



Journal of
**Fisheries and
Aquatic Science**

ISSN 1816-4927



Academic
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Preservative Effect of Quants Water to Reduce Lead Acetate Toxicity (LC₅₀, 96 h) on *Capoeta fusca*

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Abstract: This study was conducted to determine the acute toxicity of lead acetate on *Capoeta fusca*. A total of 580 fishes with mean length of 12.28±0.14 cm and mean weight of 16.64±0.52 g. were divided into 15 control and treatment groups of fish. The fish were kept in 20 L aquariums and the procedure designed in static condition according to the Organization Economic Cooperation and Development (OECD) method. Mortality rate was recorded in 96 h and lead acetate LC₅₀ was calculated by standard statistical method. LC₅₀ of 10.992, 10.594, 9.338 and 7.575 mg L⁻¹ were determined at 24, 48, 72 and 96 h post exposing respectively. In addition, minimum and maximum lethal concentrations of lead were determined as 4 and 12.5 mg L⁻¹ and MAC was 0.7575 mg L⁻¹. Lead acetate in soft water (Hardness 10 mg L⁻¹) was highly toxic for fish but in hard water (Hardness: 310 mg L⁻¹) had a little toxicity. High trend of lead for interaction with minerals such as calcium and carbonates is the major reason of this phenomenon. Lead toxicity is decreased with increase of water hardness and this is the cause of fish tolerance against some heavy metals pollution in natural environment. Lead intoxicated fish showed abnormal behaviors, restless and rapid circling.

Key words: *Capoeta fusca*, lead acetate, LC₅₀ 96 h, water hardness

INTRODUCTION

Environmental pollution due to heavy metals such as lead has been long recognized at the global level. There are many different sources of pollutants (Shah and Altindag, 2005) which results in a considerable magnitude of heavy metals to be accumulated for aquaculture life. When fish are exposed to elevated metal levels in an aquatic environment, they can absorb the bioavailable metals directly from the environment via the gills and skin or through the ingestion of contaminated water and food. Metals in the fish are then transported by the bloodstream, which brings it into contact with the various organs and tissues (Coetzee, 1998).

The surface water following rainfall as well as water transferring through metal (in particular) could be a cause of pollution for aquaculture that can accumulate and as a result, it is deposited in tissue of the aquatic organisms and consequently resulting in serious effects on metabolic, physiologic and structural systems of them. When metals reach sufficiently high concentrations in body cells they can alter the physiological functioning of the fish (Grosell *et al.*, 2006). Aquatic organisms accumulate metals to concentrations many times higher than is present in water. Advancement in technology as well as increase in population have led to environmental concerns relating from indiscriminate dumping of refuse and discharge of industrial effluents, petroleum waste

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water and crude oil spills replete with most common heavy metals in our environment (Wills, 2000). Generally speaking, fish are more sensitive than human to pollutant in water such that the water which is of value for drinking in human may be dangerous for fish (Noga, 2000). Therefore, there will be of great importance to undertake a study to determine the amount of metal pollutants to control, manage and better conserve of pollution. With respect to increasing trend of industrialization and human activities which has been occurred around the different Birjands' Quanats, of heavy metal in aquatic ecosystems and their probable accumulation, knowing the toxic effects and heavy metal on aquatic life is of great value. The fate of heavy metals introduced by human activities into aquatic ecosystems have recently become the subject of wide spread concern, since beyond the tolerable limits they become toxic (Koller *et al.*, 2004). Fish exposition to metals will result in their death. Otherwise, it will insert in organisms food chain (Shah and Altindag, 2005). Water pollution due to human activities is the main cause of poisonous in fish population (Rogers and Wood, 2004). Metals therefore, tend to accumulate in the aquatic environment and thence in fish, either directly from the surrounding water or by ingestion of food (Kumar and Mathur, 1991). In addition to direct influence of lead, a long exposition to it could raise a major and wide effects such pituitary gland function and oocyte growth disorders, anemia, decreasing activity of alpha aminolevulinic acid dehydratase in erythrocytes, increasing sensitivities to pathogenic agents, nervous disorders and scoliosis (Grosell *et al.*, 2006). Birjand is located in south of Khorasan province in Iran and it is one of the desert regions. There are no any permanent rivers in the province. However, there are valuable sources of native fish population in their Quanats. In the present research, a study was undertaken on one of the family fish of *Capoeta* genus. A few researches have been carried out on this species of fish due to the fact that it has not been widely spread in different continents (Grosell *et al.*, 2006). Black fish (*Capoeta fusca*) belongs to Cyprinidae family, which has been reported only from the east of Iran (Coad, 1998). This species of fish has been recognized of great importance from the genetic conservative point of view (Moshkani and Poorkasmani, 2004).

