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Study the Acute Toxicity of Lead Nitrate Metal Salt on Behavioral Changes of the Milkfish (*Chanos chanos*)

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Abstract: The environment is impacted by ongoing pollution, caused by both natural factors and human activities such as industrialization and mining. Heavy metals are a major problem because they are toxic and tend to accumulate in living organisms. In this study, the 96-h LC_{50} value of lead nitrate [$Pb(NO_3)_2$] was determined in milkfish. The experimental treatments consisted of Pb concentrations of 110.0, 180.0, 250.0, 320.0, 390.0, 460.0 and 530.0 mg/l. In addition, the behavioral changes resulting from each treatment were recorded. The physicochemical parameters of the sea water used in the experiment were as follows: hardness 193.4 mg/l as $CaCO_3$, pH 7.6 to 8.1, dissolved oxygen concentration 6.7 to 7.8 mg/l and salinity 37.8 to 38.8 psu. The data obtained were statistically evaluated with an EPA computer program based on the Finney probit analysis method. The study used a static bioassay test system and found log LC_{50} and 96-h LC_{50} values of 2.63 and 426.49 mg/l, respectively. The behavioral changes observed in the experiment included hyperactivity, loss of balance, vertical and downward swimming patterns, convulsions, attraction to the surface and increased mucus secretion. In addition, high concentrations of lead produced symptoms that included schooling, lack of movement, difficulty in breathing, gathering around the ventilation filter, lack of body coloration and bloody fins. No behavioral changes or deaths were observed in the control group during the experiment. The results of the study showed that acute lead toxicity severely affects mortality and normal behavior. Exposure to lead may therefore be deleterious for milkfish populations.

Key words: Heavy metal % Toxicity % LC_{50}, Lead % Behavior % *Chanos chanos*

INTRODUCTION

The rapid industrial development of coastal regions during recent decades has created some problems for aquatic ecosystems. One of these problems is pollution by heavy metals. Heavy-metal contamination has been reported in aquatic organisms [1]. Aquatic organisms are used extensively for biological monitoring of deviations in the environmental levels of anthropogenic pollutants. This method of environmental monitoring can serve to identify potential problems before the health of a system is critically altered or compromised [2]. Fish are widely used to evaluate the health of aquatic ecosystems and biological, behavioral and physiological changes serve as biomarkers of environmental pollution [3]. High concentrations of heavy metals (e.g. Pb) in the environment cause serious impairment in metabolic, physiological, behavioral and structural systems. These metals may affect organisms directly, by accumulating in the body, or indirectly, by passing to the next trophic level through the food web. One of the most serious results of the persistence of heavy metals is the biological amplification that results from transmission through the food web [4]. Lead found in the environment, urban, industrial and agricultural waste waters and its occurrence in the air, which is transported to the streams and rivers by runoffs where fish and other aquatic organisms take it up and incorporate it in their body [5, 6].

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The 96-h LC_{50} test paradigm is used to measure the susceptibility and survival potential of organisms exposed 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 [7]. The heavy metals that are toxic to many organisms at very low concentrations and that are always harmful to living beings are mercury, cadmium and lead [8].

Fish absorb dissolved or available metals and can therefore give reliable indications of metal pollution in an aquatic ecosystem [9]. The milkfish, *Chanos chanos*, is considered to be a suitable organism for monitoring heavy metal contamination. It is well suited for this purpose because of its feeding behavior and bottom feeding habits and because it is a benthopelagic fish. This study is the first investigation about of the effect of lead on milkfish (*Chanos chanos*). This report presents the results of the first determination of the 96-h LC_{50} value of Pb and lead-related behavioral changes in *Chanos chanos*.

