GERF Bulletin of Biosciences
June 2013, 4(1):14-18

Research Article **Open Access**

ISSN: 2229-6433

Acute toxicity of copper sulphate to African catfish, (*Clarias gariepinus*)

Adil A. Wani*, M. Sikdar-Bar and Hilal Ahmad Khan

Department of Zoology, Dr. Harisingh Gour University, Sagar (M.P), India

Abstract

In this study, 96 hrs LC_{50} values of copper sulphate ($CuSO_4.5H_2O$), a highly toxicant heavy metal, on African catfish, *Clarias gariepinus* of average weight 98.43 ± 24.09 g and length 20.5 ± 2.5 cm was determined. The acute toxicity tests were performed according to the static non-renewable bioassay procedure. The experimental design consisted of a control and six concentrations (24, 31, 38, 44, 50 and 55 ppm) of copper sulphate, with two replicates per group and twenty fishes in each replicate. The 96 hour LC_{50} value based on probit analysis was found to be 40.86 ppm; the lower and upper lethal confidence limit for copper sulphate indicated a wide range between 37.47 to 44.58 ppm. Susceptibility of catfish, *Clarias gariepinus* to lethal effect of copper sulphate was found to be duration and concentration dependent as mortality was increased with an increase in its concentration. Results indicated that copper sulphate is toxic to fish, to moderate extent even at lower concentrations. Therefore, the present investigation may provide useful guidance that can be exploited by the aquaculturists to formulate the safety levels of copper sulphate in water bodies.

Key words: Copper sulphate, *Clarias gariepinus*, 96 hrs LC_{50}

Introduction

Metal concentrations in aquatic organisms appear to be of several magnitudes higher than concentrations present in the ecosystem (1). This is attributed to bioaccumulation, whereby metal ions are taken up from the environment by the organism and accumulated in various organs and tissues. Metals also become increasingly concentrated at higher trophic levels, possibly due to food-chain magnification.

Aquatic toxicity tests are used to detect and evaluate the potential toxicological effects of chemicals on aquatic organisms. Since these effects are not necessarily harmful, a principal function of these tests is to identify chemicals that can have adverse effects on aquatic organisms. These tests provide a database that can be used to assess the risk associated with a situation in which the chemical agent, the organism, and exposure conditions are defined. They can identify potential environmental problems before the health of a system is critically altered or compromised (2). Copper is a micronutrient essential for all living organisms required in small amounts, and important life functions cannot function properly in its absence (3). Copper is taken up from the environment, either in the form of food or directly from the water column, binds to α-globulin and is transported to the various tissues of the body (4). Copper ions are quite toxic to fishes and had already been reported by many

*Corresponding author: waniadil@rocketmail.com

workers (5, 6, 7).

Copper sulphate is often used as an algaecide in commercial and recreational fish ponds to control growth of phytoplankton and filamentous algae, and to control certain fish diseases (8). However, above a specific concentration, copper is toxic to fishes including such cultured species as salmonids, cyprinids and catfishes (9). Thus, treating plankton with copper compounds may lead to copper bioaccumulation reaching a toxic level in fish. The toxic effect of copper is related to its capacity for catalyzing oxidative reactions, leading to the production of reactive oxygen species (10).

Fishes are ideal organisms to monitor aquatic systems because they occupy positions towards the apex of food pyramids and may, therefore, reflect effect of heavy metals on other organisms including human beings as well as direct stresses on themselves (11). This study investigates the toxic effects of copper sulphate on the *Clarias gariepinus* by determination of 96-hour LC_{50} value.

Materials and Methods

Alive, healthy and disease free specimens of catfish, *Clarias gariepinus* of either sex belonging to a single population were purchased on order from the local fish market of Sagar. Before introducing those in aquariums, fishes were treated with 0.01% $KMnO_4$ solution for 15 min to obviate any dermal infection. Fishes of average weight 98.43±24.09 g and length 20.5±2.5 cm were distributed in 120 L capacity aquariums and were unfed during the first 2 days to adapt to change in environment. Fishes were then kept for a period of fifteen days for acclimatization of laboratory conditions.

