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TOXICOLOGICAL ASSESSMENT OF COPPER SULPHATE ON CHANNA ORIENTALIS AND HETEROPNEUSTES FOSSILIS: LC50 VALUES AND MORTALITY ANALYSIS

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Abstract: The adverse effects of heavy metals, particularly copper sulphate, released from industrial effluents on aquatic life are well-documented. In this study, we determined the LC50 values of copper sulphate for two freshwater air-breathing fish species, Channa orientalis (Bloch) and Heteropneustes fossilis (Bloch). The LC50 values for Channa orientalis were determined to be 33 ppm at 24 hours, 32 ppm at 48 hours, 21 ppm at 72 hours, and 7.5 ppm at 96 hours. For Heteropneustes fossilis, the corresponding LC50 values were 15 ppm at 24 hours, 13 ppm at 48 hours, 9.5 ppm at 72 hours, and 5 ppm at 96 hours. We observed a significant increase in mucus secretion at higher concentrations of copper sulphate as compared to lower concentrations. The potential mechanisms of fish mortality under these conditions are also discussed in detail.

Keywords: Copper sulphate toxicity, LC50 values, Channa orientalis, Heteropneustes fossilis

Introduction

Copper sulphate is widely utilized to control cyanobacteria (blue-green algae) in pond water. These cyanobacterial blooms can cause nocturnal oxygen depletion, elevated carbon dioxide levels, and significant diurnal pH fluctuations. The toxic effluents of the industries, if discharged without proper treatment into the water bodies, can harm the aquatic life. Therefore, it is necessary to know the toxic concentration of various industrial wastes. Employing bioassay methods, a biologist can easily measure the degree of toxicity of any waste, and assess the extent to which it should be diluted or treated, before discharging into the water bodies.

Toxicity is examined by two methods: acute toxicity, which assesses short-term effects, and chronic toxicity, which evaluates long-term effects.

Acute toxicity tests are employed to ascertain the concentration of a harmful substance that

causes a detrimental impact on a particular proportion of fish during a brief timeframe. Typically, acute toxicity is assessed for 24, 48, 72, and 96 hours, with 96 hours being the most appropriate. Mortality, which refers to death, is often a readily identifiable and clearly significant negative outcome. A mortality rate of 50% within a 96-hour timeframe is the most consistent and reliable indicator of the harmfulness of a poisonous substance to a population of test organisms.

Doudoroff et al. (1951) conducted bioassay methods to determine the acute toxicity of industrial contaminants to fish. Henderson and Tarzwell (1957) developed a bioassay method to regulate the impact of industrial effluents. Several researchers have conducted bioassay tests to assess the LC50 values of various substances, including herbicides, insecticides, fertilizers, and other chemicals, specifically about fish, like Arora et al., (1972); Anees, (1975); Ansari, (1981); and Prasad (2013 & 2015).

Acute toxicity studies are valuable because they yield prompt and meaningful data within a brief timeframe, allowing for the easy maintenance of the parameters being studied. In recent years, the use of LC50 values has increased as a method to measure the toxicity of toxicants and assess their impact on organisms over a specified time frame.

Although several methods have been adopted by various workers for the bioassay of toxicants but static bioassay method was performed by the majority (Doudoroff et al.,1951; Henderson et al. 1957, Wong et al., 1977; Prasad and Sahani, 2024). An attempt has been made to determine the LC50 values of copper sulphate for the fishes Channa orientalis (Bl.) and Heteropneustes fossilis (Bl.). This particular chemical has been chosen for the study because it is used in huge quantities in various industries.

Methods

Healthy adult specimens of *Channa orientalis* and *Heteropneustes fossilis* were collected and identified according to Shrestha (1981). The length and weight of *C. orientalis* ranged from 14.5 to 16.8 cm and 35 to 50 grams, respectively, while *H. fossilis* ranged from 9.4 to 11.3 cm in length and 10.5 to 12.5 grams in weight. The fish were treated with a 0.1% potassium permanganate ($KMnO_4$) solution to eliminate any potential infections. After receiving this treatment, the fish were acclimatized for a period of fourteen days in a laboratory setting, placed in glass aquaria that were twenty litres in capacity and supplied with tap water. They were fed with fish food daily, but feeding was stopped 24 hours before the beginning of the experiments. Table 1 provides an extensive summary of the tap water's physico-chemical parameters.

