LC\textsubscript{50} Determination and Copper and Cadmium Accumulation in the Gills of Kutum (Rutilus frisii kutum) Fingerlings

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ABSTRACT

Water pollution by metals and subsequent fish contamination are considered to be severe problems with detrimental ecological consequences. The purpose of the present study was to determine the median acute toxicity (LC\textsubscript{50}) of copper (Cu) and cadmium (Cd) in kutum, Rutilus frisii kutum, fingerlings. Also, using sub-lethal tests, metal accumulation and gill ion changes were evaluated. Fingerlings (1.1 ± 0.25 g) were exposed to different concentrations of Cu\textsubscript{SO}\textsubscript{4} \cdot 5H\textsubscript{2}O and CdCl\textsubscript{2} \cdot 2H\textsubscript{2}O in the static bioassay OECD test. In definitive tests, fish were exposed to nominal concentrations of 0.127, 0.229, 0.330, 0.432, 0.534, and 0.636 mg/l of Cu and 3.938, 6.893, 9.847, 12.801, 15.755, and 18.709 mg/l of Cd. Results from a Probit analysis showed that 96-h LC\textsubscript{50} values were 0.45 and 12.22 mg/l for Cu and Cd, respectively. Semi-static sub-lethal tests were conducted with nominal concentrations of 0, 12.7, 25.4, 50.9, and 101.8 μg/l of Cu and 0, 0.1, 0.25, 0.5, and 1 mg/l of Cd for 4 days. Significant accumulations were observed in gill Cu/Cd levels in all treatments in comparison to the controls (\( P < 0.05 \)) with a maximum average of 22.32 ± 1.25 μg Cu/g gill wet weight at 12.7 μg Cu/l treatment and 6.18 ± 0.44 μg Cd/g gill wet weight at 1.0 mg Cd/l treatment. Finally, no significant (\( P > 0.05 \)) changes were observed in gill sodium and calcium contents during Cu/Cd exposure. In conclusion, Cu is more toxic than Cd for kutum, and it has higher uptake in the gills.

Keywords: Copper, cadmium, sub-lethal, kutum

INTRODUCTION

Environmental pollutants such as heavy metals, pesticides, and other organic materials have detrimental effects on aquatic organisms. Metals as non-degradable pollutants are considered serious threat for aquatic environments by entering through different anthropogenic and natural sources (Moore 1991). Copper (Cu) and cadmium (Cd) are two existent metals in aquatic ecosystems, and numerous studies focus on different aspects of their toxicity for aquatic biota. They are cumulative pollutants, which exert a wide range of biochemical, physiological and genetic alterations in fish and other aquatic organisms (Heath 1995; Khangarot and Rathore 2003; Di Giulio and Hinton 2008; Eyckmans et al. 2012). Nowadays, the extensive use of Cu in aquaculture, agriculture, industry, and mining has resulted in its release and subsequent increase in receiving waters (Khangarot and Rathore 2003; Guardiola et al. 2012). Similarly, Cd as a non-essential heavy metal is used widely in industry for producing paints, dyes, cement and phosphate fertilizers causing increased amounts in aquatic ecosystems (Jarrup 2003; Burger 2008).

LC\textsubscript{50} tests measure the susceptibility and survival potential of organisms to a particular toxic substance. Pollutants with higher LC\textsubscript{50} values are less toxic because greater concentrations are required to induce mortality in organisms (Eaton et al. 2005). In addition, in most natural waters, metals are usually present only at sub-lethal concentrations, and such a contamination in aquatic environments is a widespread problem particularly in rivers and estuaries. Pollution of rivers, lakes, coastal, and marine waters by metals like Cu and Cd is widely observed in aquatic environments which leads to a considerable increase of their concentrations in aquatic organisms (Simpson 1981; Ravera 1984; Ray 1984; Harrison 1986; Lopes et al. 2001; Jarrup 2003; Agus et al. 2004; Burger 2008).

