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Acute Toxicity of Lead Nitrate and Copper Sulphate in Caspian Roach (*Rutilus rutilus caspicus*)

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Abstract: The aim of present study was to determine the LC_{50} value of a lead and copper in Caspian Roach fingeling. Experimental fishes were procured from Gorgan (Iran) which were measured an average length 8.2±0.45 cm and weight 4.1±0.29 g. There after, they were kept sterile aquaria and the fish were fed with commercial pelleted food. A total of 27 aquariums for with a capacity of 60 L each stocked with 30 fingerlings were used in the experiments for each metals. About 24 aquaria for 8 concentrations of each metal (Pb and Cu). Composed the 24 treatments while 3 other aquaria were used as control. For each treatment, three replications were conducted. Physicochemical parameters like D.O., hardness, chlorine, etc., of aquaria water and the mortality rate of fish were monitored daily. The results indicated that lethal concentration (LC_{50} 96 h) for lead and copper are 276.167 and 2.440 ppm, respectively. Hence, concluded that copper is more toxic than lead for Caspian Roach (*Rutilus rutilus caspicus)*.

Key words: Lead, copper, Caspian Roach , *Rutilus rutilus caspicus*, LC_{50} 96 h, Iran

INTRODUCTION

Coastal seawater is easily contaminated by heavy metals due to human activities with heavy metal contamination reported in aquatic organisms (Olojo *et al*., 2005). The problem has become more serious for aquatic species that live close to the coastline where heavy metals tend to accumulate (Migliarini *et al*., 2005). The fact that increasing use of contaminating chemicals in many industrialised parts of the world makes the development of ecotoxicity measurement techniques an absolute necessity (Brandao *et al*., 1992). 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 (Farombi *et al*., 2007).

In recent years, toxicological studies have gained a fresh momentum and have emerged as a major field of research owing to the gravity of the situation and increasing diversity of aquatic pollutants. The toxicity of this metal on aquatic organisms is influenced by chemical features of water such as pH and hardness (Mance, 1987) and its bioaccumulation is directly related to its concentration in seawater (Sadiq, 1992). 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 (Shuhaimi-Othman *et al*., 2010) 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 beings. But if the intake is too much, it will cause toxicity (Fan *et al*., 2002). 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.

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 (Rani *et al*., 2011). The 1st step is the acute toxicity test on algae, fish, etc., in order to show

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the potential risks of these chemicals (OECD, 1993). 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 (Spacie and Hamelink, 1985). The LC_{50} 96 h 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 (Shuhaimi-Othman *et al*., 2010). 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 Caspian Roach.

MATERIALS AND METHODS

Metal toxicity tests were conducted in the laboratory conditions. Juvenile Caspian Roach selected for this study were obtained from the fish seed hatchery in Gorgan, Iran. Caspian Roach measuring 8.2±0.45 cm in length and weighing 4.1±0.29 g 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 and lead were prepared by dissolving analytical grade lead nitrate ($Pb(NO_3)_2$ from Merck) and copper sulphate (($CuSO_4$)$5H_2O$ 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 lead nitrate and copper sulphate.

The physico-chemical characteristics of the water were analyzed as per the procedure of APHA (1995). The mean values for the water qualities tested were as follows; temperature 24±1°C, pH 7-7.5, dissolved oxygen 7.8±0.2 mg L^{-1} and the experimental medium was aerated in order to keep the amount of oxygen not <4 mg L^{-1}, alkalinity 225±2.58 mg L^{-1} as $CaCO_3$, total hardness 452±3.5 mg L^{-1}, photoperiodicity 12 L:12 D, Turbidity 2.

LC_{50} 96 h determination: For determination of the LC_{50} (Lethal Concentration) values, used 27 aquaria and 8 concentrations for each metal (lead and copper). Fallowing a range finding test, 8 Pb (100, 200, 220, 240, 260, 280, 300 and 320 mg L^{-1}) and Cu (0.5, 0.75, 1, 1.5, 2, 2.5, 3 and 3.5 mg L^{-1}) concentration were chosen for common carp for each metal-treated and control tree replications were conducted. The number of dead fish was counted every 12 h and removed immediately from the aquaria. The mortality rate was determined at the end of 24, 48, 72 and 96 h. Acute Toxicity test was conducted in accordance with standard methods (APHA, 1995). In this study, the acute toxic effect of lead and copper on the Caspian Roach was determined by the use of Finney's Probit Analysis LC_{50} Determination Method. 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

LC_{50} of lead ($Pb(NO_3)_2$): Susceptibility of Caspian Roach to the impact of lead toxicity was found to increase in mortality with an increase in the concentration of lead whereas in the control mortality was virtually absent (Table 1).

Results according to SPSS18 analysis showed the median lethal concentration (LC_{50}) of lead to Caspian Roach for 96 h of exposure was 276.167 ppm, the lower and upper lethal confidence limits for lead nitrate indicate a wide range of 269.412-283.055 ppm within which the concentration response for 96 h exposure (Table 2). There was a gradual decrease in the slope function corresponding to the increase in the exposure period from 24-96 h. Observations on upper and lower confidence limits reveal a decreasing trend from 24-96 h.

