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Full Length Research Paper

Effects of copper sulfate and lead nitrate exposure on caspian sea kutum survival and behavior

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The aim of present study was to determine the LC_{50} value of in Caspian sea kutum. The results indicated that median lethal concentration (LC_{50}) of Caspian sea kutum (*RUTILUS FRISII KUTUM*) for 24, 48, 72 and 96 h of exposure are 2.944, 2.756, 2.562 and 2.310 ppm, respectively and median lethal concentration (LC_{50}) of lead to Caspian sea kutum (*R. FRISII KUTUM*) for 24, 48, 72 and 96 h of exposure as 315.841, 298.456, 281.419 and 268.065 ppm, respectively. LC_{50} increased with decrease in mean exposure times for both metals. Physiological responses like rapid opercular movement and frequent gulping of air was observed during the initial stages of exposure after which it became occasional. All these observations can be considered to monitor the quality of aquatic ecosystem and severity of pollution. Hence, concluded that copper is more toxic than lead for Caspian sea kutum (*R. FRISII KUTUM*).

Key words: Copper sulfate, lead nitrate, *Rutilus frisii kutum*, Caspian sea kutum, physiological responses, median lethal concentration (LC_{50}).

INTRODUCTION

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).

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 there is increasing use of contaminating chemicals in many industrialized parts of the world makes the development of Eco toxicity measurement techniques an absolute necessity (Brandão 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

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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 (Sadik, 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 organism's 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 thus producing damage to the structure and function of particular organism and ultimately to its survival (Rani et al., 2011).

These toxicity tests are necessary to predict the safe concentration of the chemicals in the environment (Johnson and Bergman, 1983). The first step is the acute toxicity test on algae, fish, etc; in order to show the potential risks of these chemical materials (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 96 h LC_{50} tests were 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 (Hilmy et al., 1985). 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 copper and lead on Caspian sea kutum.

MATERIALS AND METHODS

Place, prepared fish and condition to experiment

Metal toxicity tests were conducted in the laboratory conditions. Juvenile Caspian sea kutum selected for this study were obtained from the fish seed hatchery in Gorgan, Iran. Caspian sea kutum measuring 8 ± 0.5 cm in length and weighing 4 ± 0.5 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. A total of 27 aquariums, each stocked with 10 fishes were used in our experiments for each metal. Stock solutions of copper sulfate and lead nitrate were prepared by dissolving analytical grade copper sulfate ($CuSO_4.5H_2O$ from Merck) and lead nitrate ($Pb(No_3)_2$ from Merck), respectively in double distilled water. 30 fishes were used per concentration of each heavy metal. Separate groups of 30 fish each served as controls for copper and lead. The physico-chemical characteristics of the water were analyzed as per the procedure of APHA (1998). The mean values for the water qualities tested were as follows, temperature 24 ± 1°C, pH 7 to 7.5, dissolved oxygen 7.8 ± 0.2 mg/L and the experimental medium was aerated in order to keep the amount of oxygen not less than 4 mg/L, hardness 275 ± 3.58 mg/L as $CaCo_3$, photoperiodicity 12 L: 12 D, turbidity 2.

Bioassay LC_{50}

Ninety-six hours acute bioassays were performed following in general OECD guidelines for fish acute bioassays (guideline OECD203, 92/69/EC, method C1) (OECD, 1993). For determination of the LC_{50} (lethal concentration) values, following a range finding test, eight Cu (0.5, 0.75, 1, 1.5, 2, 2.5, 3 and 3.5 mg/L) and Pb (100, 200, 220, 240, 260, 280, 300 and 320 mg/L) concentrations were chosen for Caspian sea kutum. For each metal-treated and Control, tree replications were conducted. Metal solutions were prepared by dilution of a stock solution with dechlorinated tap water. A control with dechlorinated tap water only was also used. 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. During the toxicity test, the fishes were not fed. Acute toxicity test was conducted in accordance with standard methods (APHA, 1998).

Statistical analysis

In this study, the acute toxic effect of copper sulfate and lead nitrate on the Caspian sea kutum was determined by the use of Finney's Probit Analysis LC_{50} determination method (Finney, 1971). Confidential limits (Upper and Lower) were calculated and SPSS18 was also used for LC_{50} value of copper sulfate and lead nitrate with the help of probit analysis.

Behavior observation

Physiological responses like rapid opercular movement and frequent gulping of air was observed during the initial stages of exposure after which it became occasional.