Table 1

Parameters	Values
Temperature	21° to 24°C.
Dissolved oxygen	8.5 to 8.7 ppm
Water hardness	65 to 70 ppm.
pH	7.2 to 7.5

Reagent grade B. D. H. copper sulphate was dissolved in distilled water to create a stock solution. To prevent precipitation, five drops of 50% HCl were applied. The stock solution was diluted appropriately to achieve the necessary concentrations, following the steps outlined in Standard Methods (APHA, 1971).

1. **Range-finding Test:** This is utilised to ascertain an approximate range for the conclusive testing. To accomplish this goal, a defined quantity of acclimated fish are subjected to varying levels of copper sulfate, and the optimal range is identified.
   [A] ***C. orientalis***: Five solutions with varying concentrations of copper sulphate (1, 25, 50, 100, and 150 ppm) were chosen. Each solution was used to introduce five independent groups of five fish. As a control, the sixth group was set aside and submerged in tap water. Every 24 hours, the fish were shifted to fresh test solutions while tap water was used as a control.

[B] ***H. fossilis***: Five solutions with varying concentrations of copper sulphate (1, 10, 40, 80, and 100 ppm) were chosen. Each solution was used to introduce five groups of five fish individually. As a control, the sixth group was kept submerged in tap water. Every 24 hours, the fish were shifted to fresh test solutions, while tap water was used as a control.

Table 2

Range-finding test of copper sulphate on *C.orientalis* and *H. fossilis*

Name of the fish	Number of fish	Concentration (in ppm)	Mortality (in %)	Selected Range
C. orientalis	5	1	Nil	1 ppm to 50 ppm
	5	25	60	
	5	50	100	
	5	100	100	
	5	150	100	
H. fossilis	5	1	Nil	1 ppm to 40 ppm
	5	10	60	
	5	40	100	
	5	80	100	
	5	100	100	

2. **Definitive Test:** The range finding test (Table 1) identified an appropriate range of 1-50 ppm. No deaths were seen at a concentration of 1 part per million (ppm), however, all fish perished within 96 hours at a concentration of 50 ppm of copper sulphate.

   [A] ***C. orientalis***: To determine the LC50 values of copper sulphate on *C. orientalis*, ten test solutions with variable concentrations (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 ppm) were created. A total of 220 fish that had adapted to the new environment were separated into eleven groups, with each group including 20 fish. Subsequently, each cohort of 20 fish was individually exposed to the aforementioned concentrations. A control group (the 11th group) was set aside in tap water. Every 24 hours, the fish were transferred to fresh test solutions while tap water was used as a control.

   [B] ***H. fossilis***: The range finding test (Table 2) identified an appropriate range of 1-40 ppm. No deaths were seen at a concentration of 1 part per million (ppm), however, all fish perished within 96 hours when exposed to a concentration of 40 ppm of copper sulphate solution.

To determine the LC50 values of copper sulphate on *H. fossilis*, ten test solutions with varying concentrations (2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 ppm) were created. A total of 220 fish that had adapted to the environment were separated into eleven groups, with each group including 20 fish. Subsequently, each cluster of 20 fish was individually exposed to the aforementioned concentrations. A control group (the 11th group) was set aside in tap water. Every 24 hours, the fish were shifted to fresh test solutions, while tap water was used as a control.

The LC50 values were determined by graphing the concentrations of a substance against the percentage of fish death.

Result and Disscusion

When the fishes, *C. orientalis* and *H. fossilis*, were exposed to test solutions containing varying concentrations of copper sulphate, they exhibited signs of excitement, including increased opercular movements, frequent swimming, and surfacing to swallow atmospheric air. However, after a few

timeframe, all the fish exhibited decreased activity and eventually rested at the bottom, accompanied by a significant increase in the production of mucus. The mucus discharge augmented according to the escalating concentration of the test solutions. After the fish died, they sank to the bottommost and then floated to the top, exhibiting no response to physical contact.

Range-finding Test

[A] *C. orientalis*: The range was found suitable from 1-50 ppm (Table 2).

[B] *H. fossilis*: The range was found suitable from 1-40 ppm (Table 2).

Definitive Test

[A] *C. orientalis*: For 24, 48, 72, and 96 hours, the LC50 values of copper sulphate were determined to be 33, 32, 21, and 7.5 ppm respectively. (Fig. 1).

