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## Original Article

# LC<sub>50</sub> and bioaccumulation of lead nitrate (Pb(NO<sub>3</sub>)<sub>2</sub>) in Goldfish (*Carassius auratus*)

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**Abstract:** Lead is a metal with no known biological benefit to organisms. The present study focused on bioaccumulation of Lead in various organs of gold fish (*Carassius auratus*). Fishes were exposed to lead nitrate [Pb(NO<sub>3</sub>)<sub>2</sub>] at a series of concentrations 0.0 mg/L (control group), 0.09, 0.15, 0.24, 0.3, 0.36 and 0.45 mg/l, which were equivalent to approximately 2, 3, 5, 6, 7 and 9% of 96 h LC<sub>50</sub> for 28 days. After 28 days of exposure, 10 fish per treatment were captured and anesthetized under aquatic solution of clove powder (200 mg/L). Fishes were euthanized and the gill, viscera and muscle tissue were sampled and weighed. Then, lead concentrations were measured in different tissues of goldfish using ICP. Viscera had the highest lead bioaccumulation potential, followed by the gill. The muscles were least preferred site for detecting the bioaccumulation of Pb. In conclusion, although lead was found in all tissues tested, Pb bioaccumulation potential is variable depending on the tissue structure.

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## Introduction

Major adverse effects on environmental quality, ecosystem integrity and human health have often been associated with mismanagement of chemical materials and the eliminations of hazardous substances. Contamination of aquatic ecosystem with heavy metal is one of the biggest environmental concerns (Agrahari, 2009). Heavy metals reach to the aquatic environment from natural and anthropogenic sources and distributed in the water bodies, suspended solids and sediments during the course of their transportation (Olajire and Imeokparia, 2001; Adeniyi et al., 2005; Aderinola et al., 2009).

Fishes are major sources of protein. They constitute major components of most aquatic habitats acting as bio-indicator of heavy metal levels in the aquatic environment. They have been recognized as good bio-accumulators of organic and inorganic pollutants (King and Jonathan, 2003). Although several

adverse health effects of heavy metals have already been known, exposure to heavy metals is increasing in many parts of the world, particularly in developing countries, though emissions have declined in most developed countries over the last century (Järup, 2010). An increase of heavy metals toxicity and its bio-accumulation in various tissues of aquatic organisms threatens the biodiversity of ecosystems and health of consumers (Adeniyi et al., 2008; Vinodhini and Narayanan, 2008a; George et al., 2011). However, accumulation of metals in fish tissues depends on many factors including environmental factors, type of heavy metals, metal concentration, time of exposure and biological characteristics of fish (Jeziarska and Witeska, 2006). Lead is one of the non-essential and toxic heavy metals which can be found as galena (PbS), cerussite (PbCO<sub>3</sub>) and anglesite (PbSO<sub>4</sub>) in earth's crusts, rocks, soil and water (Jalili et al., 2002). Lead may reach to the environment due to anthropogenic

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activities such as mining, coal burning, cement industry, daily usage of gasoline and gas oil, batteries and paints. Since lead may enter aquatic ecosystems through discharge of industrial waste waters, such as painting, dying, battery manufacture units and oil refineries (Ramesh et al., 2009).

Lead, like most heavy metals, find ultimately its way into surface waters influencing the health of aquatic animals such as fishes. Environmental pollution with heavy metals can cause serious risk such as genetic abnormality, physiological and biochemical problem and behavioral disorder in aquatic organisms (Scott et al., 2003). Among the aquatics inhabiting the polluted waters, fishes are sensitive to heavy metal pollution compared to other aquatics (Alinnor, 2005). Lead damages various body organs including the nervous, reproductive systems and excretory systems (kidneys) causing dysfunction of blood cells (Frumkin and Geberding, 2007; Vinodhini and Narayanan, 2008b).

Unfortunately, lead has been recently detected in groundwater and surface water supplies in different areas of Iran, arising concern about its effects (Charkhabi et al., 2005; Kazemi et al., 2013). As most fishes are the food source for human, bioaccumulation of lead in different tissues of these organisms may threaten the human's health. Therefore, the purpose of the present study were to investigate the Pb bioaccumulation potential in the viscera, gills and muscles of goldfish exposed to sub-lethal concentrations of lead nitrate.

