Acute Toxicity and Accumulation of Iron, Manganese and, Aluminum in Caspian Kutum Fish (Rutilus kutum)

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ABSTRACT

Background: Iron, manganese, and aluminum are three abundant metals on earth and their concentrations have increased in aquatic environments as a result of natural and industrial activities. This study was undertaken to report the median acute toxicity (LC50) and accumulation of the sub-lethal concentration (10% 96-h LC50) of iron (Fe), manganese (Mn) and aluminum (Al) in kutum (Rutilus kutum) fingerlings .

Methods: For the 96-h LC50, the fish were exposed to concentrations of 105, 111, 117, 123, 129 and 135 mg/l of Fe and 40, 45, 50, 55, 60, and 65 mg/l of Mn and 18, 22, 26, 30, 34 and 38 mg/l of aluminum for 4 days. For sublethal exposure, they were exposed to mediums with concentrations of 12.3, 5.4 and 2.9 for Fe, Mn, and aluminum, respectively. Metal concentrations were determined by atomic absorption spectrophotometry in the gill tissues.

Results: Probit analysis showed the 96-h LC50 values of 122.98, 54.39, and 28.89 mg/l for Fe, Mn, and aluminum, respectively. Sub-lethal tests were conducted with nominal concentrations of 12.3, 5.4, and 2.9 mg/l of Fe, Mn, and aluminum for four days, respectively. Significant accumulations were observed in gills for all tested metals as compared to the control groups in short-term exposure ($P<0.05$.(

Conclusion: Obtained results clearly show that aluminum is the most toxic metal among tested ones for kutum fingerlings and it has the highest branchial AF value during sub-lethal exposure.

Keywords: Aluminum, Fishes, Iron, Lethal Dose 50, Magnesium, Water Pollution.

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INTRODUCTION

Today, aquatic animals are exposed to different concentrations of metals in aquatic environments. These metals tend to accumulate in their bodies which could influence all aspects of organisms' life [1, 2]. Generally, there are four possible routes for metals to enter a fish: food, gills, drinking water, and skin adsorption and the most important one is uptake through gills that have a prominent role in ion uptake and homeostasis [3].

Iron is an essential micronutrient for teleosts. It has a flexible redox activity, and therefore, is an integral component of cellular respiration [4]. Manganese is an essential trace element for living organisms which is involved in numerous metalloenzymes [5]. Aluminum, on the other hand, is proposed as one of the most toxic metals, especially in acidified waters [6, 7]. Fishes are more sensitive to aluminum toxicity than aquatic invertebrates [8]. Although aluminum and some metals are essential micronutrients for fishes, they can be toxic in excess concentrations .

LC50 tests measure the susceptibility and survival potential of organisms during exposure to particular toxic substances. Pollutants with higher LC50 values are less toxic for organisms because greater concentrations are required to result in mortality [9]. Metals are usually dispersed at sub-lethal concentrations around the world. Pollution of rivers, lakes, and coastal and marine waters by metals is frequently

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reported, and it has resulted in their accumulation in the tissues of aquatic organisms [10-13]. Likewise, some investigations have documented water and sediment pollution with metals in southern Caspian Sea [11, 14-20]. Rutilus kutum, a commercial species of the Caspian Sea, as an anadromos fish spends part of its life stages in polluted areas, and there is a possibility for juvenile contamination. This study was done to determine the 96-h LC50 values of Fe, Mn, and aluminum in kutum fingerlings and then, to study the accumulation of their single sub-lethal dose (10% 96-h LC50) in juveniles gills during a 4-day exposure period.

MATERIALS AND METHODS

Acute toxicity test

The bioassay was conducted in aquaculture laboratory of Shahid Rajaee Fish Hatchery Center, Sari, Iran in July, 2009. Kutum fingerlings (Rutilus kutum) with average body weight of 1.5±0.3g were obtained from the same center. Acclimation to the laboratory conditions was performed in the stock tanks for 2 weeks. For pilot experiments, the fish were transferred from the stock tanks to the 20 L experimental ones each containing 10fish/10 L. The tanks were aerated and natural photoperiod was adopted. The fish were starved for 24 hours prior to the commencement of experimentation. Then they were exposed to various concentrations of metals in range-finding tests for 4 days and the concentrations that resulted in mortality of fish within the range of 5% to 95% were recorded. Thereafter, for definitive tests, the fish were treated with 105, 111, 117, 123, 129, and 135 mg/L of Fe ($FeSO_4 \cdot 7H_2O$), 40, 45, 50, 55, 60, and 65 mg/L of Mn ($MnCl_2.4H_2O$), and 18, 22, 26, 30, 34, and 38 mg/L of aluminum ($Al_2(SO_4)_3.18H_2O$) concentrations for 4 days based on a static bioassay test following the No.203 protocol of OECD [21]. The toxicity of Fe, Mn, and aluminum to R. kutum was estimated as the median lethal concentrations (LC50). Water characteristics was as fallow: dissolved oxygen: 7.4±0.3 mg/L; temperature: 22.5±0.8°C; pH: 8.1±0.2; and water hardness: 273±12 mg $CaCO_3$/L. The fish were considered dead when gill opercula and body movements ceased, and in these conditions, they were immediately gathered. The 96-h LC50 values were calculated by the Probit Analysis test .

