ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(1): 172-175

www.fisheriesjournal.com
Received: 13-11-2015
Accepted: 16-12-2015

P Pandari Reddy
Department of Zoology,
Osmania University,
Hyderabad-500007, India.

R Jagadeshwarlu
Department of Zoology,
Osmania University,
Hyderabad-500007, India.

G Sunitha Devi
Department of Zoology,
Osmania University,
Hyderabad-500007, India.

Correspondence
G Sunitha Devi
Department of Zoology,
Osmania University,
Hyderabad-500007, India.

Determination of lethal concentration (LC_{50}) 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 LC_{50} 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: LC_{50}, 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; Zyadah 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 algaecide for controlling phytoplankton in fish ponds and lakes as well as a herbicide, used in aquatic weed control since1882 (Effler *et al.*,1980) [7]. Fishes 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 LC_{50} 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 ($CuSo_4$, $5H_2O$) 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 LC_{50} with SPSS Statistical Software.

3. Results

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

Table 1: Physical and chemical parameters of the test water

S.No	Parameters	Values of test water
1	pH (Electrometric method)	7.4
2	Temperature (^{0}C) (Field method)	29
3	Dissolved Oxygen (mg/L) (Iodometric method)	8.2
4	Total Hardness CaCO3 (mg/L) (EDTA titration method)	223
5	Alkalinity (mg/L) (Titration method)	250

The LC_{50} 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.

Table 2: LC_{50} value of *Sarotherodon mossambica* exposed to different concentrations of $CuSo_4$ for 96 hours

S. No	Concentration of CuSo4(mg/L)	Log Concentration	No. of Fishes Exposed	No. of Fishes died at 96hr	Probit Kill%	Percent Kill%
1	56	1.75	10	1	3.72	10
2	57	1.76	10	3	4.48	30
3	58	1.76	10	5	5.00	50
4	59	1.77	10	7	5.52	70
5	60	1.78	10	9	6.28	90

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 $CuSo_4$ toxicity data and probit kill.

Figure-2 shown the median LC_{50} 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; LC_{50} value was estimated to be 58 mg/L with SPSS Statistical Software.

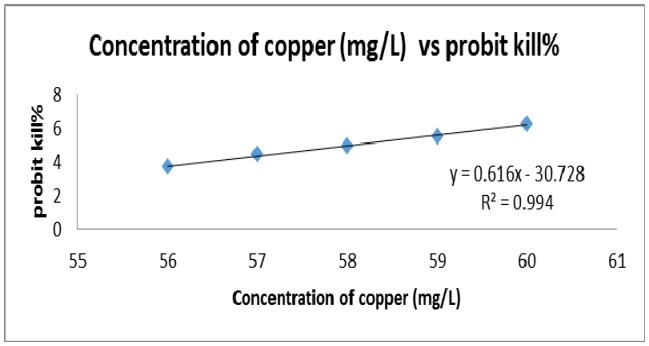

Fig 1: Graph of Concentration of $CuSo_4$ vs Probit kill

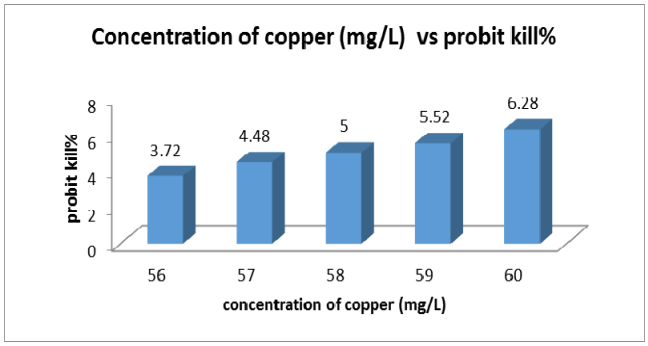

Fig 2: The median LC_{50} value of $CuSo_4$

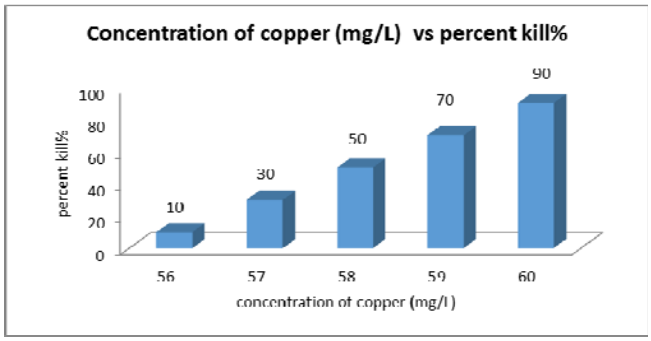

Fig 3: Concentration of $CuSO_4$ vs Percent kill

4. Discussion

In the present investigation, the 96 hr LC_{50} value for copper was found to be 58 mg/L. Results of present studies (Table-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) [17, 22].