MATERIALS AND METHODS

In order to study the effects of acute lead acetate poisonous on *Capoeta fusca*, a total of 580 black fish from a Quanat around the Birjand were taken using a basket and along with oxygen injection they were transferred to the faculty laboratory. The average size and weight of the fish were 12.28 cm (SD = 0.14) and 16.64 g (SD = 0.52) respectively. Fish were kept in the aquarium for adaptability. For determining mortality limits of lead acetate as well as survival experiment, treatments and replications were considered based upon OECD (Stoskopf, 1993). The experiment was undertaken in three stages. At the first stage, used water of hardness 310 mg L⁻¹ (Quanat water) were treated for 10 fish with two doses of 50 and 500 mg L⁻¹ lead acetate resulting in 0 and 100% mortality. At the second stage, the same two dosages were also utilised for water with hardness of 10 mg L⁻¹. Mortality limits were considered to be at 12 levels including 0, 0.125, 0.25, 0.5, 1, 2, 4, 12.5, 50, 75, 100, 300 and 500 mg L⁻¹. Ten fish were used in each aquarium and similar conditions for survival analysis were provided so that the control treatment was compared with the other treatments. In this study, the maximum concentration of lead acetate was 500 mg L⁻¹, which was decreased by 12 levels. The rate of mortality of fish was recorded once a day and for a period of 4 days. At the third stage of the experiment, poisonous determination was carried out using 14 treatments and control group under static condition in which water, airing and lighting factors within the aquariums were fixed under the standard method. In this stage, 10 fish were used. Upper and lower limits obtained from mortality determination test were divided into equal parts using mathematical series and then each part was examined and the mortality rate was measured once a day for a period of 4 days. The concentrations considered at this stage were 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 mg L⁻¹. During the

Table 1: Some of physical and chemical variables of experimental aquariums water

Parameter	Temperature (°C)	pH	Soluble oxygen	Nitrite	Nitrate	Total hardness
			-----(mg L^{-1})-----			
Quantity	25.08±0.17	7.93±0.08	5.6±0.49	0.14	0.07	10 or 310

experiment, soluble oxygen, acidity, total hardness, nitrite, nitrate and water temperature were recorded daily (Table 1). Furthermore, fish behaviour was monitored for different hours. After recording mortality rate during 96 h, LC_{50} of lead acetate was determined applying probit analysis (Finney, 1990) and using excel graphing the comparison was made.

RESULTS AND DISCUSSION

With respect to the results obtained from first and the second stages regarding comparison of mortality rate for water hardness and mortality range, it was found that the concentration limits were 4 and 12.5 mg L^{-1} (Table 2). At the stage 3, the toxicity of lead acetate as well as mortality rate of fish was determined for individual days (Table 3). Based on the results, it was revealed that the concentration mortality (LC_{50}) of lead acetate on exposed fish was 7.757 mg L^{-1} during 96 h (Table 4). Furthermore, Maximum Allowable Concentration (MAC), Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) of the lead acetate were calculated from the LC (Table 5). It was also found that the maximum allowable concentration of lead to be 10% of the LC_{50} , 96 h. In fact, MAC is a toxin concentration for which there are no any serious and toxic effects on fish life during the experiment period. On average, the LOEC was the same as LC_{10} , 96 h. LOEC is the least concentration of the poisonous for which there is statistical effects on fish life during the experimental period. NOEC is determined as there is no observed effects from the toxic effects on the fish life and it could be supposed as the same as MAC (Sanai, 2007; Zamini, 1996). Restlessness, rotation and excitability were observed as the clinical abnormal behavior when the lead acetate toxicity was undertaken.