[B] *H. fossilis*: For 24, 48, 72, and 96 hours, the LC50 values of copper sulphate were determined to be 15, 13, 9, and 5 ppm respectively. (Fig: 2)

Discussion:

Upon introducing the test-fishes into the test solutions, they exhibited signs of excitement, with an increase in opercular movements, frequent swimming, and a tendency to come to the top to suck atmospheric air. Fish subjected to dinitrophenol showed an increase in their oxygen intake and metabolic rate of respiration, as noted by Hall *et al.* (1933), and Ambrose (1959). In addition, they noted the swift flow of blood that resulted in the fish's quick movements. Verma *et al.* (1980) have similarly noticed similar alterations in fish behaviour when exposed to pesticide and detergent solutions. Srivastava and Srivastava (1977) have shown that urea causes irritation and increased swimming activity in *C. orientalis*. These are likely the key factors driving the fast movements of *C. orientalis* and *H. fossilis* fish in copper sulphate solutions.

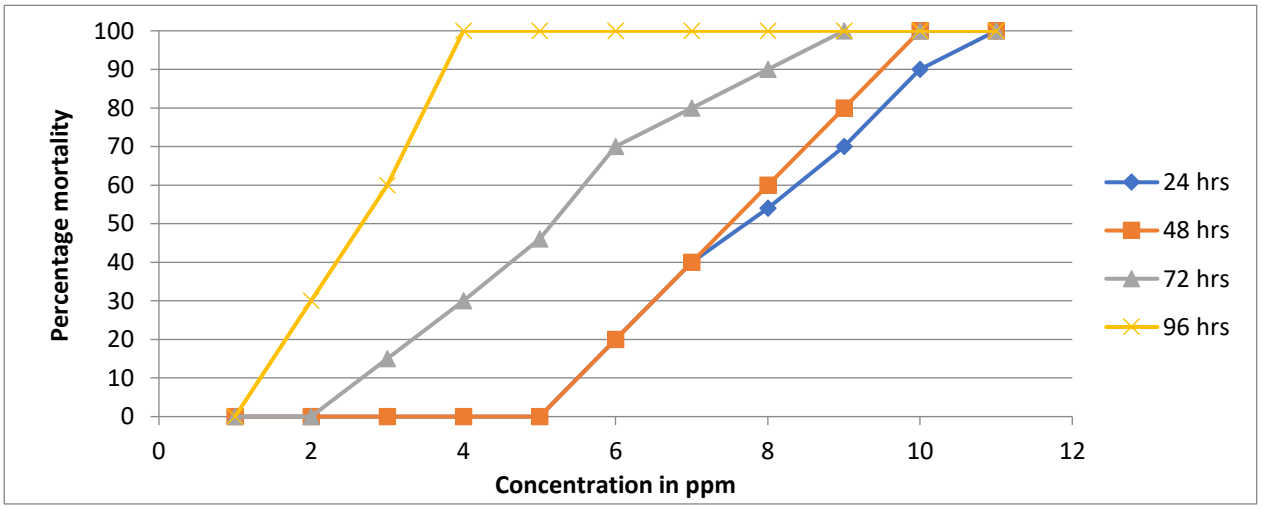

Figure 1. LC50 values of copper sulphate for C. orientalis.

