Acute Toxicity and the Effects of Copper Sulphate (CuSO$_4$.5H$_2$O) on the Behavior of the Black Fish (*Capoeta Fusca*)

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ABSTRACT

Background: The development of toxicity tests regarding toxic responses of different fish species could be more effectively used in predictive toxicology and risk assessment. In this study lethal concentrations (LC50-96 h) values of copper sulphate; an important toxic industrial pollutant, on *Capoeta fusca* were determined. Behavioral changes at different concentrations of CuSO$_4$ were determined for the *C.fusca*.

Methods: The sample fishes were collected from Qanat in Birjand and were transported to the laboratory in polythene bags. The exposure time of fish to CuSO$_4$ was 96 hours. Mortalities were recorded at 24, 48, 72, and 96 hours of exposure, and the dead fish were removed regularly from the test aquariums. Physicochemical parameters, such as dissolved oxygen, pH and Total hardness of aquaria were monitored daily.

Results: The LC$_{50}$ values for CuSO$_4$ at 24, 48, 72, and 96 h of exposure, were 43.62, 12.6, 7.66, and 6.85 mg/L, respectively. The median LC$_{50}$ value of CuSO$_4$ for *C.fusca* was found to be 6.928 mg/L by EPA method and estimated to be 6.787 mg/L with SPSS statistical software.

Conclusion: The mortality decreased with time, and most of the deaths occurred during the first 24 h. In addition, behavioural changes increased with increased concentration. This metal is an important constituent in industrial effluents discharged into freshwaters. The results obtained in this study clearly revealed the fact that it is necessary to control the use of a heavy metal such as copper.

Keywords: Acute Toxicity Tests, *Capoeta Fusca,* Copper Sulphate, Lethal Dose 50.

INTRODUCTION

Heavy metals are important environmental pollutants. Metal contamination of the environment results from both natural sources and industrial activities. Metals in soil and water may enter the food cycle with an additional contribution from air. Further potential sources of human exposure include consumer products and industrial waste as well as the working environment (1). The aquatic media are contaminated not only from the air but also from the land itself. The major factors contributing to heavy metal contamination are household and industrial waste containing either organic or inorganic matter (2). Acute toxicity test of single compounds is continually released into the aquatic ecosystems from industrial and residential areas, representing a potential risk to the aquatic biota (3). In order to manage aquatic ecosystem it is important to know the biological status of the system, especially

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when evaluating the impact of a chemical stressor on the biota. Bioavailability is a dynamic process with two different phases: a physico-chemically driven desorption and a physiologically driven uptake (4). Atmospheric deposition, erosion from the geological matrix, or from anthropogenic sources, such as manufacturing discharges, and mining wastes can introduce heavy metals into the aquatic ecosystem (5). When heavy metals enter the aquatic ecosystems, their mechanisms can cause stress effects, due to their ability to accumulate (6). Most of the heavy metal ions exhibit toxicity through the formation of coordination complexes and clusters in the animal cells. Low concentration of heavy metals may cause a chronic stress that might not kill the fish by itself but decrease its size and body weight, therefore reducing their capability to fight for food and habitat. Fish have a tendency to bio-accumulate heavy metals and human beings can be at serious risk through contamination of the food chain (7). Among different species, fishes are the creatures that cannot escape from the detrimental effects of these pollutants (8). As a result, fishes are generally used as indicators for trace metals contamination in the aquatic ecosystem because they take the place of higher trophic chain and are significant food sources (9-10). Some heavy metals such as zinc and copper are important in small quantities for biological processes in aquatic plants and animals and occur naturally in many river systems; however, when they are discharged in large quantities from sewage or agricultural runoffs, they can be extremely harmful. Copper, in the ionic forms Cu^{2+}, $Cu_2OH_2^{2+}$, and $CuOH^+$, is toxic to fish (11). Copper, an essential metal for organisms, may become extremely toxic for aquatic animals as its concentration in water increases. This study investigates the toxic effects of copper sulphate on the standard test species, black fish (*Capoeta fusca*), by determination of 96-hour LC_{50} values and evaluates behavioral disorders of the black

fish exposed to different concentration of the toxicant.