After acclimatization experimental fishes were selected at random and were kept in a static system of water. The feeding was stopped one day prior the exposure to $CuSO_4$ and fishes were not fed throughout the test. The acute toxicity tests were performed according to the static non-renewable bioassay procedure (12) as described in the previous study (13). The experimental design consisted of a control and six concentrations (24, 31, 38, 44, 50 and 55 ppm) of copper sulphate ($CuSO_4.5H_2O$), two replicates per group and with twenty fishes in each replicate. Fishes showing no respiratory movement and response to tactile stimuli were considered as dead and removed immediately. The 96 hrs LC_{50} was computed with probit analysis using statistical SPSS 16 package (14). During the exposure in different concentrations of copper sulphate, the behavioural changes of the fishes were also recorded.

Results and Discussion

The 96 hrs LC_{50} values provide a useful means of comparing the relative acute lethal toxicity of specific toxicants to organisms under specific conditions. Initially, copper sulphate exposure to fishes showed restlessness, rapid body movement, and difficulty in respiration displayed by fishes moving to the surface to gulp air, intense opercula movement, accumulation of mucus on body, loss of equilibrium by swimming sideways, finally fishes collapsed and died. From the results of mortality readings, the 96 hrs LC_{50} value and 95% confidence limits for copper sulphate based on probit analysis was found to be 40.86 ppm (Table-1 and Fig.-1). The lower and upper lethal confidence limits indicated a range between 37.47 to 44.58 ppm (Table-2). Susceptibility of catfish, *Clarias gariepinus* to lethal effect of copper sulphate was duration and concentration dependent as mortality increased with an increase in its concentration.

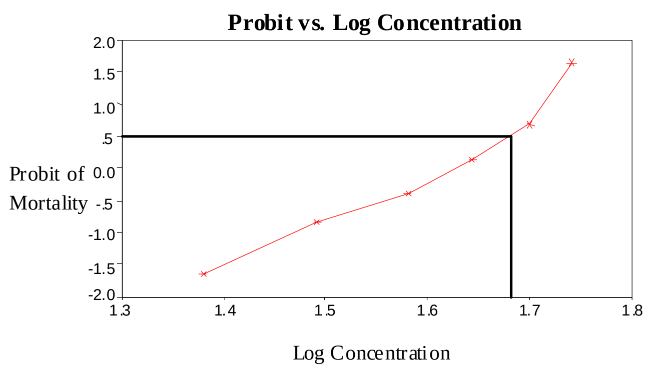

Fig. 1: Showing the linear relationship between probit response and log sub-lethal concentration of copper sulphate on *Clarias gariepinus.*

In present study, we observed that, there was a corresponding increase in mortality response of the test fishes with increased time and exposure of copper sulphate. Forgoing results of Finney's probit analysis revealed 40.86 ppm as LC_{50} value of copper sulphate exposed to *Clarias gariepinus* for 96 hrs. The results of this investigation support the observations, in this regard, shown by Osman and co-workers

Table 1: Showing the mortality of *Clarias gariepinus* at 96h after treatment of different copper sulphate concentrations.

$CuSO_4$ Conc. (ppm)	Log Conc.	Mortality	No. of fish exposed	Expected Responses	Residual	Probability
24	1.38	01	20	0.994	0.006	0.04842
31	1.49	04	20	3.887	0.113	0.19433
38	1.58	07	20	8.205	-0.205	0.41025
44	1.64	11	20	11.822	-0.178	0.59111
50	1.70	15	20	14.708	-0.708	0.73538
55	1.74	19	20	16.458	-0.542	0.82291

Table 2: Confidence limits for effective concentration

Probit	Concentration (ppm)	95% Confidence Limits	
		Lower	Upper
0.10	27.09917	21.42389	30.75566
0.15	29.31444	24.03412	32.75512
0.20	31.20351	26.29886	34.48208
0.40	37.67937	33.99955	40.88238
0.50	40.86719	37.47484	44.58082
0.60	44.32473	40.85207	49.15328
0.70	48.34843	44.34777	55.12595
0.80	53.52371	48.41539	63.57143
0.90	61.63022	54.27602	78.04491
0.99	86.14921	70.31773	128.61895

