

## Toxicity of Copper and Mercury to Caspian Roach *Rutilus rutilus caspicus*

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### Abstract

Copper (Cu) and mercury (Hg) enter aquatic ecosystems from different sources threatening health of aquatic organisms such as fish. As such, in this investigation, determining Cu and Hg toxic concentration in Caspian roach (*Rutilus rutilus caspicus*), was undertaken. Acute toxicity of Cu and Hg were determined in fish specimen under semi-static condition. Caspian roach fry (~ 1 g) were exposed to Cu or Hg at 6 concentrations (0.2, 0.27, 0.39, 0.47, 0.59 and 0.66 mg L<sup>-1</sup> total Cu and 0.22, 0.26, 0.3, 0.37, 0.44 and 0.52 mg L<sup>-1</sup> Hg). Control fish were exposed to water with no Cu or Hg. Mortality was recorded at 24-h-intervals for 96 h. Total Cu-LC<sub>50</sub> values found to be 0.43 mg L<sup>-1</sup> for 24 and 48 h and 0.42 mg L<sup>-1</sup> for 72 and 96 h. The lowest observed effect concentration (LOEC) values for total Cu were 0.39 mg L<sup>-1</sup> for 24 and 48 h, and 0.20 mg L<sup>-1</sup> for 72 and 96 h. On the other hand, free-Cu-LC<sub>50</sub> values found to be 0.22 mg L<sup>-1</sup> for 24 and 48 h and 0.21 mg L<sup>-1</sup> for 72 and 96 h. LOEC values for free Cu were 0.20 mg L<sup>-1</sup> for 24 and 48 h, and 0.10 mg L<sup>-1</sup> for 72 and 96 h. 24-72 h LC<sub>50</sub> values for Hg were 0.37 mg L<sup>-1</sup>, while 96 h LC<sub>50</sub> found to be 0.33 mg L<sup>-1</sup>. Likewise, LOEC values for Hg were 0.26 mg L<sup>-1</sup> for 24-72 h, and 0.22 mg L<sup>-1</sup> for 96 h. The results showed LOEC values for Cu and Hg in Caspian roach were lower than these metal concentrations in Caspian Sea water, suggesting little risk of acute toxicity in the sea. Chronic toxicity test is suggested to illustrate the effect of current levels of contamination in Caspian roach.

Keywords: *Copper, Mercury, Caspian roach, Toxicity*

### 1. Introduction

Aquatic habitats are under the menace of the heavy metal contamination. Heavy metals are not degradable and are accumulated across the food pyramid climax. Cu and Hg are two of the most toxic metals for fish. Cu is an essential microelement for living organisms, however at higher concentrations it is very toxic for aquatic life. Cu is introduced to water bodies from industrial waste or use of Cu sulphate as therapeutic or algacide agent. Unlike

Cu, Hg is an unessential element which is introduced mostly from erosions, volcanic eruptions and also from industrial activities (Gochfeld, 2003).

Caspian roach is one of the most valuable inhabitants of the Caspian Sea. However, Caspian roach population has recently declined in the Caspian Sea because of overfishing, degraded habitat and pollution. Consequently, artificial production of Caspian roach fry and fingerlings (0.5-3 g in weight) has been undertaken in order to enrich and rehabilitate stock population at the sea through releasing fish into the rivers flowing into the Caspian

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Sea. Previous studies have showed Cu and Hg contamination of the Caspian Sea (Agah et al., 2006; Abtahi et al., 2007; Taghipour and Azizi, 2011). Thus, there was the potential for fish contamination with Cu and Hg. This investigation was undertaken to investigate the acute toxicity of Cu and Hg in the Caspian roach. The results could help the authorities to describe the sound water quality criterion for these metals to conserve Caspian roach, this highly prized endangered species.