Sub-lethal exposure

For sub-lethal tests (10% of 96-h LC50 value), the fish were randomly distributed in 18, fiberglass tanks (each metal by 3 triplicates and 3 controls) with a density of 10 fish/tank to perform the 96-h sub-lethal tests. They were exposed to mediums with concentrations of 12.3, 5.4, and 2.9 for Fe, Mn and aluminum, respectively and 0.0 mg/L for controls. The water was changed on a daily basis to keep metal concentrations near the nominal ones. 18 fish from experimental and control groups were captured and sacrificed according to ethical codes after 96-h exposure. Then, the gills were removed, washed with 0.9% NaCl solution, and maintained at -20 °C until metal analysis.

Analysis

Gill tissue was put in test tubes and digested in concentrated super pure nitric acid (Merck, Darmstadt, Germany). Then the tubes were placed in a 100 °C oven. After complete digestion, the samples were cooled in room temperature and filtered (Whatman 42). Ultra pure water was added to each sample to reach a volume of 10 ml. Metal concentrations were determined by atomic absorption spectrophotometry. The analytical procedure was verified by measuring standard reference materials. The concentration of metal in gill was reported as µg/g wet weight (W.W). Accumulation factor (AF) was calculated according to the equation mentioned by Kim et al. [22].

Statistical Analysis

Data were presented as mean ± SE and were analyzed by student's t-test to compare differences between metal-exposed treatments and controls ($P<0.05$). Statistical analyses were performed using SPSS software (ver. 17.0, SPSS Company, Chicago, IL, USA). P-values of less than 0.05 were considered significant.

RESULTS

Results of the 96-h LC50 experiments are presented in Table 1. Under the stated experimental conditions, the 96-h LC50 values of Fe, Mn, and aluminum for R. kutum were found to be 122.98, 54.39, and 28.89 mg/L, respectively.

Table 1. Median acute toxicity (LC_{50}) of Fe, Mn, and aluminum to fingerlings of kutum *(R. kutum*).

LC_{50}	Metal		
	Fe	Mn	Al
96-h	122.98 (120.36-125.59)	54.39 (52.26-56.52)	28.89 (27.28-30.52)

There was no mortality in the control groups during all lethal and sub-lethal tests. Fish behaviors and swimming patterns were normal in all control groups. During exposure to acute concentrations, juvenile fish became excited and swam erratically, but these changes become normal later. Copious mucus secretion on gill surfaces was evident in both lethal and sub-lethal tests. All tested metals could significantly accumulate in gills of exposed fish compared to the control groups (Figure 1, $P<0.05$). The highest metal bioaccumulation was observed in Fe, and it was 0.476 µg/g gill wet weight. Accumulation factors (AF) of Fe, Mn, and aluminum in fish gills are shown in Figure 2 and the highest one (0.043) was observed for aluminum while the lowest one (0.0135) was indicated for Mn.

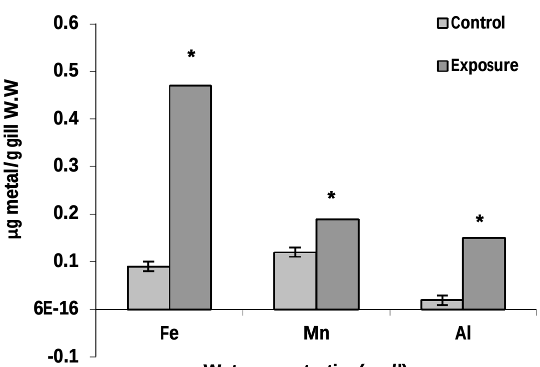

Figure 1. Metal concentrations (µg/g gill wet weight) in the gills of *R. kutum* fingerlings exposed to water borne sub-lethal concentration of Fe, Mn, and aluminum for four days (mean±SE, n=15-18). The significant difference between experimental groups and controls were analyzed by student's *t*-test. Asterisks denote significant differences between treatments and controls ($P<0.05$).