### Materials and methods

**Fish:** Goldfish (mean weight  $16.66 \pm 3.09$  g) were purchased from local sellers and transported to the laboratory. Fish were acclimated to the laboratory conditions for 2 weeks before the initiation of experiments. Fishes were randomly allocated to 12 closed 200 L recirculating tanks supplied with oxygenated water with constant dissolved oxygen ( $6.5 \pm 0.5$  mg/L), temperature ( $22 \pm 2$  °C), pH ( $7.4 \pm 0.2$ ), water hardness ( $150 \pm 5$  mg/L CaCO<sub>3</sub>) and photoperiod (16L:8D). During acclimation and at all

experiments, fishes were fed with commercial aquarium fish diet at the manufacturer's recommended rate (2% of their body weight twice a day). Fishes were starved for 1 day before the start of the experiments, and 24 h before sacrifice.

**Acute toxicity experiments:** One hundred and eighty goldfish, *Carassius auratus*, were used in acute toxicity tests. The acute toxicity test was conducted following the Organization for Economic Cooperation and Development (OECD) Guideline No. 203 under static-renewal test conditions. The lead solutions used in the experiment were prepared using dissolving lead nitrate [Pb(NO<sub>3</sub>)<sub>2</sub>] (Merck Co., Germany) with 99.5% purity in distilled water at nominal concentrations of 0 (control), 2.5, 5, 10, 15, 20 mg/L. Ten acclimatized specimens were randomly selected and allocated to each glass aquarium (100 L) with three replicates with 30 individuals being tested at each lead concentration. During the 96 h acute toxicity experiment, water in each glass aquarium was aerated and had the same conditions of the acclimation period (dissolved oxygen  $6.0 \pm 0.5$  mg/L, temperature  $22 \pm 2$  °C, pH  $7.4 \pm 0.2$ , water hardness  $225 \pm 5$  mg/L CaCO<sub>3</sub>). For the static-renewal tests, the specimens were exposed to the metal 96 h with replacement of the test solution every 24 h (all stock solutions were made immediately prior to use). The water was changed daily to reduce the build-up of metabolic wastes to keep concentrations of Pb(NO<sub>3</sub>)<sub>2</sub> close to the nominal levels. Fish mortality was recorded at 0, 24, 48, 72, and 96 h after exposure to Pb(NO<sub>3</sub>)<sub>2</sub>. Fishes were considered dead when the gill opercula and body movement ceased. Dead fish were immediately removed using a dip net. LC<sub>50</sub> values were calculated using the Probit Analysis (Banaee et al., 2011).

**Sub-lethal toxicity experiments:** 315 gold fish were randomly allocated to 21 aquaria (100 L), with 7 treatments and three replicates and sub-lethal toxicity tests were performed over 28 days. Every tank containing 15 fishes were exposed to test solutions with the following concentrations of lead nitrate [Pb(NO<sub>3</sub>)<sub>2</sub>]: 0.0 (control), 0.09, 0.15, 0.24,

Table 1. Median Lethal Concentrations of Pb(NO<sub>3</sub>)<sub>2</sub> to goldfish.

Exposure time to Pb(NO <sub>3</sub> ) <sub>2</sub>	LC <sub>50</sub> (mg/L)	Range
24 h	16.93±1.29	14.74-20.20
48 h	12.47±0.94	10.72-14.57
72 h	7.92±0.68	6.57-9.32
96 h	5.02±0.54	3.93-6.14

Table 2. Lead bio-concentrations of different organs at exposure times of 28 days.

