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Full Length Research Paper

Effect of lead nitrate on survival rate and chronic exposure on growth performance of grass carp (*Ctenopharyngodon idella*)

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The acute toxicity of lead nitrate to grass carp (*Ctenopharyngodon idella*) juveniles was assessed in a static renewal bioassay for 96 h. In addition, an experiment was conducted to determine the growth performance during 60-day sublethal ($Pb(NO_3)_2$) exposure. The results indicated that median lethal concentration (LC_{50}) of lead nitrate to Grass carp for 96 h of exposure was 246.455 µ. The chronic exposure to sublethal concentration of lead nitrate to the studied fish showed a significant decrease in final body weight in comparison to control group. The lead nitrate also had significantly decreased effect on body weight in comparison to the control. Also, the food conversion ratio (FCR) was significantly increased in comparison to control ($P < 0.05$). The lead nitrate also caused a significant decrease in the survival rate ($P < 0.05$).

Key words: Lethal concentration (LC_{50}), lead nitrate, growth, grass carp, *Ctenopharyngodon idella*.

INTRODUCTION

Heavy metals have long been recognized as serious pollutants of the aquatic environment. The accumulation of metals in the aquatic environment has direct effect on man and aquatic ecosystem. While the metals were required for metabolic activities in organisms lies in the narrow range between their essentiality and toxicity (Fatoki et al., 2002). Heavy metal contamination usually causes depletion in food utilization in fish and such disturbance may result in reduced fish metabolic rate and hence cause reduction in their growth (Javed, 2005a). Growth is a sensitive and reliable endpoint in chronic toxicological investigations (De Boeck et al., 1997).

The present work was design to investigate acute toxicity and toxic effect of lead on the growth performance of Grass carp under chronic sublethal concentrations to evaluate its potential to growth in contaminated water. 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). Metals are non-biodegradable and considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals (More et al., 2003).

Lead occurs in environment in a wide range of physical and chemical forms that influence the behavior of fish adversely at concentration higher than normal. Most of the lead in the environment is in the inorganic form and exists in several oxidized states (Jackson et al., 2005). Pb is the most stable ionic species present in the environment and is thought to be the form in which the maximum bioaccumulation of Pb occurs in aquatic organisms. However, the toxicity of Pb depends upon many factors including fish age, pH and hardness of the water (Nussey et al., 2000). Also lead is not necessary for the biological functions of animals even at low concentrations. It is being

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discharged to aquatic systems mainly from petroleum, chemistry, dyes and mining industries, which have toxic effects and can cause mortality to aquatic animals (Sorensen, 1991; Heath, 1995).

Chronic lead poisoning has similar toxic effects in fish as in mammals, these include hematological and neural disorders and tetanic spasms together with some morphological changes such as darkening in caudal fin, deformation of vertebrate, anomalies in pigment formation and covering of the gills by a mucus layer (Tulasi et al., 1992; Shah, 2006b).

MATERIALS AND METHODS

Uniform juveniles of grass carp were obtained from the Institute of Pond Fish Culture in Gorgan (Agh Ghala), Iran. They were weighted (initial weight 4.3 ± 0.5 g). The total length was also measured accurately (8.2 ± 0.44 cm). Fish were fed with aquatic plant food (*Lemna* species) at least twice a day before the experiments, and the fish were not fed during the experiments. Nutritional compositions of experimental diets (*Lemna* spp.) are given in Table 1. Proximate composition of diets was carried out using the Association of Analytical Chemists AOAC (2000) methods. Protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method; crude fat was determined using petroleum ether (40 to 60 bp) extraction method with Soxhlet apparatus and ash by combustion at 550°C. Physicochemical parameters of water, temperature, pH, total hardness, dissolved O_2, total NH_3, Na, K and CO_2 of the treated and control media were monitored on daily basis by following the methods of American Public Health Association (APHA) (1998). However, water temperature (24 ± 1°C), pH (7 to 7.5) and hardness (275 ± 2.58 mg L^{-1}) was kept constant throughout the study.

