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# Determination of Lethal concentration (LC50) of copper sulphate in Labeo Rohita.

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**ABSTRACT**
The Present study determination of (LC50 Values) acute toxicity of Indian major cart *Labeo Rohita* obtain the test organisms 160 Fish fingerlings from the Lake irrespective of sex with the help of local Fisherman, the fish divided into four groups to maintain the aeration all the daily observed and dead fishes removed immediately fishes were rinsed with 0.1% and 4k Mn o4 to avoid infection and were acclimatized to two weeks for laboratory prior to experiments 8 experimental groups (with 3 replicates) and experimental dose of copper sulphate 20mg/L, 40mg/L, 60 mg/L, 80mg/L, 100mg/L, 120mg/L, 140mg/L, 280mg/L along with control (0 mg/L). Physicochemical parameters such as pH (7.2), Turbidity, Biological Oxygen demand (BOD)(3.17), Chemical Oxygen Demand (COD)(3.47), Hardness(240.75), Alkalinity(80.25). The LC50 value at 96 hr was found to be 52.04 mg/L to *Labeo rohita*. Copper concentration was more toxic to *Labeo rohita*.

**Keywords**: LC50, Toxicity, CuSo4. 5H2o, *Labeo rohita*

## 1.INTRODUCTION:

Heavy metals are natural environmental components and considered potential marine pollutants. Large quantity of these heavy metals accumulated as a result of land based activity in the aquatic ecosystem. [Javed et al., 2017, Shah et al., 2020.] Now-a-days heavy metal residues have became a matter of serious concern because of their continuous increase I air and aquatic environment (Abah et al., 2016, Javed and Usmani 2019).
The contamination of aquatic system with heavy metals is regarded to be dangerous not only for aquatic fauna but also for the human as the consumer of fish for food (Sabullah et al., 2015).
The Lake located in Patancheruvu, North, Western of Hyderabad covers area 17.53 N 78.27 o E, part of the catchments of Nakkavagu stream, a tributary of the Manjeera river. The bulk drug manufacturing industries one of the main polluters in the cluster. The lake water polluted by untreated industrial effluents from the estates are let out into the open ground or local streams. Water sample collected from the 9:00 AM to 10:00 AM.
Fish Fingerlings from the local fisherman.Fresh water and marine fish aquaculture are sources of food nutrition, income and livelihood for communities around the world(Luis et al., 2019 Carraschi and cruz 2019) Malheiros et al., 2020 Copper sulphate (CuSo4 )has been used as a therapeutic to reduce infections caused by parasites in fish aquaculture (Owarati et al., 2020). Copper sulphate chemical agent which acts as an algicide and fungicide and is used globally in agriculture and aquaculture. (Moore et al., 1905). Copper sulphate dissolved in water splits into copper and sulphate ions . Ionic copper plays an important role in cellular metabolism by comprising apart of the active sides of many proteins (Waiwood et al., Afaghi et al., 2020).
Several studies have demonstrated that the acute toxicity of copper sulphate decreased with increased exposed time (Dalahaut et al., 2020).

**Fig 1. Labeo rohita collection site in Sakhi lake of Patancheruvu**

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## 2.MATERIALS AND METHODS:

Sample Collection: Healthy fish **(Figure 1)** of *Labeo rohita* (weight: 12.0 ± 1.5 g, length: 6.2 ± 0.5 cm) were collected from site in sakhi lake of Patancheruvu, Hyderabad. Then, 5 L capacity oxygenated bags were used to transport from sakhi lake of Patancheruvu to the Department of zoology, University College of Science, Osmania University-Hyderabad, Telangana.

### 2.1 Fish Collection and acclimatization:

*Labeo rohita* of same age group fingerlings procured from the Sakhi Lake Sangareddy and were acclimatized in the tank with 1000 Litres water capacity 10 to 15 days to the experiment. The Physicochemical parameters of the table shown in table 1. During acclimatization fish were fed with artificial feed pellets twice daily 2 to 3% of body weight.

