Determination of lethal concentration (LC₅₀) of copper to *Sarotherodon mossambica*

**P Pandari Reddy, R Jagadeshwarlu, G Sunitha Devi**

**Abstract**

The present study was conducted to evaluate the acute toxicity of copper to *Sarotherodon mossambica* by static bioassays. The average weight and length of fish used in the present investigation were 23-25 g and 12-13 cm, respectively. pH, Temperature, Dissolved Oxygen, Total hardness, Alkalinity of test water was measured daily. The five groups of fishes were exposed to the wide range of copper with different concentrations of 56, 57, 58, 59 and 60 mg/L. All the exposed fishes were daily observed and dead fishes were removed immediately. The mortality was recorded on daily basis. The LC₅₀ value at 96 hr was found to be 58 mg/L to *Sarotherodon mossambica*. The data obtained were statistically evaluated using Finney’s probit analysis method. Survival time decreased with increasing concentration of copper. Copper concentration was found to be more toxic for *Sarotherodon mossambica*. Further study needs the processes by which these chemicals affect Haematological and Biochemical changes of the fish.

**Keywords:** LC₅₀, Copper, Toxicity, *Sarotherodon mossambica*.

1. **Introduction**

In present day’s industrial development is increasing and industrial effluents containing heavy metals, entering into aquatic environment causes biochemical disturbances in fishes. Heavy metals may affect organisms directly by accumulating in their bodies or indirectly by transferring to the next trophic level of the food chain (Shah, Altindag, 2004) [31]. Copper is a biologically essential heavy metal that occurs naturally, because of its abundance and availability, Cu was one of first metals to be worked by humans 7000 to 8000 years ago (Schroeder et al., 1966). Among heavy metals, copper plays a vital role in normal physiological regulatory functions of cardiovascular and nervous systems (Rai AN, Ullah A, 2015) [32]. Copper is also an integral part of various enzymes and it protects cells against destruction by oxidation (Hogstrand and Haux, 2001) [11]. Input of Cu into aquatic systems is primarily the results of industrial discharges from metal mines, smelters, municipal sewage, and agricultural pesticides and fertilizers (Eisler1998a) [6].

Heavy metals that reach the aquatic bodies deteriorate the quality of life sustaining water and cause damage to both flora and fauna (Nriagu and Sprague, 1987; Mason, 1996; Kotsanis and Georgudaki, 1999; Zydah and Abdel Bakey, 2000; Geogudaki and Kotsanis, 2001; Verma et al., 2005) [23, 20, 18, 29]. Both flora and fauna including human beings have suffered a loss on account of copper pollution of water resources throughout the globe(Dharam Singh, Kamlesh Nath, Trivedi SP,2008) [33].

Copper sulphate is widely used as an algicide for controlling phytoplankton in fish ponds and lakes as well as a herbicide, used in aquatic weed control since1882 (Effler et al., 1980) [7].

Fish are the simple and reliable biomarker of copper pollution of aquatic bodies (Taylor et al., 2000; Lodhi et al., 2006) [28, 19].

2. **Materials and Methods**

The present investigation was conducted in research laboratory, Department of Zoology, University college of Science, Osmania University, Hyderabad. The acute toxicity of copper for *Sarotherodon mossambica* was determined in terms of 96-hr LC₅₀ and lethal concentrations.

**Fish Collection and Acclimatization**

*Sarotherodon mossambica* of same age group were procured from the Fish Seed Hatchery, Vijayawada and were acclimatized in the tank with 1000 L water capacity for one week prior.
to the experiment. The water used for the present experiments was unchlorinated tap water and the physico-chemical parameters of water are shown in the table-1. During acclimatization, fish were fed with artificial fish feed pellets twice daily.

**Physico-chemical Parameters**
The experiment was conducted in glass aquaria with 30 liters of water capacity. Prior to the start of the experiment, it was assured that all aquariums were properly washed with distilled water to remove any sort of impurities and dust particles. Acclimated fish were not fed 24-hr prior to the start of the experiment and throughout acute toxicity tests. The investigation was performed in controlled laboratory conditions at pH (7.4), Temperature (29 °C) and Total hardness (223 mg/L). All the physico-chemical parameters, Temperature (Field method), pH were recorded individually in each test container at exposure times of 24, 48, 72 and 96 hr. Dissolved oxygen (Wrinkler’s method), Alkalinity (Titration method), Total Hardness (EDTA titration method), were performed by following APHA (2009)[2] on daily basis.

