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Effect of Lead Nitrate on the Survival Rate and Growth Performance of Caspian Sea Kutum (*Rutilus frisii kutum*)

Esmail Gharedaash, Mohammad Reza Imanpour and Vahid Taghizade

Department of Fishery,
Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract: In this study 96-h LC_{50} value of lead nitrate was determined and the growth performance of Caspian Sea kutum (*Rutilus frisii kutum*) fingerlings was determined during exposure to sub lethal concentrations of lead nitrate ($Pb (NO_3)_2$) for 60-day. Experimental fishes were procured from Gorgan (Iran), which were measured an average length 8±0.5cm and weight 4±0.5g. Thereafter they were kept in sterile aquaria and the fish were fed with commercial pelleted food. 24 aquaria and 8 concentrations of lead nitrate were composed for treatments while 3 aquaria were used as control. For each treatment, three replicates were conducted. Another experiment was conducted to determine the growth performance; fish were transferred into fiberglass aquaria of 200 L water capacity. The treated fish were kept in the aquaria containing sub lethal concentrations of lead nitrate (13.4 and 26.8 mgL^{-1}) and grown for 60 days, while control fish were placed in metal free water. The results indicated that median lethal concentration (LC_{50}) of lead nitrate to Caspian Sea kutum (*Rutilus frisii kutum*) for 96h of exposure is 268.065 ppm. The chronic sublethal lead nitrate exposure to the fish exerted that larvae had significantly decreased final body weight in comparison to control treatment. The lead nitrate also had significantly increased on specific growth rate (SGR) and significantly decreased on body weight increased in comparison to the control. The food conversation ratio (FCR) was not significantly increased in comparison to control ($p>0.05$). Hence, we could conclude that lead nitrate is toxic for Caspian Sea kutum (*Rutilus frisii kutum*).

Key words: LC_{50} · Lead Nitrate · Growth · Body Weight · *Rutilus frisii kutum*

INTRODUCTION

Wide usage of heavy metals in various industries increases their levels in natural ecosystems. Heavy metals are important pollutants affecting all parts of ecosystems since their biological half-lives are long and they are being accumulated cumulatively [1]. The metals are of special concern because of their diversified effect and the range of concentration stimulated toxic ill effect to the aquatic life forms. Industrial wastes constitute the major source of metal pollution in natural water [2]. Aquatic systems are exposed to a number of pollutants that are mainly released from effluents discharged from industries, sewage treatment plants and drainage from urban and agricultural areas. These pollutants cause serious damage to aquatic life [3, 4]. The rapid increase in human population has escalated the demand for quality food, like fish, in the world. To fulfill the food requirements, fish assume greater importance because, it contains high quality proteins, fats and minerals. Another feature of fish is its ability to convert raw materials into high quality proteins more efficiently than other terrestrial animals such as sheep, goats, cows etc. Fish is an indicator to measure the freshwater contamination by heavy metals because they occupy different trophic levels in an aquatic ecosystem.

Metals are non-biodegradable and considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals [5]. Metals are unique among pollutants, which cause adverse health effects, in that they occur naturally and in many instances are ubiquitous in the environment. Heavy metals have long been recognized as serious pollutants of the aquatic environment. The accumulation of metals in an aquatic environment has direct impact on man and aquatic ecosystem.

Corresponding Author: Esmail Gharedaashi, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Lead is not necessary for the biological functions of animals even at low concentrations. It is being discharged to aquatic systems mainly from petroleum, chemistry, dye and mining industries, which has toxic effects and can cause mortality to aquatic animals [6, 7].

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 [8-10].

Heavy metal contamination usually causes depletion in food utilization in fish and such disturbance may result in reduced fish metabolic rate and hence causing reduction in their growth [11]. Growth is a sensitive and reliable endpoint in chronic toxicological investigations [12]. The present work was design to investigate acute toxicity of lead, and toxic effect of lead on the growth performance of kutum under chronic sub lethal concentrations to evaluate its potential to growth in contaminated water.

MATERIALS AND METHODS

Juvenile Caspian Sea kutum selected for this study were obtained from the fish seed hatchery in Gorgan, Iran. Caspian Sea kutum measuring 8±0.5cm in length and weighing 4±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 during this period.

Physicochemical parameters viz. 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 APHA [13]. However, water temperature (24±1°C) pH (7-7.5) and hardness (275±2.58mgL^{-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 27 aquaria that each stocked with 10 fishes were used in experiments for lead. 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 OECD guidelines for fish acute bioassays (guideline OECD203, 92/69/EC, method C1) [14]. For determination of the LC_{50}/96h (lethal concentration) values, following a range finding test, eight 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 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 [13]. In this study the acute toxic effect of lead on the Caspian Sea kutum 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 lead with the help of probit analysis.

Growth Performance: Thereafter to investigate toxic effect of lead on the growth performance of kutum under chronic sub lethal 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 liters) 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}/96h concentration for lead nitrate (13.4 and 26.8 mgL^{-1}) was used as sub lethal level for kutum. 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 percent body weight (3 times a day)) with the feed of digestible energy 2.90 kcal g^{-1} and 35% digestible protein. The treated fish were kept in the aquaria 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 kutum 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.