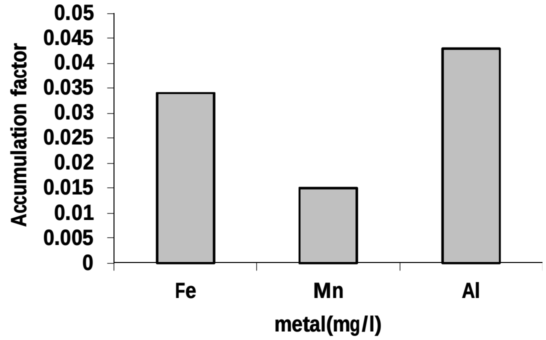

Figure 2. Accumulation factor (AF) in gills of *R. kutum* fingerlings exposed to water borne sub-lethal Fe, Mn, and aluminum concentrations for four days (n=15-18).

DISCUSSION

Acute toxicity

The susceptibility of fish to a particular metal is a very important factor for LC50 determination and subsequent values. Obtained results for 96-h LC50 values showed that aluminum has the lowest LC50 value and, thus, it is the most toxic metals for kutum among tested ones in this study. Its 96-h LC50 value was about 0.24 and 0.53 folds lower than Fe and Mn, respectively. It is well-documented that interaction between aqueous aluminum and the gill surface is central for mechanism of acute aluminum toxicity in fishes [23-25]. In addition, the toxicity of tested metals for R. kutum increased with both increasing concentration and exposure time .

The 96-h LC50 values of Fe, Mn and aluminum are different in various fish species. Generally, species differences were documented in susceptibility of fishes to a particular metal. Alam and Maughan [26] reported that 96-h LC50 values of Fe for common carp (Cyprinus carpio) ranged from 0.56 to 1.36 mg/L for 3.5 cm fish and 1.22 to 2.25 mg/L for 6.0-cm fish. Obtained LC50 values showed that kutum fingerlings are more sensitive to Mn than some other fishes. For example, 96-h LC50 value of Mn was found to be 3230 mg/L for Colisa fasciatus [27] which was higher than the obtained value in this study. Moreover, Anandhan and Hemalatha [28] estimated the 96-h LC50 of 56.92 ppm for aluminum chloride in zebra fish (Brachydanio rerio) that was much higher than our results for kutum. Gundersen et al. [29] reported LC50s for aluminum exposures of rainbow trout in the pH range of 8.0-8.6. The LC50s at all pHs were approximately 0.6 mg/L (range: 0.36-0.79 mg/L) as dissolved aluminum (i.e., filterable through a 0.4-μm filter), and were similar in both acute (96-hour) and long-term (16-day) exposures at hardness levels ranging from 20 to 100 mg/L (as calcium carbonate). Environmental conditions, such as oxygen, temperature, pH, hardness, salinity, and other metallic ions as well as fish age, may modify metal toxicity to the fish [30- 33]. It should be mentioned that during the present study, both relatively high water hardness (273±12 mg CaCO3/L) and pH (8.1±0.2) affected the obtained results as these water physicochemical characteristics mitigate metal bioavailability for fishes, and, thus, they reduce metal toxicity. It is a well-established fact that waterborne calcium and magnesium have a protective effect against metal toxicity [34-37]. Accordingly, Witeska and Jezierska [31] stated that increased water mineral content (hardness and salinity) could reduce metal toxicity for fishes.

Sub-lethal exposure

There was no mortality in R. kutum fingerlings exposed to sub-lethal metal concentrations during the 96-h exposure period. This shows that the selected concentrations were truly sub-lethal for kutum fingerlings under the stated laboratory conditions. Sub-lethal metal challenges resulted in statistically significant accumulations of metals in fish gills in the experimental groups compared to the controls. It should be stressed that even 10% of LC50 dose (as maximum acceptable concentration) can accumulate in fish tissue in high water hardness and even during short-term exposure (four days). This tissue burden might affect some aspects of juvenile life. In addition, increase in the mucus secretion was similar to the results obtained by Hmoud [38] in catfish during cadmium exposure that could affect metal uptake.

AF is a useful factor to compare the metal body burden of an organism with the degree of exposure dose [22]. Although aluminum had the lowest concentration in this study, its AF was the highest which shows its better potential for passing through gills. It is documented that gills are the primary target organ for aluminum uptake in fishes [39]. At pH 6.5 to 8.0, there is little data available on aluminum effects. It is known, however, that it binds to the gill surface, causing swelling and fusion of the lamellae, and increases diffusion distance for gas exchange [40]. The resulting damage leads to loss of membrane permeability, reduced ion uptake, loss of plasma ions, and changes in blood parameters related to respiration [41, 7].

CONCLUSION

In conclusion, our results show that aluminum is the most toxic metal among the tested ones for R. kutum. Aluminum had the highest AF during the 4-day sub-lethal exposure. With regard to the obtained bioaccumulations, we should be concerned about tissue burdens especially in osmoregulatory organs in non-lethal metal concentrations even at the short-term exposures.

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