The results obtained from the present research revealed that the average toxicity concentration (LC_{50}) of lead acetate on fish during 96 h was 7.757 mg L^{-1} . According to the earlier research it could be concluded that quality of the water such as temperature, soluble oxygen, hardness rate and pH affects on solubility, absorption and lead toxicity and the other heavy metals (Stoskopf, 1993). Based on comparison of LC_{50} of lead acetate with toxicity classification (Table 6), it can be concluded that when the rate of water hardness is 10 mg L^{-1} , lead acetate is poisonous for black fish of Quana. According to the Table 1 toxicity rate of 500 mg L^{-1} could be tolerated by the black fish even though the water hardness was 300 mg L^{-1} therefore, it could be concluded that LC_{50} of lead acetate for hard water is higher than 500 mg L^{-1} . Furthermore, lead acetate is of lower toxicity for water with 310 mg L^{-1} white shows that there is an affinity for the lead to react with water soluble such as carbonate and calcium. In fact, calcium is an important ion to make hard water. The research on acute toxicity of lead on trout indicate that organic carbon had a great affect on short time attachment of lead to gills (Macdonald *et al.*, 2002). Based on this, high concentration of lead in natural ecosystems has no effect on the aquatic life (Sanai, 2007). Lead is an enormous metal in the nature causing toxicity. Similar to treatment of lead toxicity using chelator, due to high magnitude of soluble in Quana and desert water, lead is not available for fish. Acute toxicity mechanism on lead acetate was studied showing that ionic regulation was disordered. Inhibition of ionic transfer could result in a decrease of Na, Chlorine and Ca in the blood plasma (Rogers and Wood, 2004). Lead reacts with sulphhydryl enzymatic systems results in the inhabitation of the enzyme. These enzymes are important for a number of vital cellular processes (Mirsattari, 1990; Shah and Altindag, 2005). The toxicity resulted from heavy metals could be treated using simple components such as BAL and EDTA. In the case of lead toxicity, application

Table 2: Lead acetate limit toxicity along with comparison between two water hardness

Lead acetate concentration (mg L ⁻¹)	Mortality percent after 96 h	
	Hardness (10 mg L ⁻¹)	Hardness (310 mg L ⁻¹)
500	100	0
300	100	0
100	100	0
75	100	0
50	100	0
12.5	100	0
4	100	0
2	0	0
1	0	0
0.5	0	0
0.25	0	0
0.125	0	0

Table 3: Determination of lead acetate toxicity and fish mortality for each day with average two replicates

Time (h)	Concentration (mg L ⁻¹)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
24	0	0	0	0	20	0	0	90	0	0	36	96	100	0	0
48	0	0	0	0	0	0	0	0	0	0	23	0	0	0	0
72	0	0	0	3	0	0	35	5	0	30	0	13	0	0	0
96	0	0	0	13	0	30	10	45	0	30	90	13	0	0	10
Total mortality	0	0	0	16	20	30	45	50	90	60	90	86	96	100	10

Table 4: Concentrate of lead acetate toxicity during 96 h for experimental fishes

Lethal concentration (mg L ⁻¹)	Time (h)			
	24	48	72	96
LC ₁₀	7.717	7.623	5.854	4.715
LC ₅₀	10.992	10.594	9.338	7.575
LC ₉₀	15.657	14.723	14.895	12.171

Table 5: Magnitude of MAC, LOEC and NOEC of lead acetate for experimental fish

	MAC	LOEC	NOEC
Concentration (mg L ⁻¹)	0.757	4.715	0.7575

Table 6: Classification of toxicity degree based upon the amount chemicals (Sanai, 2007)

LC ₅₀ (mg kg ⁻¹)	<15000	5000-15000	500-5000	50-500	1-50	0.25-1	>0.25
Toxic grade	Without effect	Non toxic	Low toxic	Median toxic	Toxic	Very toxic	Very high toxic

of chelators such as CaNa²⁻ EDTA could act as ionic exchanger in which calcium make stable lead chelator with exchanger calcium. Therefore, combinational form of the metal would be nonionic and stable and it is of lower toxicity (Mirsattari, 1990). Rogers and Wood (2004) indicated that lead accumulation in fish gills decreases as the water calcium concentration increases. This means that calcium could decrease the absorption of lead from water by the fish gills. In other words, calcium and magnesium could reduce the effect of lead toxicity through a competition with lead and the other heavy metals (Noga, 2000). The uptake of aqueous lead across the gills into the bloodstream is the primary mode of uptake in freshwater fish (Coetzee, 1998). Calcium upload from the gills is undertaken primarily through passive transportation from a group of channels which are sensitive to the apical voltage located in chloride cells in fish gills cells. After that, calcium is transported to lateral-basal membrane by attachment to a specific protein. It is then transferred to the blood stream either active or through Ca-ATPase enzyme or Na, Ca exchanging mechanisms. Lead could affect calcium entrance to the organism body. This effect could be prompt using calcium competition inhabitation in the apical