Table 1: Showing the correlation between the lead nitrate concentrations and the mortality rate on time 96h of *Kutum*

Concentration(mg L^{-1})	N	Mortality rate on time (96h)
0	30	0
100	30	0
200	30	0
220	30	1
240	30	5
260	30	13
280	30	19
300	30	25
320	30	30

Table 2: Lethal concentration ($LC_{1\text{-}99}$) of lead nitrate on time 96h for Kutum

Point	Concentration(mg L^{-1})	(95% confidence limits)
LC_1	205.120	(185.599-217.770)
LC_5	223.483	(208.958-233.332)
LC_{10}	233.330	(221.249-241.790)
LC_{15}	239.973	(229.429-247.610)
LC_{50}	268.065	(261.706-274.525)
LC_{85}	296.156	(288.299-307.123)
LC_{90}	302.800	(294.088-315.333)
LC_{95}	312.647	(302.517-327.653)
LC_{99}	331.118	(318.050-351.041)

Table 3: Growth parameters and survival rate of kutum (*Rutilus frisii kutum*) in experimental treatments (trial 1-2) and control

	Treatments		
Growth Indices	Control Free of metal	T1 13.4 mg L^{-1} lead nitrate	T2 26.8 mg L^{-1} lead nitrate
Initial weight (g)	3.90±0.06	3.93±0.07	3.90±0.05
Final body weight (g)	4.74±0.11^a	4.71±0.08^{ab}	4.63±0.06^b
Body weight increased (g)	0.84±0.01^a	0.79±0.02^b	0.73±0.02^c
Specific growth rate for weight (% BW day^{-1})	0.32±0.01^a	0.30±0.01^{ab}	0.28±0.01^b
Feed Conversion Ratio (%)	0.60±0.01^a	0.63±0.02^a	0.68±0.02^a
condition Factor	0.82±0.01^a	0.82±0.01^a	0.83±0.01^a
Survival rate (%)	96.18±2.18^a	94.28±4.28^a	91.99±5.71^a

Groups with different alphabetic superscripts in the same row differ significantly at $p<0.05$ (ANOVA)

RESULTS

LC_{50}/96h of Lead for Kutum: 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 1).

Table 1 shows the relation between the lead concentration and the mortality rate for 96h of kutum. Results according to SPSS18 analysis showed that the median lethal concentration (LC_{50}) of lead nitrate to kutum for 96h of exposure is 268.065 ppm (Table 2).

In this experiment the behavior of fish remarkably changed due to the treatment of lead nitrate when compared to the control. 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 treated kutum.

Growth Performance: The results clearly showed that the lead nitrate had harmful effects on the growth parameters on Kutum. The feeding and growth parameters of Kutum are presented in (Table 3). The chronic sublethal lead nitrate exposure to the fish exerted that larvae had significantly decreased final body weight in T2 when that comparison to control. The lead nitrate also had significant effects on specific growth rate (SGR) and body weight increased in comparison to the control. The food conversation ratio (FCR) was not significantly increased in comparison with the control ($p>0.05$). Between the two different concentrations of lead nitrate to Kutum, the greatest effect appeared to be obtained in treatments T2 (concentration 26.8 mgL^{-1} of lead nitrate). This is particularly false for specific growth rate, where the highest was obtained in the experimental control treatment but not significantly by T1 (13.4 mgL^{-1} of lead nitrate) (Table 3).

DISCUSSION

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 [16]. 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 [17].

The present study was initiated to find the susceptibility of the kutum to potentially hazardous lead nitrate on the survival and growth performance. The results showed that Median lethal concentration (LC_{50}) of lead nitrate to kutum for 96h of exposure is 268.065 ppm.

The median lethal concentration 96h (LC_{50}) value of lead in other aquatic organisms was reported as 300 ppm for lead as in *Tench tinca* [18], which were higher than present study. The toxicity reported by other studies differs from this study probably due to different species used, aged, size of the organism, test methods and water quality such as water hardness, as this can affect toxicity [19, 20]. Toxicity of metals may vary depending upon their permeability and detoxification mechanisms [21].

Toxic effect of lead on the growth performance of Kutum under chronic sublethal concentration showed that the lead nitrate had harmful effects on the growth parameters on Kutum. These results are in accordance with the findings of [22] that reported the fish (*C. mrigala*) stressed with sub lethal concentration of lead showed significantly lower weight increment (42.20±35.52g) than control fish (55.55±29.47g) [22].

Also these results are in accordance with the findings of Kim and Kang reported that reduced growth rate of rockfish (*Sebartes schlegeli*) due to Cu stress and there was an inverse relationship between growth and Cu exposure [23]. Hayat *et al*. exposed the fingerlings of three major carps viz. *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 [24].

Also these results are in accordance with the findings of Mohanty *et al.* that determined the effect of copper on survival, growth and feed intake of Indian major carp, *C. mrigala* for 60 days. They observed that feed intake in fish reduced significantly ($p<0.001$) at all the Cu treatments. Significant difference was recorded in feed conversion ratios of treated (0.65) and control (0.55) fish. The control fish exhibited significantly better (0.26) feed conversion ratio than the treated fish (0.50) [25]. Javed observed low feed conversion ratios in major carps (*C. catla, L. rohita* & *C. mrigala*) due to exposure of these fish to water-borne zinc [11].

Chronic sub lethal pb exposure to the fish, kutum exerted significant impact on its weights, body weight increased, specific growth rate (SGR). 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 early warning indicators of this heavy metal toxicity in Caspian Sea kutum.

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