

Acute Toxicity and the Effects Of Copper Sulphate [CuSo₄.5H₂O] on the Behavior of the Gray Mullet [Mugil Cephalus]

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Abstract

In this study, 96h LC₅₀ values of copper sulphate [CuSo₄.5H₂O], a highly toxicant heavy metal, on gray mullet, Mugil Cephalus of average weight mean weight 7.42 g; mean length 6.51cm was determined. At the first, for rang finding test fish were exposed to CuSo₄ at several selected concentrations 1, 5, 10, 15, 20 and 30 mg/L, then then fish exposed to five concentrations control, 35, 40, 45, 50, 55 and 60 ppm of CuSo₄ for LC₅₀-96h. Experiment was carried out in triplicate and 21 fish per each treatment, Physicochemical properties of water were measured continuously throughout the experiment. The temperature, pH, dissolved oxygen and salinity were 31°C, 7.75, 8.14 mgO₂ L⁻¹ and 37 ppt respectively. Number of mortality and behavioral responses of fish were recorded after 24, 48, 72 and 96 h. LC₅₀ values were determined with probite analysis. The 96 hour LC₅₀ value of CuSo₄ to the fish was found to be 39.68 ppm. In addition, behavioural changes increased with increased concentration. 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: Cupper Sulphate; Mortality; Acute Toxicity; Marine fish; Lethal Dose 50;

Introduction

Toxic pollutants including heavy metals are ubiquitous in polluted aquatic environment. Heavy metals are continually released in to the aquatic environment from natural processes such as volcanic activity, weathering of rocks and industrial processes [24]. Today, aquatic animals are exposed to different concentrations of metals in aquatic environments. These metals tend to accumulate in their bodies which could influence all aspects of organisms' life [5]. Cu is an essential microelement for living organisms, however at higher concentrations it is very toxic for aquatic life [1]. Cu is introduced to water bodies from industrial waste or use of Cu sulphate as therapeutic or algaecide agent [23]. Copper in the form of copper sulphate is used as an algaecide and as a therapeutic chemical for various ectoparasitic and bacterial infections [20]. Median lethal dose [LC₅₀] tests can measure the susceptibility and survival potential of animals to particular toxic substances such as heavy metals [2].

Higher LC₅₀ values are less toxic because greater concentrations are required to produce 50% mortality in animals. Copper salts [copper hydroxide, copper carbonate and copper sulphate] are widely used in agriculture as fungicide, algaecide and nutritional supplement in fertilizers. They are also used in veterinary practices and industrial.

aims of the present study were to investigate acute effects of Cupper Sulphate as potential dangerous additives to assess mortality effects of these heavy metal on a valuable cultured fish of Iran, Mugil cephalus. Mugilidae are widely distributed in the Egyptian inland waters [3]. They are the main source of fish food and are economically important in inland fisheries and aquaculture resources in Egypt [6]. Mugil cephalus has a promising market potential in Europe, East and South Asia [7]. It is also an important aquaculture species in Iran. The consumer demand stimulated the development of intensive aquaculture of these species in Asian countries [8].

Materials and Methods

Fish and condition

For this experiment 168 healthy specimen of gray mullet [Mugil cephalus] [mean weight 7.42 g; mean length 6.51cm] were captured from the coastal water of Chabahar. The animals were transported to aquaculture research center of Offshore Fisheries Research Center, in a many containers equipped with an oxygen capsule and were acclimatized for a period of seven days at 30°C under a constant 12:12 L:D photo period. Before toxicological tests, fish were acclimated to laboratory conditions [pH 7.75; DO 8.25 mg O₂ L⁻¹] for a minimum of 1 week in a 300-L tank with sea water. Acclimatized fish were fed daily a formulated feed. Dead fish were immediately removed with special plastic forceps to avoid possible deterioration of water quality [21].

Rang Finding Test

All fishes were exposed to CuSo₄ at several selected concentrations [1, 5, 10, 15 20, 30 ppm] for determine CuSo₄ dose

in acute toxicity test. Groups of 21 fish were exposed to different concentrations of CuSO₄ for 96 h in fiberglass tank. Test medium was not renewed during the assay and no food was provided to the animals. Values of mortality were measured at time 0, 24, 48, 72 and 96 h.

Behavior observation

Behavioral changes of the fish before and after the application of the toxic compound were monitored. Physiological responses like rapid opercular movement and frequent gulping of air was observed during the initial stages of exposure after which it became occasional. The dead fish were removed from the tank every 12 h during the experiments.

Acute toxicity tests

According to results obtain from range finding test, Acute toxicity tests were carried out in order to calculate the 96h-LC for Copper Sulphate. Concentration for Acute toxicity tests were [control, 35, 40, 45, 50, 55 and 60 ppm]. Mortality was recorded after 24, 48, 72 and 96h and LC₅₀ values and its confidence limits [95%] were calculated by Boudou and Ribeyre [9]. Percentages of fish mortality were calculated for each Copper Sulphat concentration at 24, 48, 72 and 96 h of exposure.