**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 (56, 57, 58, 59 and 60 mg/L) with an increment of 1 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 7-hr of exposure.

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

**Statistical Analysis:** Finney’s Probit analysis method was used to calculate the 96-hr LC₅₀ with SPSS Statistical Software.

### 3. Results
The physicochemical properties of the test water were shown in table-1.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Values of test water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH (Electrometric method)</td>
<td>7.4</td>
</tr>
<tr>
<td>2</td>
<td>Temperature (°C)</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>Dissolved Oxygen (mg/L)</td>
<td>8.2</td>
</tr>
<tr>
<td>4</td>
<td>Total Hardness CaCO₃ (mg/L)</td>
<td>223</td>
</tr>
<tr>
<td>5</td>
<td>Alkalinity (mg/L)</td>
<td>250</td>
</tr>
</tbody>
</table>

The LC₅₀ value for Copper sulphate, calculated by Finney’s probit analysis method and SPSS Statistical Software at 96 hours of exposure was shown in table-2.

### 3.2 Table 2: LC₅₀ value of *Sarotherodon mossambica* exposed to different concentrations of CuSO₄ for 96 hours

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration of CuSO₄(mg/L)</th>
<th>Log Concentration</th>
<th>No. of Fishes Exposed</th>
<th>No. of Fishes died at 96hr</th>
<th>Probit Kill%</th>
<th>Percent Kill%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56</td>
<td>1.75</td>
<td>10</td>
<td>1</td>
<td>3.72</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>1.76</td>
<td>10</td>
<td>3</td>
<td>4.48</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>58</td>
<td>1.76</td>
<td>10</td>
<td>5</td>
<td>5.00</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>59</td>
<td>1.77</td>
<td>10</td>
<td>7</td>
<td>5.52</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>1.78</td>
<td>10</td>
<td>9</td>
<td>6.28</td>
<td>90</td>
</tr>
</tbody>
</table>

The observed percentage of mortality of *Sarotherodon mossambica* for copper in static tests continuous for different hours and different concentrations were shown in table-2 and Figure-1. Displaying the probit line graph of the CuSO₄ toxicity data and probit kill.

![Fig 1: Graph of Concentration of CuSO₄ vs Probit kill](image)

The LC₅₀ value for Copper sulphate, calculated by Finney’s probit analysis method and SPSS Statistical Software at 96 hours of exposure was shown in table-2.

![Fig 2: The median LC₅₀ value of CuSO₄](image)

Figure-2 shown the median LC₅₀ value of Copper sulphate for *Sarotherodon mossambica* which was found to be 58 mg/L by Finney’s probit analysis method. According to figure-3; LC₅₀ value was estimated to be 58 mg/L with SPSS Statistical Software.
In general, water hardness is found beneficial by reducing alkalinity. It was found that at low pH mercury was more toxic compared to higher pH, which might be due to acid toxicity itself causing bicarbonate loss in the body fluid (Das, Sahu, 2005) [4]. 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) [26]. It seems that two factors, water hardness and pH levels, could affect the acute toxicity of copper sulphate on the fish Sarotherodon mossambica. Mortality was also related to the retention time of CuSO4 in water, i.e. the more the retention time of the CuSO4 in the water, the more 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 the fish decreased as the time of toxicity exposition increases (Ebrahimpour et al., 2010) [5].

According to the results of the present, the LC50 values decreased with time, and about 50% of all mortalities occurred at the first 24 hours. It was found that there was a positive relationship between the mortality and concentration levels; when the concentration level increased, the mortality rate increased as well. However, there was a negative relationship between the mortality time and concentration level; when the concentration level increased, the mortality time decreased. We employed Finney’s probit analysis method of data evaluation for acute toxicity bioassay.

5. Acknowledgements

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6. References

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~ 175 ~