RESULTS

Acute toxicity of copper and lead showed that mortality is directly proportional to the concentration of the heavy metal copper and lead while the percentage of mortality

Table 1. Showing correlation between the copper concentration and the mortality rate on time (24 – 96 h) of Kutum.

Concentration (mg L^{-1})	N	Mortality rate on time (24 - 96 h)			
		24	48	72	96
0.00	30	0	0	0	0
0.50	30	0	0	0	0
0.75	30	0	0	0	0
1.00	30	0	0	0	1
1.50	30	1	1	2	3
2.00	30	4	5	6	8
2.50	30	11	13	15	18
3.00	30	16	19	21	26
3.50	30	21	24	28	30

is virtually absent in control (Tables 1 and 3).

LC_{50} of cooper for kutum

Table 1 shows the relation between the copper concentration and the mortality rate for 24, 48, 72 and 96 h of kutum. Results according to SPSS18 analysis showed that the median lethal concentration (LC_{50}) of copper to kutum for 24, 48, 72 and 96 h of exposure are 2.944, 2.756, 2.562 and 2.310 ppm, respectively. A gradual decrease in slope function corresponding to an increase in the exposure period from 24 to 96 h was noticed. Observations on the upper and lower confidence limits revealed a decreasing trend from 24 to 96 h (Table 2).

LC_{50} of lead for kutum

Susceptibility of kutum 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 3).

Results according to SPSS18 analysis showed the median lethal concentration (LC_{50}) of lead to kutum for 24, 48, 72 and 96 h of exposure as 315.841, 298.456, 281.419 and 268.065 ppm, respectively. There was a gradual decrease in the slope function corresponding to the increase in the exposure period from 24 h to 96 h. Observations on upper and lower confidence limits reveal a decreasing trend from 24 to 96 h. Also evident was that an increase in exposure period influences increase in mortality (Table 4).

Behavioral changes

The behavior of fish remarkably changed due to the treatment of lead and copper when compared to the control. The various locomotary responses exhibited by fish due to sub lethal concentrations of lead and copper during initial stage of exposure included restlessness, erratic and fast swimming, abrupt change in position and direction, jumping and overall hyperactivity were noticed. The fish showed surfacing tendency throughout the experimental period. Physiological responses like rapid opercular movement and frequent gulping of air was observed during the initial stages of exposure after which it became occasional. Neurological symptoms like jerking movements, frightening and loss of balance were not observed in lead and copper treated kutum. Hence it was concluded that copper is more toxic than lead for kutum.

DISCUSSION

The present study was initiated to find the susceptibility of the kutum to potentially hazardous heavy metals like copper and lead. Median lethal concentration (LC_{50}) of copper to kutum for 24, 48, 72 and 96 h of exposure are 2.944, 2.756, 2.562 and 2.310 ppm, respectively and median lethal concentration (LC_{50}) of lead to kutum for 24, 48, 72 and 96 h of exposure were 315.841, 298.456, 281.419 and 268.065 ppm, respectively. Higher percent of mortality occurred with increase in concentration and exposure period of copper and lead.

The median lethal concentration 96 h (LC_{50}) value of copper and lead in other aquatic organisms was reported as 300 ppm for lead as in *Tench tinca* by Shah and Altindag (2005), which were higher than present study. The LC_{50} for *R. sumatrana*, for 24, 48, 72 and 96 h for Cu were 54.2, 30.3, 18.9 and 5.6 µg/L and For *P. reticulata*, LC_{50} for 24, 48, 72 and 96 h for Cu were 348.9, 145.4, 61.3 and 37.9 µg/L, respectively (Shuhaimi-Othman et al., 2010), which were lower than present study. The 24 h LC_{50} of Cu was reported as 1.17 mg/L for *P. reticulate* (Park and Heo, 2009), which were lower than present study. Gomes et al. (2009) reported that with juvenil

Table 2. Lethal concentration (LC_{1-99}) of copper on time (24 - 96 h) for Kutum.