Numerous investigations point out the behavioral changes in fish exposed to lethal or sub-lethal concentrations of metals like Cu and Cd (Sloman et al. 2003; Vutukuru et al. 2005). In addition, metals accumulate in different organs of aquatic animals, and produce deleterious effects on fish (Asagba et al. 2008; Isani et al. 2009; Javed 2012). However, their accumulation levels in living organisms depends on species, the size of individuals, type of tissue or organ, and the metal itself (Lloyd 1992; Finn 2007). Generally, the uptake of water-borne Cu and Cd in freshwater fish occurs mainly through the gills, and this organ has crucial role in ion uptake and homeostasis (McGeer et al. 2000). Water-borne Cu/Cd can accumulate in fish gill cells, and then, affect the function of branchial pumps like Na\textsuperscript{+}K\textsuperscript{+}-ATPase and Ca\textsuperscript{2+}-ATPase. These pumps are active in chloride cells, and have vital role in fish osmoregulation (Shephard and Simkiss 1978; Pelgrom et al. 1995; Wong and Wong 2000).

The Caspian Sea, the largest continental water body on the earth (Dumont 1998), is the habitat for numerous commercial fishes, but some investigators have documented the accumulation of contaminants like metals in Caspian Sea fish populations (Moore et al. 2003; Agus et al. 2004; Anan et al. 2005). Moreover, chemical contamination is described as one of the most significant factors influencing the commercial fish populations in Caspian Sea (Karpinsky 1992). Accordingly, some investigations have documented water and sediment pollution in southern parts of the Caspian Sea (De Mora et al. 2004; Charkhabi et al. 2005; Parizanganeh et al. 2006; Saeedi and Karbassi 2006; Parizanganeh et al. 2008; Saeedi et al. 2010; Bagheri et al. 2011) that some commercial fishes spend part of their larval and fingerling stages in such polluted environments.

Kutum, Rutilus frisii kutum is a species with great ecological and commercial value, and its stocks in the Caspian Sea are replenished through artificial breeding. Every year more than hundreds of millions fries are produced by Ira-
nian Fisheries Organization, and then, fingerlings are released into the southern rivers and estuaries of the Sea (Farabi et al. 2007; Abdolhay et al. 2010) in which they probably experience different sub-lethal concentrations of pollutants like metals. The first objective of the present study was to determine the LC50 values of Cu and Cd and the second objective was to study their accumulations and gill sodium (Na+) and calcium (Ca2+) changes during sub-lethal exposure in kutum fingerlings.

**MATERIALS AND METHODS**

**Fish**

The kutum fingerlings with average body weight of 1.1 ± 0.25 g were obtained from the Shahid Rajaee Fish Hatchery Center, Sari, Iran. Fish were transferred to aquaculture laboratory of the same center on July, 2008. Fish acclimated to the laboratory conditions with ambient photoperiod in the 1000 l stock tanks for 2 weeks before the experimental use. The fish were fed 3% of body weight by commercial food once daily in the morning (at 9:00 a.m.).

**Acute exposure**

Cu and Cd stock solutions were prepared by using CuSO4·5H2O and CdCl2·2H2O, and were stored at 4°C. Before commencing the experiments, stock solutions were diluted to the desired concentrations. Following a two week acclimation period and for pilot experiments, fish were transferred from the stock tanks to the 20 l experimental ones based on a static bioassay test following the OECD No. 203 protocol (OECD 1992). Each tank contains 10 fish/1 l of oxygeanated well water with maintaining constant dissolved oxygen at 7.6 ± 0.2 mg/l, temperature at 23.5 ± 0.9°C, pH at 8.1 ± 0.2 and water hardness at 257 ± 8 mg CaCO3/l. The fish were starved for 24 h prior to and during the experiment. Healthy kutum fingerlings were exposed to various concentrations of metals for range-finding tests to choose concentrations that resulted in mortality of fish within the range of 5 to 95%.