MATERIALS AND METHODS

This study was carried out in the Limnology Laboratories of Department of Environmental Sciences, Faculty of Agriculture, Birjand University. Birjand is the capital of South Khorasan province in the east of Iran. The province has a dry climate with significant difference between day and night temperatures with an annual rainfall of 172 mm. The basin area received a total rainfall of 76 mm in 2006 and 55 mm in 2007. Birjand is an important regional centre of agriculture and pasturage. During October 2011, *Capoeta fusca* samples (12) which belong to the family Cyprinidae, were collected from one Qnat in Birjand, Iran. Live fish were transported to the laboratory in polythene bags with Qanats water. Prior to the experiment the fish were acclimatized for 10 days to laboratory conditions in five pre-cleaned glass aquariums filled tap water. The fish were fed with commercial pelleted food at least once a day during this period.

Acclimated fish were not fed for 1 day before the start of experiments until the end of the 96-h experimental period. Thus, the volume of waste matter was minimized in order to not affecting their living condition. Care was taken to keep the mortality rate of fish not more than 5% in the last four days before the experiment was started. The fish were exposed to copper sulphate ($CuSO_4$) in the aquarium systems. The aquariums were fitted with artificial aerator to maintain oxygen levels. The exposure time of fish to copper sulphate was 96 h, without adding any food. Sets of 10 fish specimens were exposed randomly to different 40 litres tanks. The average wet weight (±SD) of fish used in experiments was 2.95 (±0.55) g. The exploratory range of concentration of test chemicals was determined with a series of range finding experiments (13). Preliminary tests were carried out to

estimate the minimum lethal and maximum nonlethal concentrations of copper sulphate. For this, eight different geometrically decreasing concentrations of copper sulphate (64, 32, 16, 8, 4, 2, 1 and 0.5 mg/L) plus a control were used. The concentration range of copper sulphate in this study was determined from 2 to 32mg/L (because of no mortality at 2 mg/L and 100% mortality at 32 mg/L). Thereafter, five different concentrations (32, 16, 8, 4 and 2(mg/L) in three-replicates were chosen. For each treatment, 10 fish specimens were used. The solutions were prepared by dissolving analytical-grade copper sulphate (Merck) in distilled water. A control was used for test with three replicates. The experiment and control water used in the investigation consisted of natural tap water, which was aerated for 48 hours to remove chlorine. No mortality was observed during the experimental period in controls. Dissolved oxygen (mg/L), temperature (C°) and pH were recorded individually in each test container at exposure times of 24, 48, 72 and 96 h. Water quality of the experimental tanks were determined according to standard procedures. Total hardness, magnesium and ammonia (mg/L) were determined before starting the experiments by photometer (Palintest, 8000). The water temperature was kept between 21 and 23 C°. Mortalities and abnormality in swimming behaviour of fish were noted at 24, 48, 72 and 96 h of exposure and the dead fish were removed regularly from the test solution. LC_{50} values were calculated from the data obtained in acute toxicity bioassays, EPA method (14), "Probit analysis program (version 1.5)" and the data was analysed with SPSS statistical software (version 16).

RESULTS

The acute toxicity of copper sulphate ($CuSO_4.5H_2O$) to the freshwater fish, *Capotea fusca* was evaluated by static bioassays and calculation of the LC_{50} (lethality concentration for 50%). The physiochemical properties of the test water and Qanats water are shown in Table 1.

The physical and chemical parameters analyzed during the bioassays showed no differences among the ranges of five concentrations, neither between test water and Qanat water, while hardness concentrations were higher in Qantas water than test water. The LC_{50} value for copper sulphate, calculated by EPA method and SPSS statistical software at 24, 48, 72 and 96 h of exposure, is shown in Table 2.

Table 3 shows the median LC_{50} value of copper sulphate on *C.fusca* which was found to be 6.928 mg/L by EPA method (Probit analysis program). In addition, according to table 4, this value was estimated to be 6.787 mg/L with SPSS statistical software.

