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Mortality Response of Silver Carp (*Hypophthalmicthys molitrix*), Gold Fish (*Carassius auratus*) and Roach (*Rutilus rutilus*) to Acute Exposure of Copper Sulphate

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Abstract: LC_{50} is the ambient aqueous chemical activity causes 50% mortality in an exposed population. In present study there were investigated acute effects of copper sulphate as potential dangerous additives to assess mortality effects of this heavy metal on some main cultured fish of Iran. Goldfish, silver carp and roach were exposed to the copper salt (0, 0.5, 1, 2, 4 and 8 ppm). LC_{50} was determined with Spss software. As there can found LC_{50} of copper sulphate in goldfish was higher than other studied species, however roach had the lowest one. The 96h toxicity tests were 3.02±0.53, 0.98±1.98 and 0.62±0.6 for the goldfish, silver carp and roach respectively. LC_{50} values indicated that copper sulphate is more toxic to cultured fish, especially roach.

Key words: Fish % LC_{50} % Copper Sulphate % Toxicity Test

INTRODUCTION

LC_{50} is the ambient aqueous chemical activity causes 50% mortality in an exposed population. These calculations are based on two important assumptions. The first assumption is that the exposure time associated with the specified LC_{50} is sufficient to allow almost complete chemical equilibration between the fish and the water. The second assumption is that the specified LC_{50}, the minimum LC_{50} that kills the fish during the associated exposure interval. Fortunately, most reliable LC_{50} satisfy these two assumptions [1]. The 96-h LC_{50} tests are conducted to measure the susceptibility and survival potential of organisms to particular toxic substances such as copper sulphate pollution. Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in organisms [2].

Acute toxicity data can help identify the mode of toxic action of a substance and may provide information on doses associated with target-organ toxicity and lethality that can be used in setting dose levels for repeated-dose studies. This information may also be extrapolated for use in the diagnosis and treatment of toxic reactions in humans. The results from acute toxicity tests can provide information for comparison of toxicity and dose-response among members of chemical classes and help in the selection of candidate materials for further work [3].

Heavy metals are one of the most relevant of aquatic ecosystems [4], also considering the growing cases of environmental accidents in the last years in to the urban waters, the aims of the present study were to investigate acute effects of copper sulphate as potential dangerous additives to assess mortality effects of these heavy metal on some valuable cultured fish of Iran, Silver Carp (*Hypophthalmichthys molitrix*), Goldfish (*Carassius auratus*) and Roach (*Rutilus rutilus*).

MATERIALS AND METHODS

Acute toxicity tests were conducted on silver carp (~ 45 g and 18 cm), goldfish (~ 15 g and 7 cm) and roach (~ 3.5 g and 7 cm). Only healthy fish, as indicated by their activity and external appearance, were maintained alive on board in a fiberglass tank. Samples transferred to a 400-L aerated tank equipped with aeration with 200 L of test medium [5].

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Copper sulphate tested concentrations were 0, 0.5, 1, 2, 4 and 8 ppm, groups of 21 fish were exposed to different concentrations of copper sulphate for 96 h in fiberglass tank. Test medium was not renewed during the assay and no food was provided to the animals. Values of mortalities were measured at time 0, 24, 48, 72 and 96 h [6].

Acute toxicity tests were carried out in order to calculate the 96h-LC_{50} for copper sulphate. Mortality was recorded after 24, 48, 72 and 96h and LC_{50} values and its confidence limits (95%) were calculated by Boudou and Ribeyre [7]. Percentages of fish mortality were calculated for each copper sulphate concentration at 24, 48, 72 and 96 h of exposure.

Also LC_{50} values were calculated from the obtained data in acute toxicity bioassays, by Finney's [8] method of ''probit analysis'' and with SPSS computer statistical software. In Finney's method, the LC_{50} 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 [8].

The 95% confidence limits of the LC_{50} values obtained by Finney's method were calculated with the formula of Mohapatra and Rengarajan [9]. Probit transformation adjusts mortality data to an assumed normal population distribution that results in a straight line. Probit transformation is derived from the normal equivalent deviate (NED) approach developed by Tort *et al*. [10], who proposed measuring the probability of responses (i.e., proportion dying) on a transformed scale based in terms of percentage of population or the standard deviations from the mean of the normal curve [4].