Also LC₅₀ values were calculated from the obtained data in acute toxicity bioassays, by Finney’s method of “probit analysis” and with SPSS computer statistical software. In Finney’s method, the LC₅₀ value is derived by fitting a regression equation

arithmetically and also by graphical interpolation by taking logarithms of the test chemical concentration on the X axis and the probit value of percentage mortality on the Y axis [10].

The LC_{1,10,30,50,70,80,90,99} values were derived using simple substitution probit of 1,10,30,50,70,80,90 and 99 respectively for probit of mortality in the regression equations of probit of mortality vs. Copper Sulphate. The 95% confidence limits for LC₅₀ were estimated by using the formula LC₅₀ [95% CL] = LC₅₀ ± 1.96 [SE [LC₅₀]]. The SE of LC₅₀ is calculated from the formula: b=the slope of the Copper Sulphate/probit response [regression] line; p=the number of Copper Sulphate used, n = the number of animals in each group, w = the average weight of the observations [9].

Results

The results from acute toxicity test as for the Copper Sulphate on gray mullet fish are shown in Table 2. There was no mortality in the control group throughout the experiment Table 1. Fish mortality was increased significantly when the concentrations and the time of exposure were increased.

Table 1: Cumulative mortality of gray mullet during acute exposure to Copper Sulphate [n=21, effective dose]

Concentration (ppm)	No. of died fishes			
	24h	48h	72h	96h
Control	0	0	0	0
35	0	3	3	9
40	0	3	6	15
45	0	9	12	15
50	0	9	15	18
55	0	12	15	18
60	3	12	15	18

As it was expected, the 96-h LC₅₀ values were decreased with prolonged exposure time. This denotes on increase in toxicity with exposure duration. Before death, fish showed rapid gill movement, nervous movements, erratic swimming, imbalance

Table 2: Lethal Concentrations (LC) of 1-99 of Copper Sulphate depending on time (24-96h) for gray mullet

point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC1	53.24	18.2 (-0.03-26.76)	18.33 (5.14-25.45)	10.5
LC10	59.26	34.01 (24.76-38.79)	31.37 (23.76-35.74)	23.6
LC30	63.63	45.47 (41.29-48.95)	40.82 (36.67-43.77)	33.1
LC50	66.66	53.41 (49.86-58.87)	47.37 (44.47-50.47)	39.68
LC70	69.68	61.34 (56.53-70.58)	53.91 (50.77-58.68)	46.26
LC80	71.51	66.14 (60.27-78.13)	57.91 (54.15-64.08)	50.24
LC90	74.05	72.8 (65.31-88.59)	63.36 (58.61-71.78)	55.76
LC99	80.08	88.61 (77.02-113.70)	76.41 (68.85-90.45)	68.86

and inability to remain upright. The 96 hour LC₅₀ value of Copper Sulphate to the fish was found to be 39.68 ppm.

Table 2: Lethal Concentrations [LC] of 1-99 of Copper Sulphate depending on time [24-96h] for gray mullet.

Discussion

Toxicity of Cu is related to gill dysfunction and sodium loss, respiration stress and oxidative stress [Roméo et al., 2000] [22]. To the present research aims to investigate Acute Toxicity Test of Copper Sulphate [CuSo₄] in gray mullet [Mugil cephalus]. In the present study, LC₅₀ values indicated that Copper Sulphate [CuSo₄] was toxic to the studied fish. The 96 hour LC₅₀ value of [CuSo₄] to the fish was found to be 39.68 ppm. A significant increase in mortality was observed in gray mullet exposed to 35 ppm of Copper Sulphate. LC₅₀ values indicated that Copper Sulphate is a toxic metal for gray mullet. LC₅₀ obtained in the present study correspond to values that have been published in the literature for other species of fish.

The median lethal concentration 96 h [LC₅₀] value of copper and lead in other aquatic organisms was reported as 300 ppm for lead in Tench tinca which is higher than the present study [16]. The LC₅₀ for R. sumatrana, for 24, 48, 72 and 96 h for Cu were 54.2, 30.3, 18.9 and 5.6 ppm and for P. reticulata, LC₅₀ for 24, 48, 72 and 96 for Cu were 348.9, 145.4, 61.3 and 37.9 ppm respectively which are lower than that achieved in the present study [17]. The 24 h- LC₅₀ of Cu was reported as 1.17 ppm for P. reticulata which are lower than that achieved in the present study reported that with juvenile Brazilian indigenous fishes, curimata Prochilodus vimbooides and piauçu Leporinus macrocephalus, 96 h- LC₅₀ of copper were 0.047 and 0.090 ppm, for curimatã and piauçu, respectively, which are considerably lower than the present study [18, 19]. Nekoubin et al 2012 reported that with Grass Carp 96 h- LC₅₀ of copper Sulphate were 1.717 ppm.

Previous studies have showed higher total Cu LC₅₀ values for Japanese flounder, Paralichthys olivaceus [8.7–12.2 ppm, 0.3 – 17 g] and red sea bream, Pagrus major [2.0–5.2 ppm, 0.5 – 13 g] compared to present study [25].

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