Table 1. Physiochemical properties of the Qanat water and test water

Parameters	Value (Qanat water)	Value (test water)
pH	8.2 ± 0.2	8.1 ± 0.1
Dissolved oxygen (mg/L)	6.3 ± 0.2	6.5 ± 0.2
Temperature (C°)	19 ± 0.2	21.5 ± 0.4
Total hardness (as $CaCO_3$, mg/L)	480 ± 6.3	360 ± 5.2
Magnesium (mg/L)	41 ± 2	33 ± 2
Nitrate (NO_3- N, mg/L)	0.43 ± 0.2	0.45 ± 0.1
Ammonia (NO_3- N, mg/L)	0.2	0.05

Table 2. Lethal concentration (LC_{50}) with 95% confidence limit (in parentheses) of copper sulphate (mg/L) estimated by EPA method and SPSS

Time (h)	EPA method	Spss method
24	43.622 (22.342 – 43.283)	42.523 (21.983 – 37.630)
48	12.608 (9.142 – 18.429)	12.420(9.106– 18.019)
72	7.660 (5.710 – 10.152)	7.574 (5.686– 9.967)
96	6.857 (5.202 – 8.888)	6.787(5.192 – 8.744)

Table 3. Estimated LC values and confidence limits by EPA method.

Point	Concentration (mg/L)	95% Confidence Limits	
		Lower	Upper
LC 1.00	1.125	0.401	1.918
LC 5.00	1.916	0.882	2.904
LC 10.00	2.545	1.336	3.642
LC 15.00	3.082	1.761	4.260
LC 50.00	6.928	5.196	9.010
LC 85.00	15.572	11.608	25.166
LC 90.00	18.861	13.641	33.026
LC 95.00	25.053	17.184	49.809

Table 4. Estimated LC values and confidence limits by SPSS.

Point	Concentration (mg/L)	95% Confidence Limits	
		Lower	Upper
LC 1.00	1.0084	0.412	1.813
LC 5.00	1.855	0.900	2.763
LC 10.00	2.471	1.357	3.479
LC 15.00	2.998	1.785	4.079
LC 50.00	6.787	5.192	8.744
LC 85.00	15.367	11.509	24.599
LC 90.00	18.644	13.534	32.250
LC 95.00	24.828	17.084	48.533

DISCUSSION

Toxicity testing has been widely used as a tool to identify suitable organisms as a bio-indicator and to derive water quality standards for chemicals. Toxicity testing is an essential tool for assessing the effect and fate of toxicants in aquatic ecosystems (15-16). Acute toxicity tests are short-term tests designed to measure the effects of toxic agents on aquatic species during a short period of their life span (17). The 96-h LC_{50} tests are conducted to measure the susceptibility and survival potential of animals to

particular toxic substances such as mercury. Higher LC_{50} values are observed in less toxic substances because greater concentrations are required to produce 50% mortality in organisms (18). Figure 1 shows as the concentration of copper sulphate increased, fish mortality also increased, which indicates a direct proportional relationship between mortality and the concentration of copper sulphate. 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 (19). Figure 1 also indicated the relationship between concentration of copper sulphate and percentage mortality rate of *Capoeta fusca* after 24 h and 96 h exposure to different concentrations of copper sulphate. The mortality decreased with time, so that most of the deaths occurred during the first 24 h. The reason might be due to rapid intoxication (19).

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 h, more of the $CuSo_4$ in water was taken up by the fish and its concentration decreased. In other words, the mortality rate of the fish decreased as the time of toxicity exposition increases (20). LC_{50} obtained in the present study compare with corresponding values that have been published in the literature for other species of fish, show different LC_{50}

of copper sulphate in different species. The 96 h LC_{50} values of copper sulphate on rainbow trout (*Oncorhynchus Mykiss*) were reported to be 0.094 mg/L by Gundogdu, 2008 (21); while Shuhaimi-Othman, 2010 (22); reported the 96 h LC50 value of copper sulphate on two freshwater fishes, *Rasbora sumatrana* (Cyprinidae) and *Poecilia reticulata* (guppy). For *R. sumatrana*, LC_{50} for 96 hours were 5.6 µg/L and for *P. reticulata* were 37.9 µg/L. Gomes et al., (2009)(23) reported that with juvenile Brazilian indigenous fishes, *curimata Prochilodus vimboides* and *piaucu Leporinus macrocephalus*, 96h-LC_{50} of copper were 0.047 and 0.090 mg/L, respectively.