MATERIALS AND METHODS

Young juvenile milkfish (*Chanos chanos*, family Chanidae) weighing 64±1.2 g, standard length 16.7±0.4 cm were collected during 2009 from the Shour-Shirin (Tiyab) estuary, located in the Kolahi region of Hormozgan province, Iran. The fish were captured by using cast nets and were transferred to the research laboratory of the Persian Gulf and Oman Sea Ecology Research Center. All specimens were washed with 0.1% $KMnO_4$ solution to remove dermal infection and pollutants, if any. The fish were allowed to acclimate to laboratory conditions for 45 days before the start of the first experiment. The pH, temperature, salinity and dissolved oxygen were continuously measured throughout the experiments to detect possible deviations from the baseline values. These measurements were needed because the addition of metal salts might change the values of these parameters. The metal salt lead nitrate [$(Pb(NO_3)_2$], Merck) served as the test compound for determination of the median lethal concentration (LC_{50}) of lead. After acclimation, the fish were divided into several groups, each containing 12 individuals. These groups were then exposed to different metal concentrations. The control group was not exposed to lead. The other groups were exposed to lead nitrate. The concentrations used for acute toxicity estimation were 110.0, 180.0, 250.0, 320.0, 390.0, 460.0 and 530.0 mg/l. The experimental design included three replicates. The stock solutions were prepared in distilled water and the required concentrations were maintained in fiberglass tanks. In the experimental aquaria, the water for the stressed groups and the water for the control group were renewed each day at the same time. The experiment did not distinguish between the sexes of the fish. The behavioral changes, morphological abnormalities and mortality of the fish were monitored during 96 h of exposure to each concentration of the toxicant. Each day, dead fish were counted and removed from the aquaria. The fish were not fed on the day before the beginning of the experiment or during the experiment. The data from the experiment were used to estimate the log LC_{50} and LC_{50} of lead. To calculate these values, the mortality observed in each treatment was determined and analyzed using a basic EPA computer program that implemented the Finney probit analysis method [10].

RESULTS

Mortality and LC_{50} Values: The mortality rates (per cent) for each concentration of lead are presented in Figure 1 and Table 1.

No mortality was observed over 96 h at lead concentrations of 110 and 180 mg/l. The lowest lead concentration at which mortality was observed was 250 mg/l. The first deaths of experimental fish were recorded in the 530 mg/l treatment after 24 h. The highest mortality, 10 fish, was observed at a concentration of 530 mg/l. Table 2 presents the numbers of fish that died and the concentrations of lead nitrate to which these fish were exposed. The data from these toxicity tests were evaluated using probit analysis. The log LC_{50} and 96-h LC_{50} values were determined and 95% confidence limits calculated. The log LC_{50} and 96-h LC_{50} for the young milkfish were 2.63 and 426.49 mg/l, respectively. The 95% confidence limits for log LC_{50} and 96-h LC_{50} were (2.59, 2.68) and (385.23, 474.75), respectively.

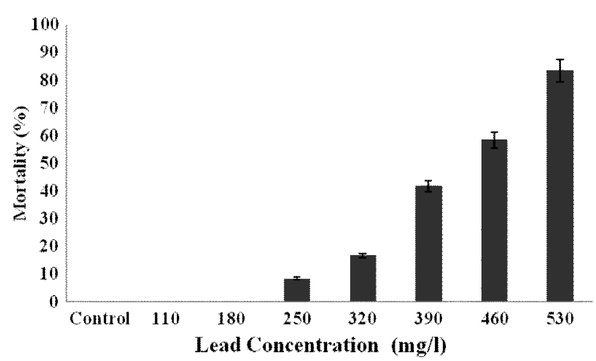

Fig. 1: The mortality rate as a percentage for each concentration of lead.