LC_{50} determination

Firstly, to investigate acute toxicity of lead, all aquaria (60 L) capacity were filled with 50 L of dechlorinated tap water. A total of 24 aquaria that each stocked with 10 fish were used in LC_{50} experiments. Stock solutions of lead nitrate were prepared by dissolving analytical grade lead nitrate $[Pb(No_3)]_2$ (from Merck) in double distilled water. 30 fishes were used per concentration of Pb. Ninety-six hours acute bioassays were performed following in general Organization for Economic Co-operation and Development (OECD) guidelines for fish acute bioassays (guideline OECD203, 92/69/EC, method C1) (OECD, 1993). For determination of the LC_{50}/96 h (lethal concentration) values, following a range finding test, seven Pb (100, 200, 240, 260, 280, 300 and 320 mg/l) concentrations were chosen for Grass carp. For each metal-treated and control, three replicates 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 (OECD, 1993). In this study, the acute toxic effect of lead on the Grass carp was determined by the use of Finney's Probity Analysis LC_{50} Determination Method (Finney, 1971). Confidential limits (Upper and Lower) were calculated and also used SPSS18 for LC_{50} value of lead with the help of probity µ analysis.

Table 1. Diet composition and proximate chemical analysis (%).

Ingredient	Percentage (%)
Protein	28
Lipid	11.4
Fiber	2.7
Ash	6

Growth performance

Thereafter, to investigate toxic effect of lead nitrate on the growth performance of Grass carp under chronic sublethal concentrations, an experiment was conducted in a completely randomized design with 3 treatments (tow concentration of lead and a control), and three replicates per treatment for a total of six fiberglass tanks (each with a capacity of 200 L), 60 fishes were used per concentration of lead. Separate groups of 60 fish each served as control for lead. 5 and 10% of LC_{50}/96 h concentration for lead nitrate (12.32 and 24.64 mg L^{-1}) was used as sublethal level for Grass carp. In control experiment set up, water with no metal was added. Throughout the experimental period of 60 days, fish were fed to satiation daily [(4% body weight (3 times a day)]. The treated fish were kept in the fiberglass tank containing sub lethal concentration of lead and grown for 60 days.

The fish were weighed individually at the beginning and at the end of the experiment. In the termination of experiment, total larvae from each tank were sampled and the final weight and length of body were measured. Growth parameters of fish were calculated based on the data of biometry of Grass carp larvae. One-way ANOVA and Duncan's multiple range tests were used to analyze the significance of the difference among the means of treatments by using the SPSS program.

RESULTS

LC_{50}/96 h of lead for grass carp

Acute toxicity of lead showed that mortality is directly proportional to the concentration of the lead nitrate while the percentage of mortality is virtually absent in control (Table 2) showing the relation between the lead concentration and the mortality rate for 96 h of Grass carp. Results according to SPSS18 analysis showed that the median lethal concentration (LC_{50}) of lead nitrate to Grass carp for 96 h of exposure is 246.455 µ (Table 3).

Growth performance

The results clearly showed that the lead nitrate had harmful effects on the growth parameters on Grass carp. The feeding and growth parameters of Grass Carp are presented in (Table 4). The chronic sublethal lead nitrate exposure to the fish exerted that larvae had significantly decreased final body weight in T1 and T2 when compared to control ($P < 0.05$). The lead nitrate also had significant effects on specific growth rate (SGR) and body weight increased in comparison to the control. The food

Table 2. Cumulative mortality of grass carp during sub-lethal exposure to lead nitrate (n = 30, each concentration).

Concentration (mg L^{-1})	N	Mortality rate (%) on 96 h
0	30	0
100	30	0
200	30	0
240	30	16.6
260	30	43.3
280	30	63.3
300	30	83.3
320	30	100

Table 3. Lethal concentrations (LC_{50}) of lead nitrate depending on time (24 to 96 h) for grass carp.