### 2.2 Experimental fish and management

This study was conducted at Department of Zoology, University college of science, Osmania University. *Labeo rohita* (12.0 ± 1.5 g ) were collected from fishponds and acclimatized in cemented tanks (294 cm X 90 cm X 87.3 cm) with flow through aerated water for seven days (Hunn et al., 1968). After acclimatization, a total of 60 fish with similar weights (12.0 ± 1.5 g) were randomly selected and shifted into glass aquaria filled with 200 L of water. Fishes were distributed equally (n = 10) into four aquaria (Control and T1, T2, T3,T4, T5, T6 T7 and T8 ) with three replicates each. The whole experiment was conducted under controlled laboratory conditions (USEPA, 2002) for 4 days. The Animal Care Committee Review Board at the Department of zoology, Osmania University, Hyderabad, Telangana state was granted ethical approval.

### 2.3 Stock Solution Preparation and LC50 Determination for Copper sulphate ($CuSO_4$)

Research-grade Copper sulphate ($CuSO_4$) (Mumbai, India) was collected from a local distributor. LC50 was determined according to the experiment conducted in an earlier study. Before starting the acute test, a stock solution of copper sulphate ($CuSO_4$) was prepared by adding 10 g of Copper sulphate ($CuSO_4$) to 1000 mL of distilled water. Then, an experiment was performed with different concentrations of metals (Copper sulphate ($CuSO_4$): 0, 20, 40, 60, 80, 100, 120, 140 and 280 mg/L) to identify the LC50 of 96 h. The stocking density of fish was 10 individual fish per 40 L of water in aquaria (12 inch × 8 inch × 8.5 inch) and the weight and length of fish were 12.0 ± 2.5 g and 6.2 ± 0.5 cm, respectively. Fish mortality for each concentration was documented at logarithmic time intervals, namely at 6, 12, 24, 48, 72 and 96 h of fish exposure. During the experiment, feed was given daily two time to fish; only aeration was provided in each tank.

### 2.4 Experimental Design for Exposure to Copper sulphate

Based on the LC50 value for 96 h of Copper sulphate ($CuSO_4$), a total of three experimental groups were selected: treatment 1 (T1)—20mg/L; treatment 2 (T2)—40 mg/L; treatment 3 (T3)—60 mg/L; treatment 4 (T4)—80 mg/L , treatment 5 (T5)—100 mg/, treatment 6 (T6)—120 mg/L., treatment 7 (T7)—140 mg/L and treatment 8 (T8)—280 mg/L. A negative control group (0.0 mg/L) was maintained simultaneously. In every group, we randomly selected 10 healthy fish, which were placed in 40 L water in glass aquaria. Each group of treatments was maintained in triplicate and fish were fed daily, two times, at the rate of 3% of their body weight. On every alternate day, 50% of water in each aquarium was siphoned to remove wastes, feed and faeces of fish. Subsequently, the same volume of water was added containing the assigned amount of Copper sulphate ($CuSO_4$). The exposure experiment lasted for 4 days and the physicochemical properties of the water, such as temperature, DO, pH, salinity, conductivity, etc., were recorded carefully to maintain the optimum quality of water for fish culture.

### Test Media:

Analytical grade copper (CuSo4, 5H2O) was used for the preparation of stock solutions that was diluted as desired. Fish were exposed for 96 hours, separately, against different concentrations of copper starting from (50.04, 51.04, 52.04, 53.04, 54.04 and 55.04 mg/L) with an increment of 1.04 mg/L for low to high concentrations, respectively.

### Acute Toxicity Test:

The experiment was carried out at stocking density of 10 fish/ aquarium. Concentration of each test media was increased gradually, level of metal concentration was maintained to 50% of toxicant concentration, while full toxicant concentrations were attained in 96-hr of exposure.

### Collection of Mortality Data:

*Labeo rohita* Fish mortalities were recorded at 24, 48, 72 and 96-hr of exposure, and dead fish were removed immediately from the test media.