Table 1: Numbers of dead fish in different concentrations of lead in 96h

	Pb concentrations (mg/l)							
Situation	Control	110	180	250	320	390	460	530
Number of fish	12	12	12	12	12	12	12	12
Number of dead fish	0	0	0	1	2	5	7	10

Table 2: Some Physicochemical Parameters of Water Monitored over the Experimental Period

Pb Concentration (mg/l)	Temperature	pH	Dissolved Oxygen	Salinity
Control	23.7	7.8	7.5	38.8
110	23.9	7.9	6.9	38.3
180	23.3	7.6	7.6	37.8
250	22.9	7.7	7.8	38.1
320	23.0	8.0	7.4	38.6
390	23.8	7.6	6.7	38.3
460	23.6	8.1	7.4	37.9
530	23.4	7.9	7.6	38.2

Table 3: The recorded Behavioral alterations in different fishes associated with different heavy metals stress

Species	Heavy metal	Behavior/Movement Comments	Reference
3-Spine stickleback	Lead nitrate	Attraction at high concentration Avoidance at low concentration	[21]
Atlantic salmon, rainbow trout	Cu & Zn	Avoidance behavior(s)	
	Zinc sulfate	53 ppb (salmon), 5.6 ppb (trout)	[22]
Salmon	Cu	Altered chemoreception and home stream recognition	[23]
Bluegill	Cd, Cr, Zn	Hyperactivity	[24]
Rainbow trout	Cu & Ni	Attraction 390 ppb (Cu), 6ppb (Ni) Avoidance 4.4 ppb (Cu), 24ppb (Ni)	[25]
Mummichog	Pb	Feeding activity and performance, predator avoidance	[6]
Rainbow trout	Heavy Metal	Avoidance behavior(s)	[26]
Rainbow trout	Cd	Altered dominance, feeding & aggression	[27]
grass carp (*Ctenopharyngodon idellus*)		loss of balance, capsizing in water and swim in that manner, gathering at the bottom, excessive mucus secretion, slowness in motion	[28]
Bloch (*Channa punctatus*)	Cr	Increasing in rate of swimming, rate of opercular activity, convulsions, loss of balance, hyperactivity	[10]
Milkfish	Cd	Increasing of mucus, convulsions, loss of balance,	[29]
Milkfish (*Chanos chanos*)	Pb	The motion of fish become extremely slow, capsizing in water, loss of balance, respiratory difficulty, motional escape in surface, increased mucus secretion and bloody fins.	[The present study]

The temperature, pH, dissolved oxygen content and salinity values measured in each aquarium are shown in Table 2.

Behavioral and Morphological Changes: The specimens showed various behavioral changes at different lead concentrations. The type, rate and duration of the behavioral changes increased with increased concentrations. In all of the treatments, fish were hyperactive and attempted to escape from the tank during the first hours at which movement occurred. The behavioral changes increased with increasing lead concentrations. Different kinds of behavioral changes were observed at different Pb concentrations. No behavioral changes or deaths occurred in the control group at any time during the experiment. All control fish were active and swam normally. Abnormal behavior was not expected to occur spontaneously in the control group. At a concentration of 110 mg/l, fish were observed to swim near the surface. The behavior of the fish in this treatment was otherwise nearly normal and similar to that of the control group. At 180 mg/l, the specimens showed respiratory difficulty, they attempted to breathe at the surface, their active movements increased, fish tried to escape from the tank and increased mucus secretion was observed. At 250 mg/l, the fish were agitated and tried to escape from the tank. Behavioral disorders, including loss of balance, respiratory difficulty and increased mucus secretion, were observed at this concentration. In this treatment, only one fish died after 4 days. At 320 mg/l, the behavior of the fish was dominated by the toxic effects of the treatment. The secretion of mucus increased and the fish capsized in the water and became motionless. The first fish died after 48 h. At 390 mg/l, the specimens initially sank to the bottom of the tank and became motionless. Moreover, fish sometimes tried to escape from the tank. Sideways swimming and loss of balance were observed after 48 h of exposure. The anal fin, the anus and the area around the eyes were bloody. The first fish died after 48 h. At 460 mg/l, the fish were observed to make sudden swimming motions during the first hours of exposure and they tried to escape from the tank. The bodies of the fish were colorless. The mouth, the area around the anus, the caudal peduncle and all of the fins were bloody. The first fish died after 36 h. At 530 mg/l, the first specimens died after 24 h.