(15) who reported 72 hrs LC_{50} value of $CuSO_4$ as 40.6 mg/l in *Oreochromis niloticus*. Tavares-Dias et al (16) evaluated 17.5 mg/l as the 96 hrs LC_{50} value of copper sulphate on juveniles of *Colossoma macropomum*. Ezeonyejiaku and colleagues (17) reported that copper was significantly more toxic to *Oreochromis niloticus* than the catfish, the 96 hrs LC_{50} values for *Oreochromis niloticus* and *Clarias gariepinus* were revealed to be 58.837 and 70.135 mg/l, respectively. The 96 hrs LC_{50} values of copper reported for *Colisa fasciatus* was 4 mg/l (18), for *N. notopterus* as 30 mg/l (19) and for *Esomus danricus* as 5.5 mg/l (20). Darwish (21) reported that a 96 hrs median lethal concentration (LC_{50}) value for copper sulphate on *Ictalurus punctatus* was 6.89 mg/l (1.75 mg/l Cu) and on *Morone chrysops* was 3.35 mg/l (1.75 mg/l Cu). This study demonstrated that sunshine bass juveniles are less tolerant of $CuSO_4$ than channel catfish fingerlings when exposed concurrently in waters from the same source. However, in contrast to our study, much lower LC_{50} values of copper were reported on *Poronotus triacanthus* as 502.95 µg/l (13) and *Danio rerio* as 73.83 µg/l (22), which suggests that these fishes are much less tolerant to toxic effect of copper. The toxicity of copper sulphate is related to the levels of copper ions in the water. Copper toxicity studies in fish have demonstrated that toxicity of copper is dependent on the concentration of free Cu^{++} (23).

Marine teleosts are more less sensitive to Cu, with reported 96 hrs LC_{50} values of 800-1000 µg/l range for spiny dog fish, *Squalus acanthias* (24), of 1140 µg/l for sheepshead (*Archosargus probatocephalus*), of 5660 µg/l for Atlantic croacker, *Micropogan undulatus* and of 2750 µg/l for pinfish, *Lagadon rhomboides* (25). Toadfish, *Opsanus beta* are even more resistant with the 96 hrs LC_{50} between 21,600 and 36,100 µg/l (26). The 48 hrs and 96 hrs LC_{50} for Cu on Mediterranean dogfish, *Scyliorhinus canicula*, was reported to be 4000µg/l and 2000 µg/l, respectively (27). The observed differences in the LC_{50} values of copper might be due to the physicochemical characteristics of the test medium, species and ages of fishes used and their susceptibility rates, which resulted in their subsequent different toxicity values. Differences in metabolic pathways among species may result in varied patterns of bio-transformation, leading to more or less toxic metabolites (28).

The lowest dose of copper sulphate that has been toxic when ingested by humans is 11 mg/kg (29). Long-term effects are more likely in individuals with Wilson's disease, a condition which causes excessive absorption and storage of copper and manifests as neurological psychiatrics symptoms and liver disease (30). Chronic exposure to low levels of copper in humans can lead to anaemia (31).

The impact of copper on the aquatic environment is complex and depends on the physicochemical characteristics of test medium (32). Therefore, the variations in the LC_{50} values for copper on fishes might be due to the variations in physicochemical characteristics of water used during experimentation. The incipient LC_{50} of copper for juvenile rainbow trout (*Salmo gairdneri*) increased when hardness and alkalinity was increased (33). Also, Wurts and Perschbacher (09) observed the 48 hrs LC_{50} of copper sulphate to channel catfish, *Ictalurus punctatus* as 48 mg/l, and found that mortality decreased as calcium hardness levels increased. Abdel-Tawwab and Mousa (34) reported that the LC_{50} of Nile tilapia, *O. niloticus* not exposed to calcium and exposed to copper was 5.03 mg Cu/l, while as LC_{50} value was increased with CaO (14.27 mg Cu/l). Merwe et al (35) reported that LC_{50} values of copper on adult *C. gariepinus* ranged from 1.29 mg/l during summer to 1.38 mg/l in winter and suggested that water temperature may alter the lethality of copper to *C. gariepinus*.

