Determination of lethal toxicity of copper to *Clarias* gariepinus

Adil A. Wani, Junaid Malik, S.M. Zuber

Department of Zoology, Government Degree College, Bijbehara (J&K), India

ABSTRACT

In this study 96 hour LC_{50} values of copper sulphate (CuSO₄.5H₂O), an 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, two replicates per group and with twenty fish in each replicate. The 96h LC_{50} value based on probit analysis was found to be 40.86 ppm; the lower and upper lethal confidence limits for copper sulphate indicate a wide range of 37.47 to 44.58 ppm. 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. 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 hour LC₅₀

I. 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 the 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 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 disease (8). However, above a specific concentration, copper is toxic to fish including such cultured species as salmonids, cyprinids and catfish (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 effects 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.

II. 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 the aquariums fish were treated with 0.01% KMnO₄ solution for 15 minutes to obviate any dermal infection. Fish of average weight 98.43 ± 24.09 g and length 20.5 ± 2.5 cm were distributed in 120L capacity aquariums and were unfed during the first 2 days to adapt to a change in environment. Fish were then kept for a period of fifteen days for acclimatization in laboratory conditions.

After acclimatization experimental fish 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 fish 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 fish in each replicate. Fishes showing no respiratory movement and response to tactile stimuli were considered as dead and removed immediately. The 96h 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 fish were also recorded.

III. RESULTS AND DISCUSSION

The 96h LC_{50} values provide a useful means of comparing the relative acute lethal toxicity of specific toxicants to organisms under specific conditions. Initially on copper sulphate exposure fish showed restlessness, rapid body movement, and difficulty in respiration displayed by fish moving to the surface to gulp air, intense opercula movement, accumulation of mucus on body, loss of equilibrium by swimming sideways, finally fish collapsed and died. From the results of mortality readings, the 96h 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 indicate a range of 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.

CuSO ₄	Log	Mortality	No. of fish	Expected	Residual	Probability
Conc.(ppm)	Conc.		exposed	Responses		
24	1.38	01	20	.994	.006	.04842
31	1.49	04	20	3.887	.113	.19433
38	1.58	07	20	8.205	205	.41025
44	1.64	11	20	11.822	178	.59111
50	1.70	15	20	14.708	708	.73538
55	1.74	19	20	16.458	542	.82291

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

Table 2: Confidence limits for effective concentration

Probit	Concentration	95% Confid	ence Limits	
	(ppm)	Lower	Upper	
.10	27.09917	21.42389	30.75566	
.15	29.31444	24.03412	32.75512	
.20	31.20351	26.29886	34.48208	
.40	37.67937	33.99955	40.88238	
.50	40.86719	37.47484	44.58082	
.60	44.32473	40.85207	49.15328	
.70	48.34843	44.34777	55.12595	
.80	53.52371	48.41539	63.57143	
.90	61.63022	54.27602	78.04491	
.99	86.14921	70.31773	128.61895	

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



In present study there was corresponding increase in mortality response of the test fish with increased exposure and time. Forgoing results of Finney's probit analysis revealed 40.86 ppm as LC50 value of copper sulphate exposed to Clarias gariepinus for 96h. The results of this investigation support the observations, in this regard, forwarded by Osman and co-workers (15) who reported 72h LC₅₀ value of CuSO₄ as 40.6 mg/l in Oreochromis niloticus. Tavares-Dias et al (16) evaluated 17.5 mg/l as the 96h 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 96h LC_{50} values for *Oreochromis niloticus* and *Clarias gariepinus* were revealed to be 58.837 and 70.135 mg/l, respectively. The 96h LC₅₀ 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 96h 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 CuSO₄ 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 96h LC₅₀ values of 800-1000 μ g/l range for spiny dog fish, *Squalus acanthias* (24), of 1140 μ g/l for sheepshead (*Archosargus probatocephalus*), of 5660 μ g/l for Atlantic croacker, *Micropogan undulatus* and of 2750 μ g/l for pinfish, *Lagadon rhomboides* (25). Toadfish, *Opsanus beta* are even more resistant with the 96h LC₅₀ between 21,600 and 36,100 μ g/l (26). The 48h and 96h LC₅₀ for Cu on Mediterranean dogfish, *Scyliorhinus canicula*, was reported to be 4000 μ g/l and 2000 μ g/l, respectively (27). The observed differences in the LC₅₀ 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 48h 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 mgCu/l, while as LC_{50} value was increased with CaO (14.27 mg Cu/l). Merwe and coworkers (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*.

V. 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.

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