### Morphological Observation

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The morphological changes or any abnormalities in exposed fish were also observed and documented throughout the culture period. Photographs of abnormalities and few morphological changes in fish were captured using a Samsung Galaxy camera.

**Statistical analysis**

All the data were analysed by one-way Analysis of Variance (ANOVA). For comparison of the mean among parameters, Duncan's Multiple Range Test (IBM SPSS version 26) was applied at $p < 0.05$ (Steel et al., 1996).

## 3. RESULT

### 3.1 Physicochemical Parameters of Culture

Different physicochemical parameters of the culture water were recorded to ensure optimum water quality for fish culture (Figure.2 and Table.1) and treatment (Table. 2) presents the properties of the water during acclimatization, LC50 determination and final exposure treatment. Almost all parameters indicated suitable conditions for fish culture. Considerable physicochemical parameter differences were not noticed during acclimatization, LC50 determination and the final exposure treatment period.

**Table 1: Physical and chemical parameters of the test water**

| S.No | Parameters | Values of test water |
|---|---|---|
| 1 | pH (Electrometric method) | 7.2 |
| 2 | Temperature (0 C) (Field method) | 23.7 |
| 3 | Dissolved Oxygen (mg/L) | 3.175 |
| 4 | Total Hardness CaCO3 (mg/L) (EDTA titration method) | 240.75 |
| 5 | Alkalinity (mg/L) (Titration method) | 80.25 |

**Table 2: LC50 value of *Labeo rohita* exposed to different concentrations of CuSo4 for 96 hours**

| S. No | Concentration (CuSo4) | Log 10 Concentration | No of fish Exposed | No of fish died at 96hr | No of fish live | Percentage Kill (%) | Probit Analysis | LC50 (mg/L) |
|---|---|---|---|---|---|---|---|---|
| 1 | 20mg/L | 1.301029996 | 10 | 0 | 10 | 0 | 0 | 52.04mg/L |
| 2 | 40mg/L | 1.602059991 | 10 | 2 | 8 | 20 | 4.16 | |
| 3 | 60mg/L | 1.77815125 | 10 | 5 | 5 | 50 | 5 | |
| 4 | 80mg/L | 1.903089987 | 10 | 6 | 4 | 60 | 5.25 | |
| 5 | 100mg/L | 2 | 10 | 7 | 3 | 70 | 5.52 | |
| 6 | 120mg/L | 2.079181246 | 10 | 8 | 2 | 80 | 5.84 | |
| 7 | 140mg/L | 2.146128036 | 10 | 10 | 0 | 100 | 8.09 | |
| 8 | 280mg/L | 2.447158031 | 10 | 10 | 0 | 100 | 8.09 | |

**Figure 2. *Labeo rohita* exposed to different concentrations of CuSo4 for 96 hours**

### 3.2 Cumulative Mortality and Determination of LC50

The cumulative mortality in percentage for Labeo rohita during the 96 h experiment is shown in **(Figure 2).** Mortality was increased gradually with the increasing concentration of CuSo4. No mortality was observed in control fish but up to 0, 20, 50, 60, 70, 80, 100% mortality was recorded at the 20, 40, 60, 80, 100, 120, 140 and 280 mg/L concentration of

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CuSo4. Moreover, 90% percent mortality was calculated at 120 mg/L exposure of CuSo4. Finally, 100% mortality occurred at the140mg/L and 280 mg/L concentration of CuSo4. From the data of 96 h mortality, the lethal concentration (LC) value was calculated using probit analysis through SPSS software. The calculated LC50 values for the 96 h study were 52.04 mg/L, respectively **.**