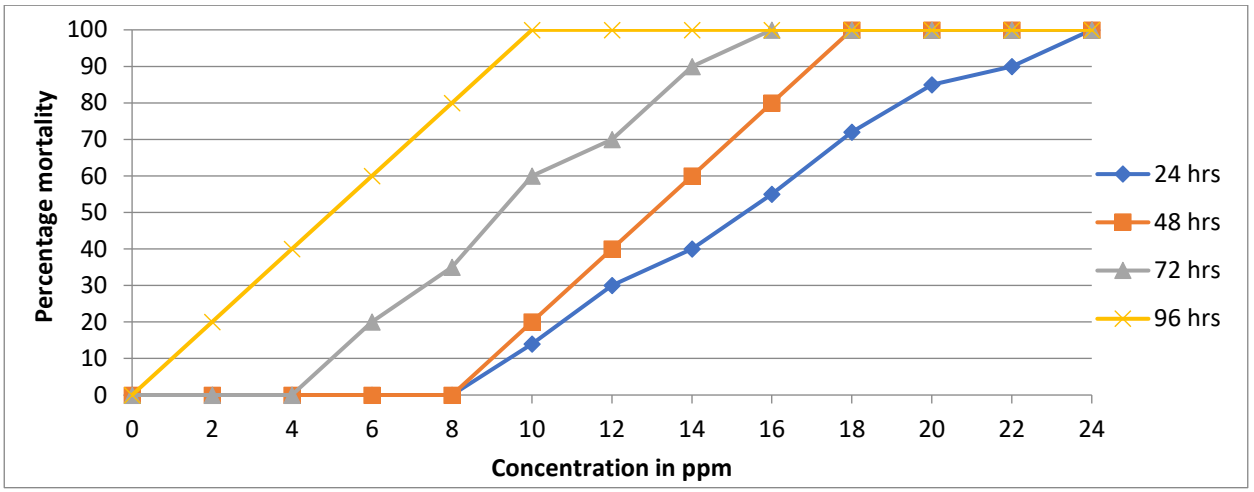

Figure 2. LC50 values of copper sulphate for H. fossilis.

The mucus is produced in large amounts. It forms a protective coating around the gills, inside the operculum, and on the body. Previous studies have yielded comparable findings for heavy metals, particularly Kudesia (1980) for Cr (III) and Srivastava (1979) for urea toxicity. The reaction between copper sulfate and the mucus-secreting gland cells in the gill epithelium triggers the production of mucus. This resulted in the inflammation in these glands. The suffocation was caused by a thick film of mucus blocking the fish's gills, which made it go to the surface to breathe in air from the atmosphere (Prasad, 2019). Earlier research, such as the investigations conducted by Srivastava *et al.* in 1979 and Carpenter in 1930, have also shown comparable results about the harmful effects of lead and urea. In addition, the presence of mucus can form a dense layer that obstructs the osmoregulatory functions of the fish's body. This is the probable cause for the mortality of fish when they are exposed to solutions containing copper sulfate.

Afterwards, the test fishes displayed lethargy, sank to the bottom, and ultimately died. According to Abel (1974), the fish perished during acute toxicity experiments as a result of many mechanisms of action. He further suggested that the mortality was probably due to alterations in membrane permeability, protein denaturation, and interference with active transport as a consequence of the detrimental impact of chemicals. It is plausible that the fatality occurred due to the same causes in this research. According to Wong *et al.* (1977), death was largely attributed to asphyxiation resulting from the interaction between heavy metal ions and certain components of the mucus produced by the gills. In addition, death was also ascribed to the blockage of metabolism.

The LC50 values of copper sulphate for *C. orientalis* were determined to be 33, 32, 21, and 7.5 ppm after exposure periods of 24, 48, 72, and 96 hours. The results for *H. fossilis* were correspondingly 15, 13, 9.5, and 5 ppm for 24, 48, 72, and 96 hours. Therefore, it is obvious that copper sulphate exhibits more toxicity towards *H. fossilis* compared to *C. orientalis*. Mount and Stephan (1969) documented that the 96-hour LC5o value of copper sulphate for *Pimephales promelas* in soft water is 84 mg Cu/L. Brungs *et al.* (1978) reported that the 96-hour LC50 value of copper for fathead minnow is 0.60-0.98 mg/L. In their study, Solbe and Cooper (1976) observed that for stone loach in hard water, the 96-hour LC50 value of copper sulphate is 0.49 mg/L. The present investigation established that the 96-hour LC50 values of copper sulphate for *C. orientalis* and *H. fossilis* were 7.5 ppm and 5 ppm, correspondingly. Thus, it becomes clear that the LC50 values are subject to fluctuation depending on the species

Conclusion

This study highlights the acute toxicity of copper sulphate on two freshwater fish species, Channa orientalis and Heteropneustes fossilis. The LC50 values indicate higher sensitivity of H. fossilis to copper sulphate compared to C. orientalis. Behavioural changes such as increased opercular movements, heightened swimming activity, and excessive mucus secretion was observed, which intensified with increasing concentrations of copper sulphate. These responses suggest significant

physiological stress and impaired respiratory functions due to the toxic effects of copper sulphate. The findings underscore the need for stringent regulation and treatment of industrial effluents containing copper sulphate to mitigate its harmful impact on aquatic ecosystems. The species-specific variations in LC50 values also emphasize the importance of tailored environmental guidelines to protect diverse aquatic species from heavy metal toxicity.

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