Toxicity studies measure a response of an organism to a biologically active substance (Alderdice, 1966) [1] 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 (Nagratnamma, Ramamurti, 1981) [21]. Toxicity testing is an essential tool for assessing the effect and fate of toxicants in aquatic ecosystem (Callow, 1993; Rand *et al*., 1995) [3, 15]. 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 (Das and Sahu, 2005) [4]. The 96 hr LC_{50} 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.

Witeska, Jeezierska (2003) [30] 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. Ebrahimpour *et al*., (2010) [9] reported that toxicity of copper metal decrease significantly with increasing water hardness. Similar types of results were also observed by Straus (2003) [13] using copper exposed fingerling of *Oreochromis aureus* revealed that toxicity increases with a decrease in total alkalinity.

In general, water hardness is found beneficial by reducing metal toxicity to fish. Rathore, Changaret, (2003) [14] found that the toxicity of mercuric chloride decreased with increasing water hardness. The 96h LC_{50} value for copper was higher in the present study than values available in the earlier studies; the reason might be due to high water hardness (120mg/L). If the water hardness was low, and the water was categorized as soft water (75 mg/L as $CaCo_3$) (Shuhaimi-Othman, 2010) [16].

In the present study, water hardness was 223mg/L and the p^H (7.4). The characteristics hardness and pH of the test water were high in the present study. Khangarot *et al.*, (1985) [24] had reported 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 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 $CuSo_4$ in water, i.e. the more the retention time of the $CuSo_4$ in the water, the more the mortality rate of the fish. At the first 24 hr, more of the $CuSo_4$ 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 LC_{50} 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

The authors are grateful Thanks to financial support of UGC-NON-NET Fellowship Osmania University and we express deep thanks to Head, Department of Zoology, Osmania University, Hyderabad.

6. References

1. Alderdice DF. The detection and measurement of water pollution biological assys, J Fish Res Bd Can. 1966; 25:1-11.
2. APHA. Standard Methods for the examination of water and wastewater. American Public Health Assoc, Washington, D.C, 2009.
3. Callow P. General principles and overview. In: P. Callow editors. Handbook of Ecotoxicology. Blackwell Scientific Publ, 1993.
4. Das S, Sahu B. Interaction of pH with mercuric chloride toxicity to penaeid prawns from a tropical estuary, East Coast of India: enhanced toxicity at low pH. Chemosphere, 2005; 58(9):1241-8.
5. Ebrahimpour M, Mosavisefat M, Mohabbati R. Acute toxicity bioassay of Mercuric chloride: an alien fish from a river. Toxicol & Environ. Chem, 2010; 92(1):169-73.
6. Eisler R. Copper Hazards to Fish, Wildlife and Invertebrates, A SYNOPTIC Review. Biological Science Report, 1998. USGS/BRD/BRS-1997-0002.
7. Effler SW, Litten S, Meyer M, Quirk M. Whole lake response to low-level Copper sulphate treatment, Water Res, 1980; 1:1489-1499.
8. Finney DT. Probit Analysis, 3rd edit. Cambridge University Press. London, 1971.
9. Ebrahimpour M, Alipour H, Rakhshah S. Influence of water hardness on acute toxicity of copper and zinc on fish. Toxicol. Ind. Health. 2010; 26:361-365.
10. Hamilton MA, Russo R, Thurston RV. Trimmed