The $LC_{1,10,30,50,70,90,99}$ values were derived using simple substitution probit of 1,10,30,50,70,90 and 99 respectively for probit of mortality in the regression equations of probit of mortality vs. copper sulphate. The 95% confidence limits for LC_{50} were estimated by using the formula LC_{50} (95% CL) = LC_{50}±1.96 [SE (LC_{50})]. The SE of LC_{50} is calculated from the formula: $SE(LC50) = 1/b\sqrt{pnw}$ Where: 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 (Hotos and Vlahos, 1998). At the end of acute test, the LOEC and NOEC were determined for each endpoint measured. In addition, the maximum acceptable toxicant concentration (MATC) was estimated for the endpoint with the lowest NOEC and LOEC [11].

RESULTS AND DISCUSSION

The mortality of studied fishes for copper sulphate doses 0, 0.5, 1, 2, 4 and 8 ppm were examined during the exposure times at 24, 48, 72 and 96 h (Tables 1-3). Fishes exposed during the period 24-96h had significantly increased number of dead individual with increasing concentration. There were significant differences in number of dead fish between the duration 24-96 in each. There was 100% mortality at 2, 4 and 8 ppm concentration within the 96 h after dosing for the roach and silver carp and no mortality at 0.5 ppm within the exposure times for the silver carp and goldfish.

Table 1: Cumulative mortality of silver carp during acute exposure to copper sulphate (n=21, each concentration)

	No. of mortality			
Concentration (ppm)	24h	48h	72h	96h
Control	0	0	0	0
0.5	0	0	0	0
1	0	6	9	13
2	5	15	18	20
4	14	20	21	21
8	18	21	21	21

Table 2: Cumulative mortality of roach during acute exposure to copper sulphate (n=21, each concentration).

	No. of mortality			
Concentration (ppm)	24h	48h	72h	96h
Control	0	0	0	0
0.5	10	12	13	13
1	12	14	15	15
2	15	19	21	21
4	18	20	21	21
8	19	21	21	21

Table 3: Cumulative mortality of goldfish during acute exposure to copper sulphate (n=7, each concentration)

	No. of mortality			
Concentration (ppm)	24h	48h	72h	96h
Control	0	0	0	0
0.5	0	0	0	0
1	0	1	1	3
2	0	1	3	6
4	0	2	7	14
8	0	6	15	21

Table 4: Lethal Concentrations (LC_{1-99}) of copper sulphate (mean ± Standard Error) depending on time (24-96h) for goldfish.

	Concentration (ppm) (95 % of confidence limits)			
Point	24h	48h	72h	96h
LC_1	-	1.12 ± 0.78	0.40 ± 0.51	0.36 ± 0.53
LC_{10}	-	4.94 ± 0.78	2.50 ± 0.51	1.07 ± 0.53
LC_{30}	-	7.71 ± 0.78	4.60 ± 0.51	2.25 ± 0.53
LC_{50}	-	9.62 ± 0.78	6.06 ± 0.51	3.02 ± 0.53
LC_{70}	-	11.5 ± 0.78	7.52 ± 0.51	3.78 ± 0.53
LC_{90}	-	14.3 ± 0.78	9.63 ± 0.51	4.88 ± 0.53
LC_{99}	-	18.1 ± 0.78	12.5 ± 0.51	6.40 ± 0.53

Table 5: Lethal Concentrations (LC_{1-99}) of copper sulphate (mean ± Standard Error) depending on time (24-96h) for silver carp

	Concentration (ppm) (95 % of confidence limits)			
Point	24h	48h	72h	96h
LC_1	0.18 ± 0.42	0.10 ± 0.77	0.06 ± 0.74	0.37 ± 1.98
LC_{10}	1.21 ± 0.42	0.76 ± 0.77	0.62 ± 0.74	0.64 ± 1.98
LC_{30}	2.94 ± 0.42	1.23 ± 0.77	1.02 ± 0.74	0.84 ± 1.98
LC_{50}	4.41 ± 0.42	1.56 ± 0.77	1.30 ± 0.74	0.98 ± 1.98
LC_{70}	5.34 ± 0.42	1.89 ± 0.77	1.58 ± 0.74	1.12 ± 1.98
LC_{90}	7.08 ± 0.42	2.37 ± 0.77	1.99 ± 0.74	1.32 ± 1.98
LC_{99}	9.47 ± 0.42	3.02 ± 0.77	2.55 ± 0.74	1.59 ± 1.98