Concentration of Pb(NO <sub>3</sub> ) <sub>2</sub> in water (mg/L)	Bio-concentration of Pb(NO <sub>3</sub> ) <sub>2</sub> in different organs (mg/g)		
	Muscle	Visceral	Gills
0	0.000±0.000	0.000±0.000	0.000±0.000
0.09	0.067±0.005	0.437±0.022	0.240±0.012
0.15	0.060±0.002	1.130±0.057	0.276±0.014
0.24	0.075±0.004	1.732±0.087	0.318±0.016
0.3	0.108±0.005	2.040±0.102	0.378±0.019
0.36	0.226±0.011	3.645±0.182	0.546±0.027
0.45	0.222±0.021	3.636±0.182	0.706±0.035

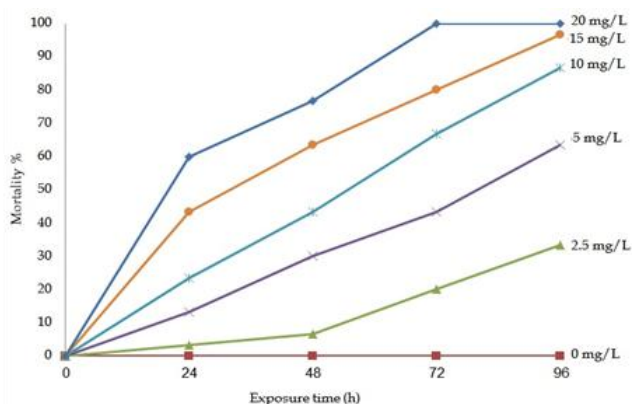


Figure 1. Changes of mortality of goldfish exposed to various concentrations of lead at different exposure times.

0.3, 0.36 and 0.45 mg/L, respectively. Sub-lethal concentrations were selected according the previous acute toxicity test. The water was changed daily to reduce the build-up of metabolic wastes and keep concentrations of lead nitrate near the nominal levels. Ten fishes per each exposure concentration were captured and anesthetized with the extract of clove powder (1:5000) 28 days after the exposure.

**Lead levels analysis in different tissues:** After dissecting the specimens, gill, viscera and muscle were separated. The samples were weighed and were placed in an electrical furnace to obtain the ashes for 8 hours at 550 °C. Then 1 g of ash was mixed with HNO<sub>3</sub> and HCl (1:1). For isolation of ash particle the

solution was filtered, mixed with deionized water and diluted to 25 ml.

Lead concentration was measured using ICP-OES-Perkin elmer (7300-BD). After calibration with standard soluble at 0.5, 1, 2, 5, 8 and 10 mg/lit, calibration diagram of lead was drawn and metal level in these prepared soluble measured. Samples were filtered using whatman filter (0.22 μm) and at last Pb concentration in each sample detected with ICP instrument. Pb levels were measured in triplicate and measurements were repeated three times.

**Statistical analysis:** Data are presented as mean ± SD. All the data were tested for normality (Kolmogorov-Smirnov test) and analyzed using a one way analysis of variance (ANOVA). The linear regression was used to examine the relationship between the concentration of lead in water and its accumulation in various tissues.

## Results

Acute toxicity of Pb(NO<sub>3</sub>)<sub>2</sub> was determined in goldfish after 24, 48, 72 and 96 hours of toxicity. LC<sub>50</sub> values significantly decreased over exposure time from 16.93 ± 1.29 mg/L at 24 h to 5.02 ± 0.54 mg/L at 96 h. Results of this study have been shown in Table 1. According to Figure 1, the toxicity of lead

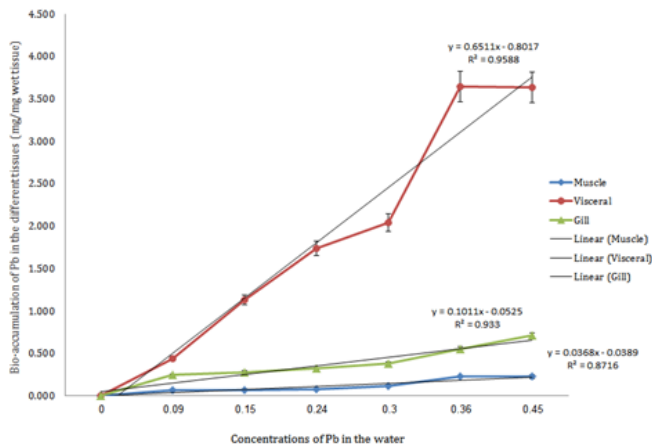


Figure 2. Linear regression between of water concentrations and tissue levels of Pb after 28 days of exposure.

nitrate on goldfish and mortality of fishes increased with increase of concentration and exposure time.