Point	Concentration (mg L^{-1})	95% confidence limits
LC_1	179.821	157.052-194.214
LC_5	199.341	182.339-210392
LC_{10}	209.747	195.665-219.170
LC_{15}	216.768	204.548-225.201
LC_{50}	246.455	239.668-253.143
LC_{85}	176.141	267.914-287.957
LC_{90}	283.162	273.972-296.814
LC_{95}	293.568	282.774-310.116
LC_{99}	313.089	298.976-335.380

Table 4. Growth parameters and survival rate of Grass carp in experimental treatments (Trial 1 to 2) and control.

Growth Index	Treatment		
	Control (Free of metal)	T1 (12.32 mg L^{-1} lead nitrate)	T2 (24.64 mg L^{-1} lead nitrate)
IW[1]	4.30±0.01	4.32±0.02	4.30±0.01
FW[2]	6.82±0.01[a]	6.28±0.02[b]	5.96±0.32[b]
WG[3]	2.52±0.02[a]	1.96±0.04[b]	1.66±0.33[b]
SGR[4]	0.77±0.01[a]	0.62±0.01[b]	0.54±0.09[b]
FCR[5]	22.94±0.47[a]	26.00±0.75[a]	34.68±2.06[b]
SR[6]	96.18±1.47[a]	89.07±1.47[a]	69.12±2.60[b]

Groups with different alphabetic superscripts at the same row differ significantly at $P<0.05$. IW = Initial weight (g), FW = final body weight (g), WG = body weight increased (g), SGR = specific growth rate for weight (% BW day^{-1}), FCR = feed conversion ratio (%), SR = survival rate (%).

conversation ratio (FCR) was significantly increased in T2 when in comparison with the control and T1 ($P < 0.05$). Between the two different concentrations of lead nitrate to Grass carp, the greatest effect appeared to be obtained in treatments T2 (concentration 24.64 mg L^{-1} of lead nitrate). This is particularly false for food conversation ratio (FCR) where the lower was obtained in the experimental control treatment, and survival rate where the highest was obtained in the experimental control treatment not significantly by T1 (12.32 mg L^{-1}) ($P > 0.05$). Of course, final body weight, body weight increased, specific growth rate and body weight gain in T1 (12.32 mg L^{-1}) were not significant by T2 (24.64 mg L^{-1}) (Table 4).

DISCUSSION

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).

The present study was initiated to find the susceptibility of the Grass carp to potentially hazardous lead nitrate on the survival and growth performance. The results showed that median lethal concentration (LC_{50}) of lead nitrate to Grass carp for 96 h of exposure is 246.455 ppm. The median lethal concentration 96 h (LC_{50}) value of lead in other aquatic organisms was reported as 300 µ for lead as in *Tench tinca* (Shah and Altindag, 2005a), which were higher than present study. 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 on their permeability and detoxification mechanisms (Darmono and Denton, 1990).

Toxic effect of lead on the growth performance of grass carp under chronic sublethal concentration showed that the lead nitrate had harmful effects on the growth parameters on Grass carp. These results are in accordance with the findings of Javed et al. (1993b) that reported the fish (*C. mrigala*) stressed with sublethal concentration of lead showed significantly lower weight increment (42.20 ± 35.52 g) than control fish (55.55 ± 29.47 g).

Also, these results are in accordance with the findings of Hayat et al. (2007) who exposed the fingerlings of three major carps: *C. catla*, *Labeo rohita* and *Cirrhina mrigala* to sublethal concentrations of manganese for 30 days. During this exposure period, all the fish species showed negative growth. Also, these results are in accordance with the findings of Javed et al. (1993b) that observed low feed conversion ratios in major carps (*C. catla, L. rohita* and *C. mrigala*) due to exposure of these fish to water-borne zinc (Javed, 2005a).

The results of these studies may provide guidance to select acute toxicity to be considered in field bio-monitoring efforts designed to detect the bioavailability of lead nitrate and early warning indicators of this heavy metal toxicity in Grass carp.

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