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Determination of LC_{50} of Copper Sulfate and Lead(II) Nitrate and Behavioral Responses of Grass Carp (*Ctenopharyngodon idella*)

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Abstract

In the present study, grass carp (*Ctenopharyngodon idella*) were exposed to copper and lead for 24, 48, 72 and 96 h. Experimental fishes which measured an average length of 8.2 ± 0.44 cm and weighed 4.3 ± 0.5 g. Seven groups (with three replicates) of experimental fish were exposed to seven concentrations of each metal. The grass carps were kept in 21 tanks each stocked with 30 fishes were used in our experiments for each metal. The median lethal concentration (LC_{50}) of copper to grass carp for 24, 48, 72 and 96 h were 2.422 mg/L, 2.222 mg/L, 2.006 mg/L, and 1.717 mg/L respectively. The median lethal concentration (LC_{50}) of lead to grass carp for 24, 48, 72 and 96 h were 293.747 mg/L, 278.019 mg/L, 260.324 mg/L, and 246.455 mg/L, respectively. In addition, the behavioral changes of grass carp at different metal concentrations were determined. LC_{50} increased as mean exposure times decreased 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. In conclusion, copper is more toxic than lead for grass carp.

Keywords: Copper, lead, grass carp, *Ctenopharyngodon idella*, LC_{50}

Introduction

Extensive uses of metals, the legacies of past contamination and new technologies, continue to pose and important ecological risk in aquatic environment [1]. Metals such as Cu, Cd, Zn, and Pb are released from natural sources as well as human activity. Impact of these metals on the environment is an increasing problem worldwide. Toxicity testing has been widely used as a tool to identify suitable organisms as a bioindicator and to derive water quality standards for chemicals. Toxicity testing is an essential tool for assessing the effect and fate of toxicants in aquatic ecosystems. Heavy metals research especially in using organisms as bioindicators, is still scarce. Therefore, it is important to conduct studies with local organisms that can be used to gain data on metal toxicity, to determine the organism's sensitivity and to derive a permissible limit for water that can protect aquatic communities [2]. Heavy metals such as copper and lead have gained wide interest in the scientific community in recent years due to their potential human health hazards. Copper in the form of copper sulfate is used as an algaecide and as a therapeutic chemical for various ectoparasitic and bacterial infections [3]. Heavy metal concentrations in plant tissues are influenced by metabolic requirements for essential micronutrients such as Cu, whilst non-essential

metals including Pb tend to be excluded or compartmentalized [4]. Copper is an essential micronutrient required in mitochondria and chloroplast reactions, enzyme systems related to photosystem II electron transport, cell wall lignification, carbohydrate metabolism and protein synthesis [5]. Lead is non-essential and higher concentrations may be toxic to some species [6].

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 biological systems producing damage to the structure and function of a particular organism and ultimately to its survival [7].

From the surrounding water, fish may absorb dissolved heavy metals that may accumulate in various tissues and organs and even be biomagnified in the food-chain/web. In the absorption process there are four possible routes for metals to enter a fish: the food ingested; simple diffusion of the metallic ions through gill pores; through drinking water; and by skin adsorption [8]. Amongst fish species, considerable differences in sensitivity to metals have been reported. Salmonids are generally sensitive to high cadmium levels [8]. Carp (*Cyprinus carpio*) and *Catla catla* have the ability to accumulate and concentrate cadmium and iron to the levels several orders of magnitude above those found in their environment [9,10]. In the acute toxicity test, juvenile fish are exposed to a range of toxicant concentrations in a static system for 96 h. A toxic effect is determined by a statistically significant decrease in the survival rate of fish exposed to the toxicant relative to the survival of fish in a control (i.e., without toxicant). Under normal circumstances, metals, which are mainly beneficial, indeed essential, such as copper, may become pollutants when present in excess by exhibiting toxic effects on the organism [11]. The most effective indications of toxic pollution are behavioral changes. Behavioral changes are often evaluated by optomotor responses [12].