**Figure 4: Concentration of CuSO4 vs Percent kill( %)**

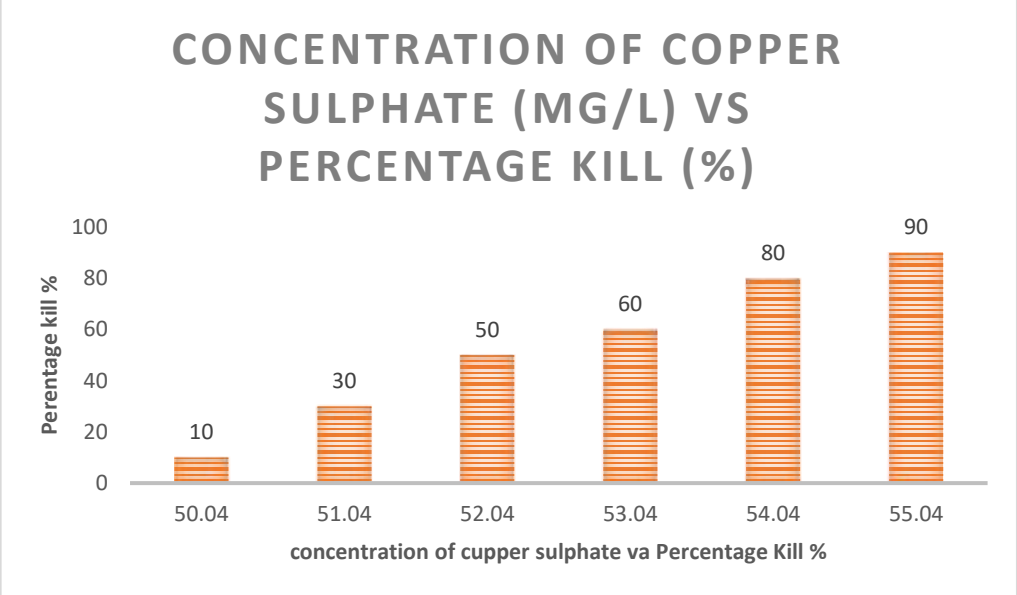
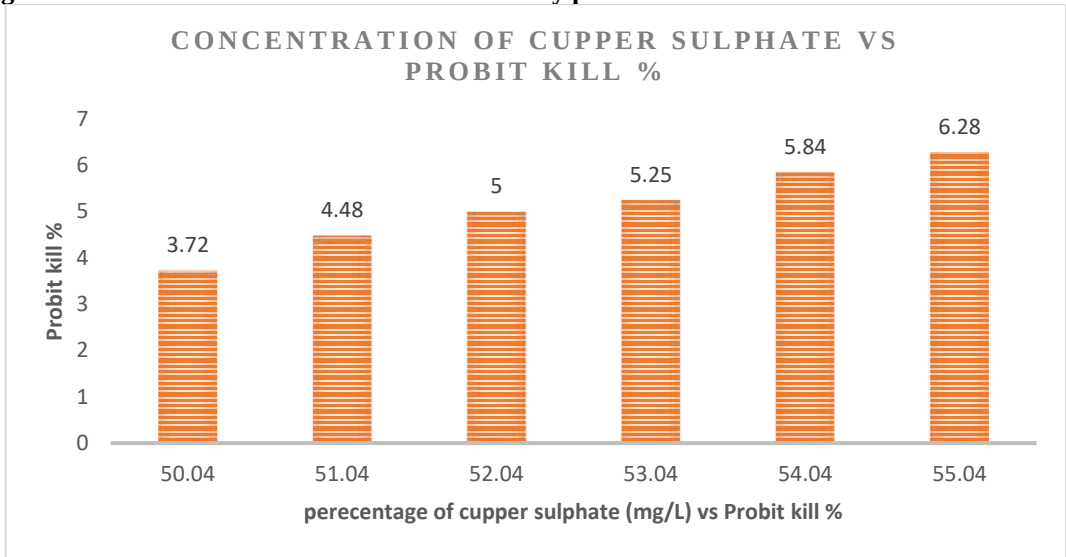



**Table 4: LC50 value of *Labeo rohita* exposed to different concentrations of CuSo4 for 96 hours**

| S.No | Concentration (CuSo4) | Probit Analysis |
|---|---|---|
| 1 | 50.04 | 3.72 |
| 2 | 51.04 | 4.48 |
| 3 | 52.04 | 5 |
| 4 | 53.04 | 5.25 |
| 5 | 54.04 | 5.84 |
| 6 | 55.04 | 6.28 |