Sub-lethal concentrations of  $Pb(NO_3)_2$  were equivalent to approximately 2, 3, 5, 6, 7 and 9% of 96 h  $LC_{50}$  value (0.09, 0.15, 0.24, 0.30, 0.36, and 0.45 mg/L of aqueous solution of lead nitrate, respectively) for 28 day toxicity testing. Therefore, no mortality and no significant changes was found in clinical indicators and feeding behavior in treated fishes during the experimental periods.

The bioaccumulation of Lead in different organs of goldfish after exposure to contaminated water with different concentrations of  $Pb(NO_3)_2$  are presented in Table 2. Bio-concentration of Pb in the viscera and the gill were higher than that of the muscle in all of treatments.

Linear regression between concentrations of Lead in water and its bio-accumulation in various tissues was graphically illustrated in Figure 2.

## Discussion

Pollutants enter the fish body through a number of routes: directly through the digestive tract due to consumption of contaminated water and food or non-dietary routes across permeable membranes such as the gill or skin (Burger et al., 2002). On absorption, pollutants are transported in the blood stream to either a storage place (*i.e* bone) or to the liver for transformation and/or storage (Nussey et al., 2000)

or excreted by the kidney, the gill or stored in an extra hepatic tissues such as fat (Dimari et al., 2008). Lead, as one of heavy metals, is toxic for wildlife, experimental animals and humans (Wirth and Mijal, 2010). Results of our research showed the direct effect of pollution levels on bio-accumulation of lead in goldfish tissues. The bioaccumulation of Pb in visceral tissue of exposed fish obviously was higher than the other tissues. It seems reasonable because the viscera organs such as the liver and digestive organs are important organs for the uptake of heavy metals from diets. Ganbi (2010) reported that Pb bioaccumulation potential in the visceral tissues is higher than the muscle. Vinodhini and Narayanan (2008a, b) found that the kidney, the liver and the spleen accumulate more metals compared with muscle tissues of common carp in metal polluted media. This result agrees with those of other authors (Adeniyi et al., 2008; Sharma et al., 2011). There are numerous proteins (e.g. metallothionein) in visceral tissue which may be bound to heavy metals (Frumkin and Geberding, 2007). The high amount adipose tissue surrounding visceral tissues may be effective in the bioaccumulation of Pb.

In fish, gills are considered to be the dominant site for contaminant uptake because of their anatomical and/or physiological properties that maximize absorption efficiency (Takarina et al., 2012). In our research gill accumulation was lower than the viscera and higher than the muscle. There are records of similar bio-concentration of lead was noted by Łuszczek-Trojnar et al. (2013).

Muscles are the edible part of fish; therefore bioaccumulation of heavy metals in muscle could be a threat to consumers. Although Ozturk et al. (2009) believed that muscle is not an active tissue in accumulation of heavy metals, our results confirmed accumulation of lead in muscles. Studies conducted by other researchers on lead bioaccumulation in the other species (Falusi and Olanipekun, 2007; korai et al., 2008; Ozturk et al., 2009; Ganbi, 2010; Victor et al., 2012) confirmed our results.

Although different bio-accumulation abilities observed in different tissues, the levels of Pb in of goldfish tissues indicated its high bioavailability. The correlation coefficients ( $r^2$ ) for three types of tissues, including gills, viscera and muscle were 0.9330, 0.9588 and 0.8716 for 28 days of exposure. The linear regression between the concentration of lead in water and its accumulation in goldfish's tissue may indicate that various tissues could serve as a useful tool for the evaluation of heavy metal exposure. Generally, positive correlation exists between Pb concentrations of the tissues and exposure levels.

Physiological differences in the function and structure of various tissues may be influenced on the bioaccumulation of metals. Yap et al. (2004) and Łuszczek-Trojnar et al. (2013) believed that different rates of accumulation and depuration of Pb in the different soft tissues were found and this might be due to different mechanisms of metal binding and regulation.

Results of the present study emphasized that lead has a high potential to be accumulated in various tissues of fish, especially in soft tissues. So, the lower the bioaccumulation of lead in the muscle tissues, when compared with other tissues, seems reasonable. The results obtained clearly demonstrate the linear relationship between  $Pb(NO_3)_2$  concentrations in water and bio-concentration of Pb in different tissues.

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