The majority of 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 grass carp.

Materials and methods

Fish

210 uniform juveniles of grass carp were obtained from the Institute of Pond Fish Culture in Gorgan (Agh Ghala), Iran. They were wed (initial weight 4.3 ± 0.5 g.). The total lengths of the fish were also measured accurately (8.2 ± 0.44 cm). They were fed with aquatic plant food (*Lemna* sp.) at least twice a day before the experiments but were not fed during the experiments.

Conditions

Metal toxicity tests were conducted under laboratory conditions. This experiment was conducted in a completely randomized design with seven treatments for each metal and three replicates per treatment for a total of twenty one fiberglass tanks (each with a capacity of 450 liters). Ten fiberglass tanks were for replacement water. The density of fish per tank was 30 fish and the water temperature was 19.46 ± 1.23 °C, pH was 7.85 ± 0.26 and dissolved oxygen level was maintained above 7.65 ± 0.55 mg/L during the experiment with an electrical air pump (by a single filtration unit). Stock solutions of copper sulfate and lead(II) 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. There were 30 fishes per concentration of each heavy metal.

LC_{50} determination

96 h acute bioassays were performed following, in general, OECD guidelines for fish acute bioassays (guideline OECD203, 92/69/EC, method C1) [13]. For determination of the LC_{50} (lethal concentration) values, the following ranges were tested, seven Cu (0.5, 0.75, 1, 1.5, 2, 2.5 and 3 mg/L) and Pb (100, 200, 240, 260, 280, 300 and 320 mg/L) concentrations were chosen for grass carp. For each metal-treated use three 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 tanks. The mortality rate was determined at the end of 24, 48, 72 and 96 h. The acute toxicity test was

conducted in accordance with standard methods [14].

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.

Statistical analysis

In this study the acute toxic effect of copper and lead on the grass carp was determined by the use of Finney's Probit Analysis LC_{50} determination method [15]. Confidential limits (Upper and Lower) were calculated and also used SPSS18 for LC_{50} value of cooper and lead with the help of probit analysis.

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

LC_{50} of copper for grass carp

Table 1 shows the relationship between the copper concentration and the mortality rate for 24, 48, 72 and 96 h of grass carp. Results according to SPSS18 analysis showed that the median lethal concentration (LC_{50}) of copper to grass carp for 24, 48, 72 and 96 h of exposure are 2.422, 2.222, 2.006 and 1.717 mg/L 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**).

Table 1 Correlation between the copper sulfate concentration and the mortality rate (%) on time (24 - 96 h) for grass carp.

Concentration (mg l^{-1})	**Mortality rate (%) on time (24 - 96 h)**				
	N	**24 h**	**48 h**	**72 h**	**96 h**
0.00	30	0	0	0	0
0.50	30	0	0	0	0
0.75	30	0	0	3.33	10
1.00	30	3.3	6.6	10	20
1.50	30	16.6	23.3	26.6	36.6
2.00	30	33.3	43.3	53.3	60
2.50	30	53.3	63.3	73.3	86.6
3.00	30	73.3	80	90	100