The dead fish found in any treatment were immediately removed and dissected. Changes apparently occurred in some of the internal organs. The gills were bloody, the kidney was blackish and the liver was yellow to brownish. Copious mucus and internal secretions were observed on gills and other organs.

DISCUSSION

Table 2 shows the values of the physicochemical parameters (temperature, pH, dissolved oxygen and salinity) of the water in each tank. The concentration of heavy metals in fish is affected by several factors. These factors include the food habits and the foraging behavior of the organism, the physical and chemical properties of the water and the rate of accumulation of the heavy metal in the body [5, 9]. This study found a 96-h LC_{50} value of Pb of 427 mg/l for *Chanos chanos*. Different mortality rates were found at different lead concentrations (Table 1, Figure 1). The 96-h LC_{50} values and mortality of fish vary among different species and different metals. [11-13] reported 96-h LC_{50} values of 0.181, 0.13 and 0.51 ppm Hg for *Barbus conchonus*, *Eutropus maculatus* and *Salmo gairdneri*, respectively. [14] found 96-h LC_{50} values of 2.5 and 28.0 ppm Cd for *Jordanella floridae* and *Mugil cephalus*, respectively. [15] found 4.1 and 3.36 ppm total and dissolved Pb, respectively, for the 96-h LC_{50} values of *Salvalinus inalis* and [16] recorded 19 ppm Pb for the 96-h LC_{50} of *Colisa fasciatus*. However, [17] found 2.4 ppm Pb for the 21-day LC_{50} of *Salmo gairdneri*. [9] recorded the 96-h LC_{50} for *Tinca tinca* as 6.5 ppm for Cd and 300.0 for ppm Pb. In contrast with these results, the present study determined a 96-h LC_{50} for *Chanos chanos* of 426.49 mg/l of Pb. [18] found that young juvenile milkfish are more sensitive to hexavalent Cr than to Cd and they obtained 96-h LC_{50} values for Cr and Cd of 22.45 mg/l and 38.9 mg/l, respectively. [19] reported that the approximate 96-h LC_{50} for Cd in juvenile *Chanos chanos* is 27.3 mg/l.

This study of milkfish subjected to different lead concentrations showed that exposure to increasing amounts of lead resulted in increased mortality (Table 1, Figure 1) and that various behavioral disorders resulted from lead exposure. These disorders included loss of balance, respiratory difficulty, slowness of motion, capsizing in water, sinking to the bottom of the tank and increased mucus secretion. These toxic effects increased as the dose increased. The available data on the effects of heavy metals on fish behavior are sparse and variable. Table 3 shows some examples of the behavior of numerous species for comparison with the results of this study. In this paper, we have compared the results of the present study with those of previous studies (Table-3). With the exception of highly reactive materials, most substances produce systemic effects. Both effects can be demonstrated for some materials. For example, lead produces effects on the skin at the site of absorption and is then transported systemically to produce its typical effects on the central nervous system and other organs. The target organ of toxicity is often not the site of the highest concentration of the chemical. Lead is concentrated in bone, but its toxicity is due to its effects in soft tissues, particularly the brain. The target organ most frequently involved in systemic toxicity is the CNS (brain and spinal cord) [20]. For this reason, exposure to lead can affect behavior. In conclusion, the results of this study clearly illustrate that the toxic effects of lead on milkfish (i.e., the LC_{50} value, behavioral changes and mortality) varied with increasing heavy metal concentrations and in response to such water conditions as temperature, pH and dissolved O_2. The study demonstrated that milkfish can be used as an effective bioindicator for acute pollutants such as lead nitrate. Continued investigation and monitoring of milkfish exposed to treatments using other heavy metals is therefore warranted.

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