## 2. Materials and Methods

### 2.1. Fish and Maintenance Condition

Total of 2000 Caspian roach fry (~1 g) were obtained from Bony Fish Propagation and Rearing Center of Sijeval (Bandar Torkaman, Iran). Fish were packed in 10 plastic containers (100 L in volume) filled with water and oxygen in 1:2 proportions. Fish were transferred to Aquaculture Research Center of Gorgan University of Agricultural Sciences and Natural Resources, within 30 min. The fish were stocked in a fiberglass tank containing 1000 L of water. Fish were maintained under continually-aerated condition in tanks, 14:10 light: dark natural light and constant temperature ( $23 \pm 1$  °C) for 20 days, during which they were fed (~1 % of body weight, once a day) by trout commercial pellet (Biomare, France; 0.8 mm in diameter). Water exchange was about 50% every other day (dechlorinated tap water of Gorgan city). Water quality parameters such as total hardness, alkalinity, magnesium, calcium, iron, potassium, free Cu and sulphate levels were determined using portable Photometers with commercial kits provided by the manufacturer (Wagtecch Portable Photometer 7100, Berkshire, UK). Dissolved oxygen (DO), pH, temperature and salinity were measured using portable multi-parameter meter (sensION 156, USA) as follows: DO > 6 mg L<sup>-1</sup>, pH = 7.7-8, salinity = 0.1 ppt, total hardness = 180 mg

L<sup>-1</sup> (CaCO<sub>3</sub>), alkalinity = 168 mg L<sup>-1</sup> (CaCO<sub>3</sub>), magnesium = 1 mg L<sup>-1</sup>, calcium = 75 mg L<sup>-1</sup>, iron = 0.01 mg L<sup>-1</sup>, potassium = 9 mg L<sup>-1</sup>, free Cu = 0.03 mg L<sup>-1</sup> and sulphate = 7 mg L<sup>-1</sup>. No mortality was observed during this period.

### 2.2. Toxicity Test

The static renewal test (Weber, 1993) was performed in order to evaluate acute toxicity of Cu and Hg. Based on preliminary tests and previous results, fish were exposed to concentrations of 0, 0.2, 0.27, 0.39, 0.47, 0.59 and 0.66 mg L<sup>-1</sup> total Cu (as CuSO<sub>4</sub>) corresponding to 0, 0.1, 0.15, 0.2, 0.23, 0.31 and 0.35 mg L<sup>-1</sup> free Cu and 0, 0.22, 0.26, 0.3, 0.37, 0.44 and 0.52 mg L<sup>-1</sup> Hg (as HgCl<sub>2</sub>), to determine 24-96 h, LC<sub>50</sub>. Three 25 L, white and cylindrical tanks were used for each concentration. Each tank was stocked with 10 fish. Fish were allowed to adapt to these tanks for 10 days under aerated condition and fed and kept as explained above. No mortality was observed during this period. Feeding was stopped 24 h before dosing. Required amounts of CuSO<sub>4</sub> and HgCl<sub>2</sub> aqueous solution was prepared as stock solution. Cu and Hg solutions were added to each tank to the set concentrations. Feeding and aeration was ceased at the dosing point and thereafter. pH, DO, total hardness and alkalinity were measured as 8.01, 7.1 mg L<sup>-1</sup>, 170 mg L<sup>-1</sup> (CaCO<sub>3</sub>) and 161 mg L<sup>-1</sup> (CaCO<sub>3</sub>), respectively. Other water chemical properties were measured only before dosing which were as follows: magnesium = 1.1 mg L<sup>-1</sup>, calcium = 72 mg L<sup>-1</sup>, iron = 0.01 mg L<sup>-1</sup>, potassium = 9 mg L<sup>-1</sup> and sulphate = 7.3 mg L<sup>-1</sup>. Mortality was recorded at 24, 48, 72 and 96 h after dosing.

### 2.3. Statistical Analysis

LC<sub>50</sub> for each period (24-96 h) was determined by probit regression using EPA Probit Analysis Program V. 1.5 for each group, separately. All data

were accepted if calculated chi-square for heterogeneity was lower than the tabular value at the 0.05 level. LOEC determined as the maximum concentration which caused no mortalities at each time point (24-96 h) (Rand, 1995).

### 3. Results

In all tests, the most number of dead fish were observed at 24 h post exposure, thus the values of 24-96 h LC<sub>50</sub> were very close (Tables 1-3). Based on total Cu, LC<sub>50</sub> values found to be 0.43 mg L<sup>-1</sup>

for 24 and 48 h and 0.42 mg L<sup>-1</sup> for 72 and 96 h (Table 1). LOEC values for total Cu were 0.39 mg L<sup>-1</sup> for 24 and 48 h, and 0.20 mg L<sup>-1</sup> for 72 and 96 h (Table 1). On the other hand, based on free Cu, LC<sub>50</sub> values found to be 0.22 mg L<sup>-1</sup> for 24 and 48 h and 0.21 mg L<sup>-1</sup> for 72 and 96 h (Table 2). LOEC values for free Cu were 0.20 mg L<sup>-1</sup> for 24 and 48 h, and 0.10 mg L<sup>-1</sup> for 72 and 96 h (Table 2). 24-72 h LC<sub>50</sub> values for Hg were 0.37 mg L<sup>-1</sup>, while 96 h LC<sub>50</sub> found to be 0.33 mg L<sup>-1</sup> (Table 3). Likewise, LOEC values for Hg were 0.26 mg L<sup>-1</sup> for 24-72 h, and 0.22 mg L<sup>-1</sup> for 96 h (Table 3).