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

Point	Concentration (mg l^{-1}), (95 % confidence limits)			
	24 h	**48 h**	**72 h**	**96 h**
LC_1	0.575 (0.019 - 0.890)	0.415 (0.094 - 0.748)	0.327 (0.123 - 0.631)	0.184 (0.017 - 0.360)
LC_5	1.116 (0.725 - 1.373)	0.945 (0.578 - 1.194)	0.819 (0.489 - 1.051)	0.633 (0.336 - 0.845)
LC_{10}	1.405 (1.093 - 1.621)	1.227 (0.930 - 1.439)	1.081 (0.809 - 1.280)	0.873 (0.626 - 1.055)
LC_{15}	1.599 (1.335 - 1.795)	1.417 (1.163 - 1.608)	1.258 (1.022 - 1.438)	1.034 (0.818 - 1.200)
LC_{50}	2.422 (2.239 - 2.650)	2.222 (2.051 - 2.421)	2.006 (1.847 - 2.180)	1.717 (1.571 - 1.873)
LC_{85}	3.246 (2.963 - 3.685)	3.027 (2.776 - 3.398)	2.753 (2.536 - 3.059)	2.399 (2.206 - 2.666)
LC_{90}	3.440 (3.125 - 3.939)	3.218 (2.938 - 3.638)	2.930 (2.689 - 3.277)	2.561 (2.347 - 2.862)
LC_{95}	3.729 (3.362 - 4.318)	3.500 (3.175 - 3.998)	3.192 (2.913 - 3.602)	2.800 (2.553 - 3.156)
LC_{99}	4.270 (3.803 - 5.033)	4.029 (3.614 - 4.677)	3.684 (3.328 - 4.218)	3.249 (2.934 - 3.713)

LC_{50} of lead for grass carp

Susceptibility of grass carp 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 grass carp for 24, 48, 72 and 96 h of exposure was 293.747 mg/L, 278.019 mg/L, 260.324 mg/L and 246.455 mg/L respectively. There was a gradual decrease in the slope function corresponding to the increase in the exposure period from 24 to 96 h. Observations on upper and lower confidence limits reveal a decreasing trend from 24 h to 96 h. Also evident was that an increase in exposure period results in an increase in mortality (**Table 4**).

Table 3 Correlation between the lead nitrate concentration and the mortality rate (%) on time (24 - 96 h) for grass carp.

Point	Concentration (mg L^{-1}), (95 % confidence limits)			
	24 h	**48 h**	**72 h**	**96 h**
LC_1	194.834 (153.546 - 215.575)	183.672 (147.939 - 203.536)	181.482 (154.325 - 198.125)	179.821 (157.052 - 194.214)
LC_5	223.810 (197.195 - 238.079)	211.311 (186.806 - 225.522)	204.578 (184.824 - 217.060)	199.341 (182.339 - 210.392)
LC_{10}	239.257 (219.820 - 250.720)	226.045 (207.157 - 237.612)	216.891 (200.877 - 227.361)	209.747 (195.665 - 219.170)
LC_{15}	249.679 (234.463 - 259.870)	235.986 (220.571 - 246.085)	225.198 (211.553 - 234.466)	216.768 (204.548 - 225.201)
LC_{50}	293.747 (282.562 - 312.378)	278.019 (268.972 - 290.231)	260.324 (252.895 - 268.306)	246.455 (239.668 - 253.143)
LC_{85}	337.815 (317.599 - 377.948)	320.053 (304.530 - 347.221)	295.449 (285.045 - 311.338)	276.141 (267.914 - 287.957)
LC_{90}	348.237 (325.538 - 393.803)	329.994 (312.446 - 361.192)	303.757 (292.023 - 322.141)	283.162 (273.972 - 296.814)
LC_{95}	363.684 (337.219 - 417.388)	344.728 (324.064 - 382.014)	316.069 (302.209 - 338.308)	293.568 (282.774 - 310.116)
LC_{99}	392.660 (358.966 - 461.794)	372.367 (345.649 - 421.283)	339.166 (321.039 - 368.913)	313.089 (298.976 - 335.380)

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

Concentration (mg l^{-1})	Mortality rate (%) on time (24 - 96 h)				
	N	24 h	48 h	72 h	96 h
0	30	0	0	0	0
100	30	0	0	0	0
200	30	0	0	0	0
240	30	3.3	6.6	3.3	16.6
260	30	10	16.6	26.6	43.3
280	30	20	30	50	63.3
300	30	33.3	53.3	70	83.3
320	30	53.3	70	90	100

The present study aimed to find the susceptibility of grass carp to potentially hazardous heavy metals like copper and lead. Median lethal concentration (LC_{50}) of copper to grass carp for 24, 48, 72 and 96 h of exposure were 2.422 mg/L, 2.222 mg/L, 2.006 mg/L and 1.717 mg/L, respectively and median lethal concentration (LC_{50}) of lead to grass carp for 24, 48, 72 and 96 h of exposure were 293.747 mg/L, 278.019 mg/L, 260.324 mg/L and 246.455 mg/L respectively. A higher percent of mortality occurred with an increase in concentration and exposure period to copper and lead.