Conclusion

From the results obtained, it can be concluded that the copper sulphate is quite toxic to *C. gariepinus*. The present investigation provides useful information that can be exploited by the aquaculturists to formulate the safety levels of copper in water bodies and to formulate safer and more effective application rates for copper sulphate when used for parasite control.

Acknowledgement

Authors are thankful to Prof. Mrs. Mangla Bhide, former HOD of Zoology, and Prof. U.S. Gupta (DSW), Dr. Harisingh Gour University, Sagar (M.P) for their valuable guidance for carrying out this work and critically reading the manuscript.

References

1. Laws EA. Aquatic Pollution- An introductory text. John Wiley and Sons, New York, U.S.A., 2000; pp. 309-430.
2. Jimenez BD, Stegeman JJ. Detoxification enzymes as indicators of environmental stress on fish. American Fisheries Society Symposium. 1990; 8: 67-69.
3. Steenkamp VE, Du Preez, HH, Schoonbee HJ. Bioaccumulation of copper in the tissues of *Potamonautes warren* (Decapoda) from industrial, mine and sewage polluted freshwater ecosystems.South African J Zoo. 1994; 29: 152-161.
4. Aaseth J, Norseth T. Copper. In: Handbook on the toxicology of metals. Vol. II: Specific metals. 2nd edition. Friberg L, Nordberg GF, Vouk VB (Eds.), Elsevier, New York. 1986; pp. 233-254.
5. Wepener V, Van Vuren JHJ, Du Preez HH. Uptake and distribution of a copper, iron and zinc mixture in gill, liver and plasma of freshwater teleosts, *Tilapia sparrmanii*. Water SA. 2001; 27: 99-108.
6. Oliveira-Filho, EC, Lopes RM, Paumgartten FJR. Comparative study on the susceptibility of freshwater species to copper-based pesticides. Chemosphere. 2004; 56: 369-374.
7. Wani AA, Sikdar-Bar M, Borana K, Khan HA, Andrabi, SSM, Pervaiz PA. Histopathological alterations induced in gill epithelium of African catfish, *Clarias gariepinus*, exposed to copper sulphate. Asian J Exp Biol Sci. 2011; 2(2): 278-282.
8. Tucker CS, Robinson EH. Channel Catfish Farming Handbook. Van-Nostrand-Reinhold, New York, USA; 1990.
9. Wurts WA, Perschbacher PW. Effects of bicarbonate alkalinity and calcium on the acute toxicity of copper to juvenile channel catfish (*Ictalurus punctatus*). Aquaculture. 1994; 125: 73–79.
10. Lopes PA, Pinheiro T, Santos MC, Mathias M, Collares-Pereira MJ, Viegas-Crespo AM. Response of antioxidant enzymes in freshwater fish populations (Leuciscus alburnoides complex) to inorganic pollutants exposure. Sci Total Environ. 2001; 280: 153-163.
11. Van der Oost R, Beyar J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environmental Toxicology and Pharmacology. 2003; 13: 57-149.
12. USEPA. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 5th edition. Washington, DC: US Environmental Protection Agent, EPA821R02012; 2002.
13. Jiraungkoorskul W, Sahaphong S, Kangwanrangsan N. Toxicity of copper in butterfish (*Poronotus triacanthus*): tissues accumulation and ultrastructural changes. Inc Environ Toxicol. 2007; 22: 92-100.
14. Finney DJ. Probit Analysis, 3rd edition. London:

Cambridge University Press; 1971.