Point	Concentration (mg L^{-1}), (95% confidence limits)							
	24h		48h		72h		96h	
LC_1	1.023	(0.414-1.396)	1.036	(0.502-1.375)	1.038	(0.572-1.342)	0.928	(0.522-1.201)
LC_5	1.586	(1.158-1.861)	1.540	(1.153-1.795)	1.484	(1.140-1.717)	1.333	(1.028-1.545)
LC_{10}	1.886	(1.547-2.116)	1.808	(1.495-2.025)	1.722	(1.438-1.922)	1.549	(1.294-1.733)
LC_{15}	2.088	(1.802-2.295)	1.989	(1.720-2.184)	1.883	(1.635-2.064)	1.695	(1.471-1.862)
LC_{50}	2.944	(2.753-3.183)	2.756	(2.586-2.949)	2.562	(2.406-2.727)	2.310	(2.165-2.463)
LC_{85}	3.800	(3.502-4.272)	3.522	(3.280-3.884)	3.240	(3.037-3.530)	2.926	(2.745-3.176)
LC_{90}	4.003	(3.670-4.539)	3.703	(3.433-4.116)	3.410	(3.176-3.731)	3.072	(2.873-3.355)
LC_{95}	4.303	(3.915-4.939)	3.971	(3.657-4.463)	3.639	(3.378-4.032)	3.288	(3.058-3.623)
LC_{99}	4.865	(4.370-5.692)	4.475	(4.072-5.120)	4.085	(3.751-4.620)	3.693	(3.401-4.131)

Table 3. Showing correlation between the lead concentration and the mortality rate on time (24 - 96 h) of Kutum.

Concentration (mg L^{-1})	N	Mortality rate on time(24 – 96 h)			
		24	48	72	96
0	30	0	0	0	0
100	30	0	0	0	0
200	30	0	0	0	0
220	30	0	0	0	1
240	30	1	2	3	5
260	30	3	5	8	13
280	30	6	9	15	19
300	30	10	16	21	25
320	30	16	21	27	30

Table 4. Lethal concentration (LC_{1-99}) of lead on time (24 - 96 h) for Kutum.

Point	Concentration (mg L^{-1}), (95% confidence limits)			
	24 h	48 h	72 h	96 h
LC_1	222.078 (185.743-240.943)	214.293 (185.793-231.077)	212.018 (190.220-225.967)	205.120 (185.599-217.770)
LC_5	249.546 (226.223-262.604)	238.948 (219.198-251.136)	232.349 (216.356-242.938)	223.483 (208.958-233.332)
LC_{10}	264.189 (247.095-274859)	252.092 (236.650-262.186)	243.187 (230.089-252.185)	233.330 (221.249-241.790)
LC_{15}	274.068 (260.516-282.789)	260.960 (248.134-269.932)	250.499 (239.211-258.568)	239.973 (229.429-247.610)
LC_{50}	315.841 (304.800-334.010)	298.456 (290.175-309.199)	281.419 (274.663-288.673)	268.065 (261.706-274.525)
LC_{85}	358.615 (338.214-395.102)	335.953 (322.483-358.199)	312.338 (303.206-325.688)	296.156 (288.299-307.123)
LC_{90}	367.494 (345.807-409.860)	344.821 (329.696-370.216)	319.651 (309.453-334.945)	302.800 (294.088-315.333)
LC_{95}	382.137 (356.981-431.814)	357.964 (340.277-388.136)	330.489 (318.572-348.806)	312.647 (302.517-327.653)
LC_{99}	409.604 (377.783-473.152)	382.620 (359.925-421.952)	350.489 (335.423-375.062)	331.118 (318.050-351.041)

Brazilian indigenous fishes, curimata (*Prochilodus vimboides*) and piaucu (*Leporinus macrocephalus*), 96 h LC_{50} of copper were 0.047 and 0.090 mg/L for curimatã and piauçu, respectively, which were lower than present study. This indicates that different organisms have different sensitivity to heavy metals. The toxicity reported by other studies differs from this study probably due to different species used, age, size of the organism, test methods and water quality such as water hardness, as this can affect toxicity (Hodson et al., 1982; McCahon and Pascoe, 1988). Toxicity of metals may vary depending upon their permeability and detoxification

mechanisms (Darmono et al., 1990).

The behavior of fish remarkably changed including restlessness, erratic and fast swimming, abrupt change in position and direction, jumping and overall hyperactivity which were noticed. The fish showed surfacing tendency throughout the experimental period. Physiological responses like rapid opercular movement and frequent gulping of air was observed during the initial stages of exposure after which it became occasional. Neurological symptoms like jerking movements, frightening and loss of balance were not observed in lead and copper treated kutum.

The effect of copper on the gill morphology, directly inducing necrosis, hypertrophy, hyperplasia and high mucus production (Mazon et al., 2002a; Fernandes and Mazon, 2003) and indirectly stimulating proliferation of chloride cells in the secondary lamellae through cortisol (Bonga, 1997), reduces the effectiveness of the respiratory surface, resulting in respiratory impairment.

Conclusion

In the present study, a comparison of LC_{50} values and behavior change indicated that copper was more toxic than lead to fishes. 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 sulfate and early warning indicators of this heavy metal toxicity in Caspian Sea kutum.

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