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Determination of LC_{50} of Lead Nitrate and Copper Sulphate in Common Carp (*Ciprinus carpio*)

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Abstract: In this study 96-h LC_{50} value of lead and copper was determined for the Juvenile common carp (*Ciprinus carpio*). Experimental fishes which were measured an average length 7.3±0.44 cm and weight 4.3±0.5 g. The common carp in experimental treatments were fed commercial pelleted food with 4 percent body weight of fish (2 times a day). Eleven groups (with three replication) of experimental fish (containing 20 fish in each group) were exposed to different concentrations of lead 0, 100, 200, 220, 240, 260, 280, 300 and 320ppm, respectively for 96 hrs. Simultaneously, eleven group of experimental fish (containing 20 fish in each group) were exposed to different concentrations of copper 0, 0.5, 0.75, 1, 1.5, 2, 2.5, 3 and 3.5ppm, respectively for 96 hrs. The result showed that lethal concentration (LC_{50}) for lead and copper are 2.624ppm and 279.557ppm, respectively.

Key words: Lead · Copper · Common Carp · (*Ciprinus carpio*) · LC_{50}96 H

INTRODUCTION

Heavy metal contamination severely interfere with ecological balances of an ecosystem and produces devastating effects on environment quality anthropogenic inputs like waste disposal directly adds to the burden of environmental degradation [1]. Coastal seawater is easily contaminated by heavy metals due to human activities with heavy metal contamination reported in aquatic organisms [2]. The problem has become more serious for aquatic species that live close to the coastline where heavy metals tend to accumulate [3]. The fact that increasing use of contaminating chemicals in many industrialized parts of the world makes the development of ecotoxicity measurement techniques an absolute necessity [4]. Assessment of toxicity on particular organism exposed to a particular toxicant will reveal facts regarding the health of given ecosystem and would eventually help us to propose policies to protect the ecosystem. Toxicity tests will reveal the organisms sensitivity to a particular toxicant that would help us to determine the permissible limit of a toxicant in an ecosystem. Heavy metals such as mercury and lead have gained wide interest in the scientific community in recent years due to their potential human health hazards [5]. The toxicity of any pollution is either acute or chronic. Although the toxicant impairs the metabolic and physiological activities of the organisms, physiological studies alone do not satisfy the complete under toxic stress. Acute toxicity test in used to determine the concentration of a test material or the level of an agent the produces a deleterious effect on a group of test organism during a short-term exposure under controlled conditions. All toxicants are capable of severally interfering with the biological systems that producing damage to the structure and function of particular organism and ultimately to its survival. Acute toxicity test constitute only one of the many tools available to the aquatic toxicologists but they are the basic means of provoking a quick, relatively inexpensive and reproducible estimate of the toxic effects of a test material [6]. The 96-h LC_{50} tests are conducted to measure the susceptibility and survival potential of organisms to particular toxic substances such as heavy metals. Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in organisms [7]. And Copper is a very important element, which could influence the metabolism of the human body and it is also a nutritional element for living

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beings. But if the intake is too much, it will cause toxicity [8]. The toxicity of any pollutant is either acute or chronic. Although the toxicant impairs the metabolic and physiological activities of the organisms, physiological studies alone do not satisfy the complete understanding of pathological conditions of tissues under toxic stress.

Majority of the studies concerning the effects of heavy metals on fish have been confined to the acute toxicity test with the death of fish as an end point. Hence, in the present study, an attempt has been made to assess the acute toxicity of lead nitrate and copper sulphate on common carp (*Ciprinus carpio*).

MATERIALS AND METHODS

Metal toxicity tests were conducted under laboratory conditions. Juvenile common carp selected for this study were obtained from the fish seed hatchery in Gorgan, Iran. common carp measuring 7.3±0.44cm in length and weighing 4.3 ± 0.5g were used for the experiment. They were brought to the laboratory and acclimatized for 14 days and The fish were fed with commercial pelleted food at least once a day during this period. All glassware and aquariums used in this experiment were washed and thoroughly rinsed with deionized water prior to use. Prior to each trial, all aquariums (60 L) capacity, were filled with 50 L of dechlorinated tap water. Stock solutions of copper sulphate and lead nitrate were prepared by dissolving analytical grade copper sulphate ($CuSo_4$ from Merck) and lead nitrate ($Pb(No_3)_2$ from Merck), respectively in double distilled water. Thirty fishes were used per concentration of each heavy metal. Separate groups of 30 fish each served as controls for copper sulphate and lead nitrate. The physico-chemical characteristics of the water were analyzed as per the procedure of APHA [9]. The mean values for the water qualities tested were as follows, temperature 24±1°C, pH 7-7.5, dissolved oxygen 7.8±0.2mg/l and the experimental medium was aerated in order to keep the amount of oxygen not less than 4 mg/l, alkalinity 225±2.58mg/l as $CaCo_3$, total hardness 452±3.5mg/l, Photoperiodicity 12 h light and day Turbidity 2.

For determination of the LC_{50} (lethal concentration) values, fallowing a range finding test, eight Pb (100, 200, 220, 240, 260, 280, 300 and 320 mg/L) and Cu (0.5, 0.75, 1, 1.5, 2, 2.5, 3 and 3.5 mg/L) concentrations were chosen for common carp for each metal-treated and control three replications were conducted. The number of dead fish was counted every 12 hrs and removed immediately from the aquaria. The mortality rate was determined at the end of 24, 48, 72 and 96 hrs. Acute Toxicity test was conducted in accordance with standard methods (APHA). In this study the acute toxic effect of lead and copper on the common carp was determined by the use of Finney's Probit Analysis LC_{50} Determination Method [10]. Confidential limits (Upper and Lower) were calculated and also used SPSS18 for LC_{50} value of lead and copper with the help of probit analysis.