Table 1: LC<sub>50</sub> and LOEC values of Cu (total) with 95% confidence limits for Caspian roach

	LC <sub>50</sub> (mg L <sup>-1</sup> )	95% Confidence Limites		LOEC (mg L <sup>-1</sup> )	Slope ± S.E	Intercept ± S.E
		Lower	Upper			
24 h <sup>1</sup>	0.43	0.40	0.45	0.39	11.7 ± 1.69	9.31 ± 0.60
48 h <sup>2</sup>	0.43	0.40	0.45	0.39	11.8 ± 1.71	9.37 ± 0.61
72 h <sup>3</sup>	0.42	0.39	0.44	0.20	10.5 ± 1.43	9.01 ± 0.53
96 h <sup>4</sup>	0.42	0.39	0.44	0.20	10.5 ± 1.43	9.01 ± 0.53

<sup>1</sup> Tubular Chi-Square = 9.488; Calculated Chi-Square = 5.835.

<sup>2</sup> Tubular Chi-Square = 9.488; Calculated Chi-Square = 4.645.

<sup>3</sup> Tubular Chi-Square = 9.488; Calculated Chi-Square = 3.601.

<sup>4</sup> Tubular Chi-Square = 9.488; Calculated Chi-Square = 3.601.

Table 2: LC<sub>50</sub> and LOEC values of Cu (free) with 95% confidence limits for Caspian roach

	LC <sub>50</sub> (mg L <sup>-1</sup> )	95% Confidence Limites		LOEC (mg L <sup>-1</sup> )	Slope ± S.E	Intercept ± S.E
		Lower	Upper			
24 h <sup>1</sup>	0.22	0.21	0.23	0.20	12.45 ± 1.83	12.24 ± 1.22
48 h <sup>2</sup>	0.22	0.21	0.23	0.20	12.57 ± 1.57	13.35 ± 1.25
72 h <sup>3</sup>	0.21	0.20	0.23	0.10	11.12 ± 1.74	12.46 ± 1.18
96 h <sup>4</sup>	0.21	0.20	0.23	0.10	11.12 ± 1.74	12.46 ± 1.18

<sup>1</sup> Tubular Chi-Square = 9.488; Calculated Chi-Square = 3.411.

<sup>2</sup> Tubular Chi-Square = 9.488; Calculated Chi-Square = 2.785.

<sup>3</sup> Tubular Chi-Square = 9.488; Calculated Chi-Square = 0.719.

<sup>4</sup> Tubular Chi-Square = 9.488; Calculated Chi-Square = 0.719.

Table 3: LC<sub>50</sub> and LOEC values of Hg with 95% confidence limits for Caspian roach

	LC <sub>50</sub> (mg L <sup>-1</sup> )	95% Confidence Limites		LOEC (mg L <sup>-1</sup> )	Slope ± S.E	Intercept ± S.E
		Lower	Upper			
24 h <sup>1</sup>	0.37	0.35	0.39	0.26	17.35 ± 2.42	12.49 ± 1.05
48 h <sup>2</sup>	0.37	0.34	0.37	0.22	13.93 ± 1.73	11.25 ± 0.79
72 h <sup>3</sup>	0.37	0.34	0.37	0.22	13.93 ± 1.73	11.25 ± 0.79
96 h <sup>4</sup>	0.33	0.32	0.35	0.22	12.03 ± 1.43	10.79 ± 0.70

<sup>1</sup> Tubular Chi-Square = 9.488; Calculated Chi-Square = 2.809.

<sup>2</sup> Tubular Chi-Square = 9.488; Calculated Chi-Square = 8.115.

<sup>3</sup> Tubular Chi-Square = 9.488; Calculated Chi-Square = 8.115.