channels of fish branches. In fact, lead could enter the body via the same way of the calcium. There is another mechanism of lower action by which lead interferes with Ca-ATPase enzyme. Therefore, lead toxicity increases when the water hardness decreases. Moreover, an increase of calcium in the ration could reduce the lead uptake. There is in fact an antagonistic relationship between calcium and lead (Rogers and Wood, 2004). The other studies have shown that calcium could decrease the lead absorption in the gastrointestinal tract of the animal through which a supporting effect against lead (Varma *et al.*, 2001). No research has been carried out on the black fish in Iran because of this kind of fish is exclusively found in some particular regions of the country. Because of this, biological aspects of black fish have been undiscovered (Grosell *et al.*, 2006). This is why other species have been compared in this study. The average of lead toxicity rate differed among various species of fish; lead accumulation in this organism depends upon to the many factors. Furthermore, previous exposition of fish to heavy metals could decrease the average lead toxicity rate resulting in an increase resistance through acclimatization. This could result in synthesizing a kind of proteins (Methallothioneins) in the organism body by which metal ions are detoxified (Shah and Altindag, 2005) which in turn resulting an increase in LC₅₀. The studies undertaken by other researchers showed that the average concentration of lead acetate in water hardness 60 mg L⁻¹ were 120.61 mg L⁻¹ for *Acipenser* and 0.428 and 0.825 mg L⁻¹ for Common whitefish and *rainbow trout* respectively during 96 h of experiment (Mohammadnejad *et al.*, 2005). In common carps which are of smaller than 3.5 cm and greater than 6 cm, the magnitude of the lead acetate were 0.44 and 0.8 mg L⁻¹, respectively suggesting that the amount of the lead increases as the size of fish increases (Alam and Maughan, 1995). The corresponding figures for some other species were found to be 50.48 mg L⁻¹ for Silver carp 72.2 mg L⁻¹ for Grass carp (Zamini, 1996), 300 mg L⁻¹ for tench (*Tinca tinca*) (in water hardness 77.5 mg L⁻¹) (Shah and Altindag, 2005), 3.36 for *Salvalinus frontalis* (Holcombe *et al.*, 1976), 19 mg L⁻¹ for *Colisa fasciatus* (Srivastava and Mishra, 1979), 11.2 for *Gmmarus pulex* in temperature 25 centigrade degree and 23.2 mg L⁻¹ in temperature 15 centigrade degree (Bat *et al.*, 2000). Therefore, black fishes are more sensitive to the lead acetate than the (*Acipenser*), ray-finned fishes and common carp. Figure 1 shows that the lead needed decreases when the toxicity dosage during different days increases. Through comparing 4 days of the experiments (Fig. 2), it reveals that LC₅₀ decreased as the number of experiment hours increased. This means that a lower of the toxin is needed for mortality when the exposition time increases. This could be due to the fact that time is one of the important factors affecting toxicity. As the fish is exposed a constant concentration of the toxin, not only the fish resistance is deteriorate but also there will be more time for the toxin to affect on fish. Therefore, it could be suggested that more investigations to be carried out on the effects of heavy metals on black fish as well as *Garra rossica* of the Quanat. Moreover, an experiment is needed to study combinational

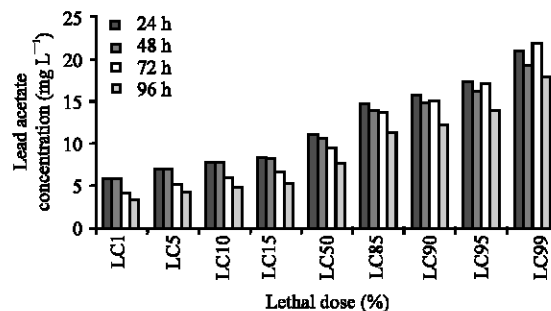


Fig. 1: Lead acetate toxicity for experimental fishes

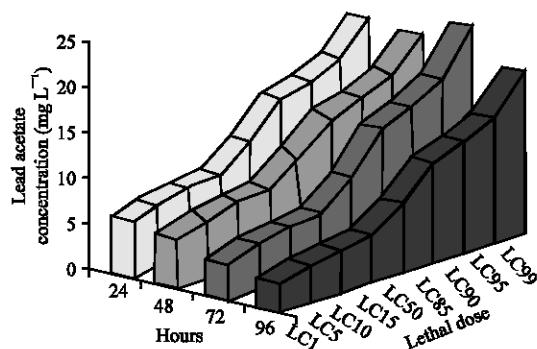


Fig. 2: Comparison of lead acetate toxicity by different dosages during experiment

effects of heavy metals, pesticides, fertilizers and the hazardous materials through which an appropriate model of toxins influences in natural ecosystems is made. Furthermore, the amount of sediments of different Quants in the region and physical and chemical properties their water is needed to be investigated.

ACKNOWLEDGMENT

This research was carried out through financial support provided by the University of Birjand for which the authors are gratefully acknowledged.

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