Table 1: Correlation between the lead nitrate concentration and the mortality rate of Caspian Roach

Conc.	No. of subjects	Observed responses	Expected responses	Residual	Probability
0.0	30	0	0.0000	0.000	0.000
100.0	30	0	0.0000	0.000	0.000
200.0	30	0	0.1670	-0.167	0.006
220.0	30	1	0.9170	0.083	0.031
240.0	30	4	3.4190	0.581	0.114
260.0	30	11	12.5570	-1.557	0.419
280.0	30	18	15.5250	1.475	0.551
300.0	30	23	23.5960	-0.596	0.787
320.0	30	28	27.8410	0.159	0.928

LC_{50} of copper ($CuSO_4$): Table 3 shows the relation between the copper concentration and the mortality rate for 96 h of Caspian Roach. Results according to SPSS18 analysis showed that the median lethal concentration (LC_{50}) of copper to Caspian Roach for 96 h of exposure is 2.440 ppm. The lower and upper lethal confidence limits for lead nitrate indicate a wide range of 2.293-2.591 ppm within which the concentration response for 96 h exposure (Table 4).

Heavy metal pollution in water is in large part, due to agricultural run-off, industrial waste and mining activities. Mining is by far the biggest contributor to metal pollution. Mine drainage water, effluent from the tailing ponds and drainage water from soil heaps continue to extrude unwanted metals into the aquatic environment (Rani and Sivaraj, 2010). Metal concentrations in aquatic organisms appear to be of several magnitudes higher than concentrations present in the ecosystem (Laws, 2000) and 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 (Wyn *et al*., 2007).

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

Point	Estimated LC values and confidence limits: Concentration (mg L^{-1})	95% confidence limits: Upper	Lower
$LC_{1.00}$	206.382	184.078	220.722
$LC_{5.00}$	226.825	210.279	237.779
$LC_{10.00}$	237.724	224.076	247.043
$LC_{15.00}$	245.077	233.264	253.415
$LC_{50.00}$	276.167	269.412	283.055
$LC_{85.00}$	307.258	298.629	319.626
$LC_{90.00}$	314.611	304.964	328.850
$LC_{95.00}$	325.509	314.194	342.682
$LC_{99.00}$	345.952	331.219	368.915

Susceptibility of Caspian Roach 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 Caspian Roach to lethal concentration of copper and lead depicts that the toxicity is dilution and duration depended. Higher present 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* (Lloyd, 1992; Finlayson and Verrue, 1982; Hansen *et al*., 2002), guppy, *Poecilia reticulate* (Singh *et al*., 2010), *Cyprinus carpio* (Mehmet *et al*., 2004), Nile tilapia, *Oreochromis niloticus* (Mulley *et al*., 2000) and rohu, *Labeo rohito* (Dardenne *et al*., 2007).

Researchers employed SPSS18 analysis for evaluating the acute toxicity response of lead nitrate and copper sulphate. Analysis gave LC_{50} 96 h value for Caspian Roach exposed to lead nitrate and copper sulphate concentrations as 276.167 and 2.440 ppm, respectively. While 95% lower and upper confidence limits for the LC_{50} for copper sulphate was 2.293

Table 3: Correlation between the copper sulphate concentration and the mortality rate of Caspian Roach

Conc.	No. of subjects	Observed responses	Expected responses	Residual	Probability
0.00	30	0	0.001	-0.001	0.000
0.50	30	0	0.015	-0.015	0.001
0.75	30	0	0.063	-0.630	0.002
1.00	30	0	0.224	-0.222	0.007
1.50	30	2	1.674	-0.326	0.056
2.00	30	7	6.848	0.152	0.228
2.50	30	17	16.219	0.781	0.541
3.00	30	24	24.857	0.857	0.829
3.50	30	29	28.911	0.089	0.964

Table 4: LC_{50} value of lead nitrate with lower an upper (95%) confidence limits

Point	Estimated LC values and confidence limits: Concentration (mg L^{-1})	95% confidence limits: Upper	Lower
$LC_{1.00}$	1.066	0.637	1.347
$LC_{5.00}$	1.468	1.417	1.687
$LC_{10.00}$	1.683	1.415	1.872
$LC_{15.00}$	1.828	1.593	1.999
$LC_{50.00}$	2.440	2.293	2.591
$LC_{85.00}$	3.052	2.871	3.306
$LC_{90.00}$	3.197	2.997	3.485
$LC_{95.00}$	3.411	3.180	3.754
$LC_{99.00}$	3.814	3.519	4.226

and 2.591 ppm, also for lead nitrate was 2.969 and 3.695 ppm, respectively. The results are in agreement with Singh *et al.* (2010) and Mehmet *et al.* (2004).

CONCLUSION

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 Caspian Roach.

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