Thereafter, in definitive tests, fish were treated in the same conditions with 0.127, 0.229, 0.330, 0.432, 0.534, and 0.636 mg/l of Cu (equivalent to 0.5, 0.9, 1.3, 1.7, 2.1, and 2.5 mg/l of CuSO4·5H2O) and 3.938, 6.893, 9.847, 12.801, 15.755, and 18.709 mg/l of Cd (equivalent to 8, 14, 20, 26, 32, and 38 mg/l of CdCl2·2H2O) concentrations. Acute toxicity of Cu and Cd to fingerlings kutum was calculated based on the Probit Analysis test (Finney 1971).

**Sub-lethal exposure**

For sub-lethal tests, fish were randomly distributed in thirty 20 l vessels according to Cinier et al. (1999). Then, 1 ml of 65% super pure nitric acid (Merck, Darmstadt, Germany) was added to each ashed sample in silica vessels. After complete digestion, ultra pure water was added to each sample to reach volume of 15 ml. Then, sample filtered (0.22 μm Cellulose acetate, Sandic, S&S, Germany), and the metal concentrations determined by ICP-OES (GBC, Integra XL). The concentration of metal in gill was reported as μg/g wet weight (WW). Also, accumulation factor (AF) was calculated based on the following definition (Kim et al. 2004):

$$\text{Accumulation Factor (AF)} = \frac{C_g}{C_w}$$

where Cg, Cc, Cw are the metal concentration in the experimental groups, controls, and water, respectively.

**Statistical analysis**

Statistical analyses were performed using SPSS software (ver. 17.0, SPSS Co., Chicago, IL, USA). Data are presented as mean ± SE. All the data were tested for normality (Kolmogorov–Smirnov test) and homogeneity (Levene’s test). Data were analyzed by One-way analysis of variance (ANOVA). Means were compared by Duncan’s multiple comparison test (P < 0.05) (Sokal and Rohlf 1995).

**RESULTS**

Results of the LC50 experiments have presented in Table 1. Under our experimental conditions, the 96-h LC50 values of Cu and Cd for *R. frisii kutum* was found to be approx. 0.45 ± 0.024 and 12.22 ± 0.64 mg/l, respectively.

As soon as the fish were exposed to acute and sub-lethal concentrations of Cd/Cu, they have become slightly excited and swam erratically, becoming normal only a few hours later. In sub-lethal test, after a 4 day exposure, there was a consistent increase in the rate of opercular movement, and also, excess mucus secretion was partly evident. It should be noted that behavioral and swimming patterns in control groups were normal, and there was not any mortality in this group during the experimental period.

According to the detailed results shown in Figs. 1 and 2, it is clear that Cu and Cd could accumulate in the gills of
exposed fish at all treatments compared to control group after 96-h sub-lethal exposure significantly \((P > 0.05)\). Also, significant difference was observed between the result of 101.8 \(\mu\)g Cu/l (400 \(\mu\)g CuSO\(_4\cdot5\)H\(_2\)O/l) dose compared to other Cu doses \((P < 0.05)\), but significant differences was not observed in gill Cd concentrations among experimental ones \((P > 0.05)\). The highest levels of Cu and Cd compounds were 22.32 ± 1.25 \(\mu\)g/g wet weight (12.7 \(\mu\)g Cu/l treatment equivalent to 50 \(\mu\)g CuSO\(_4\cdot5\)H\(_2\)O/l) and 6.18 ± 0.44 \(\mu\)g/g wet weight (1 mg Cd/l treatment equivalent to 2 mg CdCl\(_2\cdot2\)H\(_2\)O/l), respectively. Accumulation factors (AF) of Cu and Cd in fish gills are shown in Fig. 3. The AFs were decreased with increasing in exposure concentration, and showing an inverse relationship between AFs and exposure concentrations. A comparison between groups revealed neither difference in gill sodium nor calcium levels during Cd/Cu exposures for 4 days \((P > 0.05, \text{Table 2})\).