Table 6: Lethal Concentrations (LC_{1-99}) of copper sulphate (mean ± Standard Error) depending on time (24-96h) for Roach

	Concentration (ppm) (95 % of confidence limits)			
Point	24h	48h	72h	96h
LC_1	0.54 ± 0.2	0.01 ± 0.6	0.01 ± 0.6	0.01 ± 0.6
LC_{10}	0.76 ± 0.2	0.07 ± 0.6	0.07 ± 0.6	0.07 ± 0.6
LC_{30}	0.95 ± 0.2	0.40 ± 0.6	0.40 ± 0.6	0.40 ± 0.6
LC_{50}	1.25 ± 0.2	0.62 ± 0.6	0.62 ± 0.6	0.62 ± 0.6
LC_{70}	3.46 ± 0.2	0.85 ± 0.6	0.85 ± 0.6	0.85 ± 0.6
LC_{90}	6.66 ± 0.2	1.18 ± 0.6	1.18 ± 0.6	1.18 ± 0.6
LC_{99}	11.0 ± 0.2	1.63 ± 0.6	1.63 ± 0.6	1.63 ± 0.6

Median lethal concentrations of 1%, 10%, 30%, 50%, 70%, 90% and 99% test were in tables 4-6. Because mortality (or survival) data were collected for each exposure concentration in a toxicity test at various exposure durations (24, 48, 72, or 96 hours), data can be plotted in other ways; the straight line of best fit is then drawn through the points. These were time-mortality lines. As there can found LC_{50} of copper sulphate in goldfish was higher than other species, however roach had the lowest one.

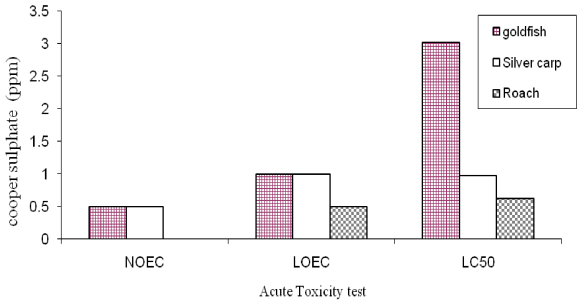

Fig. 1: Acute toxicity testing statistical endpoints of copper sulphate

Toxicity Testing Statistical Endpoints are in Fig 1. LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) were same for all studied fishes, however LC_{50} (the median Lethal Concentration) had significant different between species. The Maximum Acceptable Toxicant Concentration (MATC) for the goldfish, silver carp and roach were 0.30, 0.09 and 0.06 ppm of copper sulphate respectively.

As in our results the *y*-axis represents percentage mortality and the *x*-axis represents concentration of copper sulphate. Both variables increased with distance from origin. The cumulative responses to copper sulphate concentrations yield the sigmoid (S-shaped) curve [4].

In determining the toxicity of a new chemical to fish, an acute toxicity test is first conducted to estimate the median lethal concentration (LC_{50}) of the chemical in water to which organisms are exposed [4]. The relationship between the degree of response of test organisms and the quantity of exposure to the chemical almost always assumes a concentration-response form. variability in acute toxicity even in a single species and single toxicant depending on the size, age and condition of the test species along with experimental factors. The differences in acute toxicity may be due to changes in water quality and test species [12].

In the present study, LC_{50} values indicated that copper sulphate is more toxic to cultured fish, especially roach. 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 and even different time, but lower value of LC_{50} for studied fish was important and confirm sensitively of aquaculture species to low copper sulphate doses. Although the LC_{50} under a defined set of environmental conditions can provide useful information, the numeric value can not used in the field, so in continue we used some biomarkers for better understanding of copper sulphate toxicity.

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