The 96 h LC_{50} for copper sulphate at this study was determined at 6.857 mg/L. Comparing our data with those available in the literature may not be meaningful because various factors may influence bioassay techniques like differences in fish (e.g., species, weight, size) and other environmental factors (24). Several important factors are known to make heavy metals biologically less active and therefore less toxic. Water hardness is well known to attenuate metal toxicity and is a major factor, which influences the toxic effects of heavy metals on fish (25). Toxicity of the metal is usually concerned with impairment of active Ca^{2+} transport in fish by the competitive blockade of epithelial Ca^{2+} channels in the gill epithelium (26).
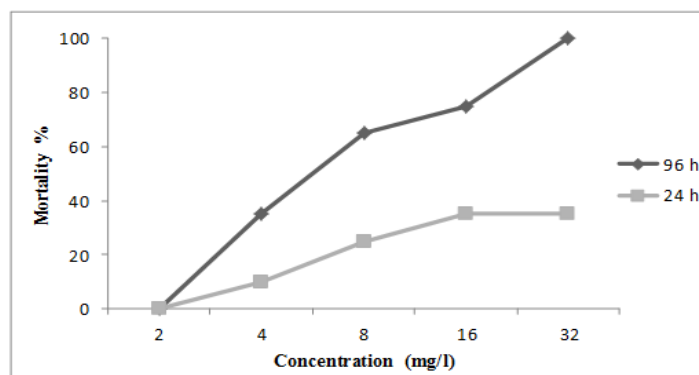

Figure 1. Mortality of *Capoeta fusca* after 24 h and 96 h exposure to different concentrations of copper

In general, higher hardness is beneficial by reducing metal toxicity to fish (27). Rathore and Khangarot, (2003) (28) found that the toxicity of mercuric chloride decreased with increasing water hardness. The 96 h LC_{50} value for copper sulphate was higher in the present study than values available in the literature; the reason might be due to high water hardness (360 mg/L). In Shuhaimi-Othman Study (2010) (22), the water hardness was low, and the water was categorized as soft water (<75 mg/L as $CaCO_3$). Gundogdu. (2008)(21), used test water with pH= 7.4 and a total hardness of 249.56 mg/L as $CaCO_3$.

In the present study, water hardness was 360 mg/L as $CaCO_3$ and the pH of the test water was higher than eight. The characteristics of the test water (hardness and pH) were higher in the present study than values available in the literature. Khangarot et al., (1985) (29) reported that the acute toxicity to the common carp fry (*Cyprinus carpio*) decreased with increasing pH 5.5–8.5. It was found that at low pH (pH<7) mercury was more toxic compared to higher pH (pH>5), which might be due to acid toxicity itself causing bicarbonate loss in the body fluid (19). At low pH, metals are usually in their most bioavailable 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 (25). Results of bioassay are still fragmentary and highly variable as the change in species, chemical, biological, and environmental factors influence the toxicity (19).

It seems that two factors, water hardness and pH levels, could affect the acute toxicity of copper sulphate on the *C. fusca* Behavioral changes are the most sensitive indication of potential toxic effects.

The test animals' abnormal behavior varied according to the test solution concentration (19). Behavioural changes were observable within the first hour. In the control and the 2 mg/L concentration of copper sulphate, groups there were no behavioral changes or deaths throughout the experiment. In low concentrations (4 mg/L), the fish showed some abnormal swimming and tended to gather at the surface. Fish exposed to concentrations of $CuSO_4$ (8 mg/L) demonstrated some abnormal behavior.

They tried to avoid the toxic water with fast swimming; the fish were observed to have breathing difficulties and tried to breathe air from the surface water. Finally, the fish swam downward and settled in the bottom of the aquarium until they died. Fish exposed to higher concentrations (16 and 32 mg/L) tried to avoid the toxic water with fast swimming and jumping; and they showed jerky movements. Lastly, they settled on the bottom of aquarium; and after some time their bellies turned upward and the fish died. The major cause of mortality might be due to respiratory epithelium damage by oxygen culminating in the formation of a mucus film over the gills of fish (19).

CONCLUSION

According to the results of this experiment, the LC_{50} values decreased with time, and about 50% of all mortalities occurred at the first 24 h. 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. Also, behavioral changes increased with increased concentration. We employed two different methods of data evaluation for acute toxicity bioassay. Our results were similar in the two methods used. The environmental contamination with this metal can represent a great threat for the

fish populations and a serious problem for the aquaculture.

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