15. Osman M, El-Fiky S, Soheir Y, Abeer A. Impact of water pollution on histopathological and electrophoretic characters of *Oreochromis niloticus*. Res J Environ Toxicol. 2009; 3: 9-23.
16. Tavares-Dias M, Ferreira J, Affonso E, Ono E, Martins M. Toxicity and effects of copper sulphate on parasitic control and haematological response of Tambaqui *Colossoma macropomum*. Bol Inst Pesca. 2011; 37: 355-365.
17. Ezeonyejiaku CD, Obiakor MO, Ezenwelu CO. Toxicity of copper sulphate and behavioural locomotor response of tilapia, *Oreochromis niloticus* and catfish, *Clarias gariepinus* species. Online J Anim Feed Res. 2011; 1: 130-134.
18. Mishra S, Srivastava AK. The acute toxic effects of copper on the blood of a teleost. Ecotox Environ Safety. 1980; 4: 191-194.
19. Barad VS, Kulkarni RS. Haematological changes induced by short-term exposure to copper in the Indian freshwater fish, *Notopterus notopterus*. The Biosca. 2010; 5: 313-316.
20. Vutukuru SS, Ch. Suma, Radha K, Juveria, Smitha J, Venkateswara J, Anjaneyulu Y. Studies on the development of potential biomarkers for rapid assessment of copper toxicity to freshwater, fish using *Esomus danricus* as model. Int J Environ. Res Public health. 2005; 2: 63-73.
21. Darwish AM. Species sensitivity to copper: Acute toxicity to channel catfish, *Ictalurus punctatus*, and sunshine bass, *Morone chrysops* x *M. saxatilis*. J Appl Aquacult. 2006; 18: 89-99.
22. Campagna AF, Fracacio R, Rodrigues BK, Eler MN. Effects of the copper in the survival, growth and gill morphology of *Danio rerio*. Acta Limnol Bras. 2008; 20: 253-259.
23. Howarth RS, Sprague JB. Copper lethality to rainbow trout in waters of various hardness and pH. Water Res. 1978; 12: 455-462.
24. De Boeck G, Hattink J, Franklin NM, Buckin CP, Wood S, Walsh PJ, Wood CM. Copper toxicity in the spiny dogfish, *Squalus acanthias*: Urea loss contributes to the osmoregulatory disturbance. Aquat Toxicol. 2007; 84:133-141.
25. Steele CW. Comparison of the behavioural and acute toxicity of copper to sheepshead, Atlantic croacker and pinfish. Mar Pollut Bull. 1983; 14: 425-428.
26. Grosell M, Wood CM. Copper uptake across rainbow trout gills: mechanisms of apical entry. J Exp Biol. 2002; 205: 1179-1188.
27. Torres P, Tort L, Flos R. Acute toxicity of copper to Mediterranean dogfish. Comp. Biochem Physiol C. 1987; 86(1): 169–171.
28. Johnson CM, Toledo MCF. Acute toxicity of endosulfan to the fish *Hyphessobrycon bifasciatus* and *Brachydanio rerio*. Archiv Environ Contamin Toxicol. 1993; 24: 151-155.
29. NIOSH, 1981-1986. National Institute for Occupational Safety and Health Registry of toxic effects of chemical substances (RTECS). Cincinati, OH: NIOSH.
30. Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson disease. Lancet. 2007; 369: 397-408.
31. TOXNET, 1975-1986. National library of medicine's toxicology data network. Hazardous Substances Data Bank (HSDB). Public Health Service. National Institute of Health, U. S. Department of Health and Human Services. Bethesda, MD: NLM.
32. Takasusuki J, Araujo MRR, Fernandes, MN. Effect of water pH on copper toxicity in the neotropical fish, *Prochilodus scrofa* (Prochilodondidae). Bulletin of Environmental Contamination and Toxicology. 2004; 72: 1075-1082.
33. Miller TG, Mackay WC. The effects of hardness, alkalinity and pH of tested water on the toxicity of copper to rainbow trout (*Salmo gairdneri*). Water Res. 1980; 14: 129-133.
34. Abdel-Tawwab M, Mousa MAA. Effect of calcium pre-exposure on acute copper toxicity to juvenile Nile tilapia, *Oreochromis niloticus*. Zagazig Vet J. (2005); 33:80-87.
35. Van der Merwe M, Van Vuren JHJ, Du Preez HH. Lethal copper concentration levels for *Clarias gariepinus* (Burchell, 1822)- a preliminary study. Koedoe. 1993; 36: 77-86.