spearmen- Karber method for media lethal concentration in toxicity bioassay. Environ. Sci. Technol. 1977; 11:714-719.
11. Hogstrand C, Haux C. Binding and detoxification of heavy metals in lower vertebrates with reference to metallothionein. Comp. Biochem. Physiol, 2001; 100:137-141.
12. Rathore RS, Khangarot BS. Effect of temperature on the sensitivity of sludge worm Tubifex tubifex (Mullet) to selected heavy metals. Ecotoxicol. Environ. Saf. 2002; 53:27-36.
13. Straus DL. The acute toxicity of copper to blue Tilapia in dilutions of settled pond water. Aquaculture, 2003; 219:233-240.
14. Rathore RS, Khangarot B. Effects of water hardness and metal concentration on a freshwater Tubifex tubifex Muller. Water, Air, & Soil Pollution, 2003; 142(1):341-56.
15. Rand GM, Wells PG, McCarty LS. Introduction to aquatic toxicology. In: Fundamental of Aquatic Toxicology: Effects, Environmental fate and Risk assessment; Taylor& Francis, 1995, 3-67.
16. Shuhaimi-Othman M, Nadzifah Y, Ahmad A. Toxicity of Copper and Cadmium to Freshwater Fishes. Proceedings of World Academy of Science, Engin Technol, 2010; 65:869-71.
17. Khangarot BS. Histopathological changes in the branchial apparatus of Punctius sopore (Ham.) subjected to toxic doses of Zinc. Arch. Hydrobiol, 1982; 93:352-358.
18. Kotsanis N, Georgdaki JL. Arsenic induced liver hyperplasia and kidney fibrosis by microinjection technique: A sensitive animal bioassay for environmental metal toxicity. Bull. Environ. Contam. Toxicol, 1999; 62:169-178.
19. Lodhi HS, Khan MA, Verma RS, Sharma VD. Acute toxicity of copper sulphate to freshwater prawns. J Environ Biol. 2006; 27(3):585-588.
20. Mason CF. Biology of freshwater pollution. 3rd edition. Longman, U.K, 1996, 1-4.
21. Nagratnamma R, Rammurthi R, Comparitive evaluation of methyl parathion toxicity to some selected freshwater organisms. Curr. Sci, 1981; 50:334-335.
22. Nilkant GV, Sawant KB. Studies on accumulation and Histopathology of gills after exposure to sublethal concentration of Hexavalent chromium and effect on the oxygen consumption in Scylla serrata (Forskal). Poll. Res, 1993; 12(1):11-18.
23. Nriagu JO, Sprague JB. Cadmium in the aquatic environment. John Wiley and Sons, Inc. 1987, 272.
24. Khangarot B, Sehgal A, Bhasin M. Effect of pH on Toxicity of Sodium Pentachlorophenate to Fry of Common Carp in Softwater. Archiv fur Hydrobiologie, 1985; 103(3):375-9.
25. Zyadah MA, Abdel Bakey TC. Toxicity and bioaccumulation of copper, Zinc and Cadmium in some aquatic organisms. Bull. Environ. Contam. Toxicol, 2000; 64:740-747.
26. Pyle G, Swanson S, Lehmkuhl D. The influence of water hardness, pH and suspended solids on nickel toxicity to larval fathead minnows (Pimephales promelas). Water, Air & Soil Pollution, 2002; 133(1):215-26.
27. Hogstrand C, Wood CM. The Physiology and Toxicology of Zinc in Fish, in Taylor AW, ed., Toxicology of Aquatic Pollution, Physiological, Molecular and Cellular Approaches. University of Cambridge, New York, NY, USA, 1996, 61-84.
28. Taylor LN, McGeer JC, Wood CM, McDonald DG. Physiological effects of Chronic copper exposure to rainbow trout (Oncorhynchus mykiss) in hard and soft water: evolution of chronic indicators. Environ. Toxicol. and chem, 2000; 19:2298-2308.
29. Verma RS, Khan MA, Tripathi R, Shukla S, Shrama VD. Heavy metal Toxicity to freshwater prawn, Macrobrachium dayanum (Crustacea- Decapoda). Aquaculture, 2005; 6(1):57-62.
30. Witeska M, Jezierska B. The effect of environmental factors on metal toxicity of fish. Fresen. Environ. Bull, 2003; 12:824-9.
31. Shah SL, Altindag A. Haematological parameters of tench (Tinca tinca) offter acute and chronic exposure to lethal and sub-lethal mercury treatments. Bull. Environ. Contam. Toxical, 2004; 73:911-918.
32. Rai AN, Ullah A. Determination of Acute Toxicity of Copper and Cobalt for Tilapia nilotica, Journal of Bioresource Management, 2015, 2(1).
33. Dharam Singh, Kamlesh Nath, Trivedi SP. Impact of copper on haematological profile of freshwater fish, Channa punctatus, Journal of Environ. Biol, 2008.