccumulation resulting from continuous release of copper containing wastes in the water.

Behavioural study:

Behavioural changes observed in the test fishes exposed to different concentrations (6-30 mg/l) of copper sulfate are shown in Table 2. Control fish were active throughout test period and showed normal movement, body balance and activity.

The exposure of fish to different concentrations of Cu showed changes in behavioural responses of test fish (Table 2). In the present study the aquatic surfacing activity and opercular activity (number of beat/ min) of *Clarias batrachus* increased with increasing the concentrations of heavy metal, copper and duration of exposure. Increased opercular activity was also found in *Channa punctatus* after exposure to heavy metal, mercuric chloride (Pandey *et al.*, 2005). Increased opercular beat may be due to the physiological adaption of fish in hypoxic conditions. Similar observations have been reported by Singh *et al.* (2011). The presence of

any toxic material in the medium induces the surfacing activity of aquatic animals due to enhanced oxygen demand for respiration (Haniffa and Augustian, 1989). In *Clarias batrachus*, surface activity increased with increase in concentration within 24 h but decreased thereafter. Increase in surfacing and gulping of surface waters appeared as an attempt to avoid breathing in the poisoned water. The loss of positive rheotaxis is a good indication of any toxic response. As a defense mechanism to neutralize the toxic effect of copper, copious amount of mucus was secreted by the fish gradually which covers the whole body, gill and buccal cavity. Chocking of gills by mucus may also be one of the reasons for frequent surfacing of fishes. Among other factors the PO₂, PCO₂ and swimming space are also responsible to elicit surfacing in fish (Singh *et al.*, 2011). The restlessness and hyperactivity in fish may occur due to the inactivation of acetylcholinesterase, leading to accumulation of acetylcholine at synaptic junctions (Fulton and Key, 2001). In the last phase of poisoning fish lost their balance and hang horizontally, finally they fell on the bottom, leading to death. Thus whole behavioural activity is affected due to the enzymatic system and loss of balance in fishes.

Morphological study:

The morphological changes in fish, *Clarias batrachus* exposed to different concentrations of copper sulfate for 96 h are shown in Table 3.

The copper sulfate treated fishes showed the excessive secretion of the mucus. The blood clotting was observed on the gill and body surface of fish. Many other changes occurred in the body of fish due to exposure of copper sulfate as gill colour changed from dark red to dull red and skin became erupted.

Thus from the present study it may be concluded that air breathing catfish, *Clarias batrachus* is sensitive to copper toxicity and can be used as indicators of copper related stress in the fish on exposure to elevated copper levels in the

Table1: Relationship between concentration of copper sulfate and percentage of mortality of fish at 96 h

S. No.	Conc. (mg/l)	Log Conc.	No. of fish exposed	No. of fish dead (96 h)	Per cent mortality	Probit mortality
1	0	0	10	0	0	0
2	9.0	0.9542	10	1	10	3.72
3	12.0	1.0792	10	2	20	4.16
4	15.0	1.7610	10	4	40	4.75
5	18.0	1.2553	10	7	70	5.52
6	21.0	1.3222	10	8	80	5.84
7	24.0	1.3802	10	9	90	6.28
8	27.0	1.4314	10	10	100	8.03

Table 2: Changes in behavioural parameters in *Clarias batrachus* exposed to different concentration of copper sulfate up to 96 h. OB: Opercular beat; SF: Surfacing; LB: Loss of Balance and MS: Mucus secretions

Dose (mg/l)	24 h				48 h				72 h				96 h			
	OB	SF	LB	MS	OB	SF	LB	MS	OB	SF	LB	MS	OB	SF	LB	MS
Control	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6 mg/l	N	N	N	N	N	N	N	N	N	N	-	N	+	+	-	+
12 mg/l	N	N	N	+	+	+	-	+	+	N	-	+	++	+	-	++
18 mg/l	+	+	N	+	+	+	-	++	++	++	--	++	+++	++	--	+++
24 mg/l	+	+	N	+	+	+	-	++	+++	++	---	++	+++	++	---	+++
30 mg/l	++	++	N	++	++	++	---	+++	+++	+++	---	+++	+++	+++	---	+++

N= normal; - = slightly decreased, -- = mildly decreased, --- = Strongly decreased, + =mildly increased, ++ = moderately increased , +++ = Strongly increased

Fig. 1: Regression line between the Probit mortality of *Clarias batrachus* and log concentration of copper sulfate.

Table 3: Effect of copper sulfate on the morphology of *Clarias batrachus* exposed for 96 h

Parameter (Colour)	Concentration of Copper Sulfate (mg/l)					
	Control	6 mg/l	12 mg/l	18 mg/l	24 mg/l	30 mg/l
Gill	Dark red	Red	Red	Dull Red	Blood Clotted	Blood Clotted
Skin	Normal grey	Grey	Dark Grey	Dull Black	Few black and white patches appears	More black and white patches appears
Fin	Grey	Light grey	Moderate grey	High grey	Grey and wounded	Grey and wounded

water as it is evident from behavioural responses as well as morphological changes.

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