**Figure 5. The calculated LC50 values for 96 h study period and The median LC50 value of CuSo4**




**4. DISCUSSION**

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Discussion In the present investigation, the 96 hr LC50 value for copper was found to be 52.04 mg/L. Results of present studies **(Table-1,2),** Clearly indicate that the rate of mortality for any fixed time increases with increase in concentration and for a particular concentration with increase in exposure time and a regular mode of action of toxicant, due to accumulation up to dangerous level leading to death. Another contributing factor causing death may be due to the damage of the gills by the heavy metal (Khangarot, 1982; Nilkaht, Sawant, 1993) . Toxicity studies measure a response of an organism to a biologically active substance (Alderdice, 1966) and are useful in determining water quality. The wide variation in sensitivity of different species to different heavy metals depends on various factors like age, sex, weight, physical stage of the animal and presence or absence of enzyme system that can degrade the pollutants . Toxicity testing is an essential tool for assessing the effect and fate of toxicants in aquatic ecosystem . The major cause of mortality might be due to respiratory epithelium damage by oxygen culmination during the formation of a mucus film over the gills of fish . The 96 hr LC50 test were conducted to measure the susceptibility and survival potential of animals to particular toxic substance such as copper. It was found that there was positive relationship between the mortality and concentration levels; when concentration level increased, the mortality rate increased as well. found that environmental conditions such as oxygen concentration, temperature, total hardness, alkalinity and presence of other metals influence toxicity levels to the fish. Increases in water temperature can enhance the uptake of metals by the aquatic organisms reported that toxicity of copper metal decrease significantly with increasing water hardness. Similar types of results were also observed by Straus (2003) using copper exposed fingerling of *Oreochromis aureus* revealed that toxicity increases with a decrease in total alkalinity.

In general, water hardness is found by reducing metal toxicity to fish. Rathore, Changaret, (2003) found that the toxicity of the mercury chloride decreased with increasing water hardness. The 96h LC50 value for copper was higher in the present study than values available in the earlier studies; the reason might be due to high hardness (120mg/L). If soft water (75mg/L as CaCo3) (Shushaimi-Othman, 2010). In the present study the water hardness was 223 mg/L and the pH (7.4). The characteristics hardness and pHof the test water were high in present study. Khangarot et al., (1985) had report that the acute toxicity to the common carp fry(Cyprinus carpio) decreased with increasing pH5.5-8.5. It was found that at low pH mercury was more toxic compared to other pH, which might be due to acid toxicity itself causing bicarbonate loss in the body fluid (Das, Sahu, 2005). At low pH, metals are usually in their most bio available form as monovalent or divalent cations. In this way ameliorating effect of low pH was attributed to H+ competition with metal ions at gill surfaces (Pyle et al., 2002). It seems that two factors, water hardness and pH levels, could effect the acute toxicity of copper sulphate on the fish *Sarotherodon mossambica.*

Mortality was also related to the retention time of the CuSo4 in the water more than the mortality rate of the fish. At the first 24 hr, more of the CuSo4 was taken up by the fish and its concentration decreased in water. In other words, the mortality rate of fish decreased as the time of toxicity exposition increases (Ebrahimpour et al., 2010).

## 5. CONCLUSION

In the present investigation the test species, *Labeo rohita* has shown differential toxicity level with the function of period. This shows that the more is the duration period the less is the concentration required. The observed percentage mortality and probit mortality of *Labeo rohita* in presently studied fish *Labeo rohita* confirm that Toxicity evaluation (LC50values) and behavioural changes in fishes are very sensitive indicators under toxicity of chemicals. The behavioural changes affecting the general health status of the fish. Therefore, the results of the present investigation might be applied as biomonitoring program guidelines for fish populations cultured in heavy-metal-polluted water.

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