It was observed that grass carp individuals displayed various behavioral changes when subjected to different metals concentrations. The behavioral and swimming patterns in the control group were normal and there were no deaths during the experimental period. The behavioral changes in fish subjected to different concentrations (1.5, 2, 2.5 and 3 mg/L of copper sulfate and 240, 260, 280, 300 and 320 mg/L of lead(II) nitrate in 72 and 96 h) of metals during the initial stage of exposure included restlessness, sudden jerks, respiratory difficulty, erratic and fast swimming, abrupt change in position and direction, capsizing in water and swimming in that manner were observed in experimental treatments. Physiological responses like rapid opercular movement, frequent gulping of air and remaining laying on one side before death were observed during the initial stages of exposure after which it became occasional. Hence, we conclude that copper is more toxic than lead for grass carp.

Discussion

The results showed that the previous body accumulation of heavy metals has a direct effect on the LC_{50} values of the respective metals in grass carp. The fish with a lower body concentration of a heavy metal had a lower 96-h LC_{50} value of the respective metal and vice versa.

The concentration of heavy metals in fish is related to several factors, such as the food habits and foraging behavior of the organism [16], trophic status, source of a particular metal, distance of the organism from the contamination source and the presence of other ions in the milieu [17], bio-magnification and/or bio-diminishing of a particular metal [18], food availability [19]., metallothioneins and other metal detoxifying proteins in the body of the animal [20], temperature, transport of metal across the membrane and the metabolic rate of the animal [21], physical and chemical properties of the water [22], and the seasonal changes in the taxonomic composition of different trophic levels affecting the concentration and accumulation of heavy metals in the body of fish [16].

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 [23]. Metal concentrations in aquatic organisms appear to be of several magnitudes higher than concentrations present in the ecosystem [24], 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 [25]. The median lethal concentration 96 h (LC_{50}) value of copper and lead in other aquatic organisms was reported as 300 mg/L for lead in *Tench tinca* [26], which is higher than the 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 mg/L and for *P. reticulata*, LC_{50} for 24, 48, 72 and 96 for Cu were 348.9, 145.4, 61.3 and 37.9 mg/L respectively [27], which are lower than that achieved in the present study. The 24 h-LC_{50} of Cu was reported as 1.17 mg/L for *P. reticulata* [28], which again is lower than the present study. Gomes *et al.* [29] reported that with juvenile 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 are considerably lower than the present study. This indicates that different organisms have different sensitivity to heavy metals.

Susceptibility of common carp to the lethal effect of heavy metals was duration and concentration dependent as mortality increased with an increase in its concentration. The LC_{50} value of copper sulfate and lead(II) nitrate divulges the susceptibility of common carp to lethal concentrations of copper and lead, and depicts that the toxicity is dilution and duration dependent. A higher mortality rate was found with increasing concentration and exposure period, hence confirming the observation made in case of salmonids, *Oncorhynchus mykis*, *Salvelinus confluentus* and *Oncorhynchus tshawytscha* [30-32], guppy, *Poecilia reticulate* [33], *Cyprinus carpio* [34]. *Heteropneustes fossilis* [35].

Conclusions

In the present study, a comparison of the LC_{50} values and behavior changes indicated that copper is more toxic than lead to fishes (especially in treatments 2.5 and 3 mg/L of copper sulfate). 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(II) nitrate and copper sulfate and early warning indicators of this heavy metal toxicity in grass carp.

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