**DISCUSSION**

**Acute toxicity**

The susceptibility of fish to a particular metal is a very important factor for LC\(_{50}\) determination and subsequent values. In this study, the toxicity of Cu and Cd for *R. frisii kutum* increased with increasing concentration and exposure time. When fish were exposed to 0.127 mg/l of Cu and 3.938 mg/l of Cd, only 6.7% and 10% of fish died after 96 h, respectively, whereas 83.3% and 86.7% of fish died after 96 h when fish were exposed to concentrations of 0.636 mg/l of Cu and 18.709 of mg/l of Cd. Results obtained from the acute toxicity tests obviously demonstrated that Cu is extremely more toxic than Cd for kutum, and the 96-h LC\(_{50}\) value of Cd is about 27.2-fold higher than Cu one. Acute toxicity tests can detect the toxic damages of special pollutants in a short period of time. They ascribe the degree of toxicity among various pollutants, and the relative sensitivities of species to a particular one (Buikema et al. 1982).

The obtained LC\(_{50}\) values showed that kutum fingerlings are more sensitive to Cu and Cd than some other fishes. Values of 1.402 mg/l of Cu for *Exomus damircus* and 17.1 mg/l of Cd for *Cyprinus carpio* have already been reported (Suresh et al. 1993; Vutukuru et al. 2005). Also, Chen et al. (2012) reported a 96-h LC\(_{50}\) of 2.55 ± 0.03 mg/l for 1-month old Juvenile tilapia * Oreochromis mossambicus* during pulsed Cu exposure. Moreover, Tsay and Yu (1981) showed a 96-h LC\(_{50}\) of 23.20 mg/l in *Anguilla japonica* (0.65 g) during copper sulphate exposure. The 96-h LC\(_{50}\) values of Cu and Cd for fish differ from species to species and according to the type of metal. However, metal toxicities depend upon various factors such as water hardness and pH (Lauren and McDonald 1986; Perschbacher and Wurts 1999). It is worth noting that in the present study, high water hardness (275 ± 8 mg CaCO\(_3\)/l) and to some extent pH (8.1 ± 0.2) have affected LC\(_{50}\) values because high water hardness minimizes the bioavailability of Cu and Cd to fish and waterborne calcium and magnesium have a protective effect against Cu and Cd toxicity (Pagenkopf 1983; Perschbacher and Wurts 1999).

**Sub-lethal exposure**

According to obtained LC\(_{50}\) values of Cu, and its higher toxicity to kutum, its concentrations in the present study were lower compared to Cd in sub-lethal tests. Besides, some of the tested Cu concentrations in sub-lethal tests occur in Iranian surface water occasionally (Varedi et al. 2010). No mortality was observed in *R. frisii kutum* fingerlings exposed to all sub-lethal Cd concentrations over a 4 day period, but one fish died in one of 3 replications at the both 25.4 and 50.9 \(\mu\)g Cu/l treatments during the 96 h sub-
transport by affecting branchial ionic pumps like Na⁺/K⁺-ATPase, Ca²⁺-ATPase, and carbonic anhydrase (Christensen and Tucker 1976; Shephard and Sinkiss 1978; Pelgrom et al. 1995; Pratap and Wendlaar Bonga 1993; Li et al. 1998; Wong and Wong 2000), and therefore, metals change the ion balances of fish osmoregulatory organs (Ay et al. 1999; Grosell et al. 2003). In oppose to our supposition for gill Na⁺/Ca²⁺ changes during Cu/Cd concentration exposure, significant differences comparing to correspondence control value was not observed. In this study, because plasmatic ionic changes were not studied in fingerlings, comparison with others plasmatic ionic data during metal exposure was impossible. It was proposed that short duration of exposure period was certainly effectual on obtained results and thus, it is likely that the sufficient time was not provided for manifestations of ionic changes in fingerling gills. In conclusion, LC₅₀ results clearly show that Cu/Cd toxicity for R. frisius kutum increased with increasing concentration and exposure time, and more importantly, Cu is more toxic than Cd for fingerlings. Also, water concentration influences the branchial metal concentrations and AF in kutum but not in a dose-dependent manner. Moreover, Cu has a greater potential for entering through the fish gills. According to the results, we need more concern around environmental metal concentrations especially in rivers and estuaries for protecting the fish larva and juveniles from metal contamination.

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