RESULTS AND DISCUSSION

Table 1 shows the relation between the lead nitrate concentration and mortality rate of common carp according to SPSS18 analysis. Mortality in control treatment, 100, 200 and 220ppm concentration of lead were virtually absent and found to be suitable for LC_{50} upper and lower confidence. according to SPSS18 analysis showed that the median lethal concentration (LC_{50}) of lead to common carp for 96 hrs of exposure is 279.557ppm the lower and upper lethal confidence limits for lead nitrate indicate a wide range of 272.717 to 286.837ppm within which the concentration response for 96 h exposure. (Table 2).

Table 3 shows the relation between the copper sulphate concentration and mortality rate of common carp according to SPSS18 Analysis. Mortality in control treatment, 0.5, 0.75 and 1ppm concentration of lead were virtually absent and found to be suitable for LC_{50} upper and lower confidence limits and fitted for regression equation along with slope function for 96hrs exposed period. According to SPSS18 analysis showed that the median lethal concentration LC_{50} values was 2.624ppm the lower and upper lethal confidence limits for copper sulphate indicate a wide range of 2.466 to 2.793ppm within which the concentration response for 96 hrs exposure (Table 4).

Table 1: Showing correlation between the lead nitrate concentration and the mortality rate of common carp.

Conc.	Number of fishes	Observed Responses	Expected Responses	Residual	Probability
0.000	30	0	0.001	-0.001	0.000
0.500	30	0	0.022	-0.002	0.001
0.750	30	0	0.075	-0.075	0.003
1.000	30	0	0.226	-0.226	0.008
1.500	30	1	1.386	-0.386	0.046
2.000	30	5	5.251	-0.251	0.175
2.500	30	16	12.786	3.214	0.426
3.000	30	21	21.392	0.392	0.713
3.500	30	26	27.151	-1.151	0.905

Table 2: Showing LC_{50} value of lead nitrate with lower and upper (95%) confidence limits

	Estimated LC Values and Confidence Limits		
		95% Confidence Limits	
Point	Concentration (mg/l)	Upper	Lower
LC 1.00	1.070	0.586	1.383
LC 5.00	1.525	1.168	1.765
LC 10.00	1.768	1.473	1.973
LC 15.00	1.932	1.676	2.117
LC 50.00	2.624	2.466	2.793
LC 85.00	3.317	3.109	3.617
LC 90.00	3.480	3.249	3.823
LC 95.00	3.723	3.455	4.131
LC 99.00	4.178	3.834	4.716

Table 3: Showing correlation between the copper sulphate concentration and the mortality rate of common carp

Conc.	Number of fishes	Observed Responses	Expected Responses	Residual	Probability
00.00	30	0	0.000	0.000	0.000
100.00	30	0	0.000	0.000	0.000
200.00	30	0	0.139	-0.1391	0.005
220.00	30	0	0.771	-0.771	0.026
240.00	30	3	2.936	0.064	0.098
260.00	30	10	7.836	2.164	0.261
280.00	30	16	15.173	0.827	0.506
300.00	30	21	22.444	-1.444	0.748
320.00	30	27	27.211	-0.211	0.907

Table 4: Showing LC_{50} value of copper sulphate with lower and upper (95%) confidence limits

	Estimated LC Values and Confidence Limits		
		95% Confidence Limits	
Point	Concentration (mg/l)	Upper	Lower
LC 1.00	208.428	186.212	222.693
LC 5.00	229.265	212.939	240.097
LC 10.00	240.373	226.991	249.572
LC 15.00	247.867	236.329	256.108
LC 50.00	279.557	272.717	286.837
LC 85.00	311.246	302.051	324.620
LC 90.00	318.741	308.465	334.079
LC 95.00	329.849	317.828	348.243
LC 99.00	333.085	320.532	352.392

Heavy metals are some of the most-active polluting substances as can cause serious impairment to circulatory, metabolic, physiological and even structural systems when high concentrations are present in aquatic ecosystems [11]. Although heavy metals are often referred to as a common group of pollution, individual metals pose different problems in freshwater environments and therefore they have to be considered separately [12].

Susceptibility of common carp to the lethal effect of experimental heavy metal was duration and concentration dependent as mortality increased with an increase in its concentration. LC_{50} value of copper sulphate and lead nitrate divulges the susceptibility of common carp to lethal concentration of copper and lead, depicts that the toxicity is dilution and duration depended. Higher percent of mortality occurred with increase in concentration and exposure period, hence confirm the observation made in case of salmonids, *Oncorhynchus mykis, Salvelinus confluentus and Oncorhynchus tshawytscha* [12-14], guppy, *Poecilia reticulate* [15], *Cyprinus carpio* [16], Nile tilapia *Oreochromis niloticus* [17] and rohu, *labeo rohito* [18].

We employed SPSS18 Analysis for evaluating the acute toxicity response of lead nitrate and copper sulphate. Analysis gave 96 h LC_{50} value for common carp exposed to copper sulphate and lead nitrate concentrations as 279.557ppm and 2.624ppm, respectively. 95% lower and upper confidence limits for the LC_{50} for, lead nitrate was 272.717ppm and 286.837ppm, also copper sulphate was 2.466ppm and 2.793ppm, respectively. Our results are in agreement with Singh [15] and Yilmaz [16]. The results of these studies may provide guidance to selection of acute toxicity to be considered in field biomonitoring efforts designed to detect the bioavailability of lead nitrate and copper sulphate and early warning indicators of this heavy metal toxicity in common carp.

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