#### 4. Discussion

Heavy metals are highly toxic to aquatic organisms. Toxicity of Cu is related to gill dysfunction and sodium loss (Grosell et al., 2007), respiration stress (Grosell et al., 2007) and oxidative stress (Roméo et al., 2000). Acute Hg exposure has been found to affect both pituitary-interrenal (cortisol elevation) and the pituitary-thyroid (T3 and T4 elevation) axis in fish (Bleau et al., 1996). It affects olfactory system (Baatrup and Doving, 1990) and brain acetylcholine esterase activity (Shaw and Panigrahi, 1990) in fish. Likewise, gill (Wobeser, 1975), liver (Kendall, 1977) and kidney (Kirubakaran and Joy, 1988; Handy and Penrice, 1993) damages have been observed in fish exposed to Hg.

Previous studies have showed higher total Cu LC<sub>50</sub> values for Japanese flounder, *Paralichthys olivaceus* (8.7–12.2 mg L<sup>-1</sup>, 0.3 – 17 g) (Furuta et al., 2008) and red sea bream, *Pagrus major* (2.0–5.2 mg L<sup>-1</sup>, 0.5 – 13 g) (Furuta et al., 2008) compared to present study. The difference might be due to difference in water salinity, as the previous study conducted in seawater. Grosell et al. (2007) suggested that Cu toxicity was due to osmotic disturbance and whole body sodium loss and as osmotic gradient between fish body and brackish water and saltwater was comparatively lower than between fish body and freshwater, Cu toxicity decreased parallel to salinity increment. Likewise, Straus and Tucker (1993) reported higher total Cu LC<sub>50</sub> values (0.95-0.98 mg L<sup>-1</sup>) for channel catfish *Ictalurus punctatus*, in freshwater. However, larger fish (fingerling) was tested in that study compared to the present one suggesting, the increment in Cu toxicity tolerance along with increase in body weight. Alam and Maughan (1992) reported similar (0.3 mg L<sup>-1</sup>) total Cu LC<sub>50</sub> values in a cyprinid species, common carp, *Cyprinus carpio*. Free Cu is very toxic (Chakoumakos et al., 1979). It was suggested that water chemistry (pH, hardness and alkalinity) is the key factor determining free Cu concentration

(Chakoumakos et al., 1979). Thus, depending on the water chemistry, Cu toxicity might change, while total Cu remain unchanged. Accordingly, it is useful to report free Cu when acute toxicity test is conducted. Chakoumakos et al. (1979) found that total Cu LC<sub>50</sub> for *Salmo clarki* decreased ~ 2 folds (0.578 vs. 0.293 mg L<sup>-1</sup>) parallel to decrease in hardness, while based on free Cu, the values decreased ~ 30 folds (0.009 vs. 0.0003 mg L<sup>-1</sup>). The present results showed about half of the total Cu remained free, Cu rendering it toxic to fish. Other toxic forms of Cu such as CuOH<sup>+5</sup> were not investigated in this study.

MacLeod and Pessah (1973) found 96 h-LC<sub>50</sub> of Hg to be 0.28 mg L<sup>-1</sup> for rainbow trout *Onchorhynchus mykiss* fingerlings, which is lower than the present results. This difference expectedly might be because of difference between the species. Alam and Maughan (1992) reported the 96 h-LC<sub>50</sub> value for common carp to be 0.57 mg L<sup>-1</sup>, which was higher than that of the present results, suggesting that common carp might be more tolerant to Hg toxicity compared to its relative species, Caspian roach. Snarski and Olson (1982) found the lower value (0.17 mg L<sup>-1</sup>) for Hg 96 h-LC<sub>50</sub> in fathead minnow *Pimephales promelas* larvae which was lower compared to present study, suggesting lower tolerance in larvae stage and/or species difference. Generally, due to difference in methods and analyses, comparison between the studies could not be conclusive.

Caspian Sea is a closed system and any introduced-contaminations accumulate in the sea body. Previous investigations have showed cumulative increase in heavy metal contamination in the Caspian Sea (Anan et al., 2002). Cu contamination in southern Caspian water was reported by Ashja Ardalan et al. (2008) to be 0.009 mg L<sup>-1</sup>. On the other hand, Hg concentration in the southwest Caspian Sea water was 0.027-0.065 mg L<sup>-1</sup> (Shahsavaripour et al. 2009). Khakpour et al. (2009) reported Cu and Hg concentrations in the water column of Gorganroud River to be less than 0.05 and

0.02 mg L<sup>-1</sup>, over the year. Both metal concentrations was lower than the LOEC values obtained in the present work, suggesting there was no risk of acute Cu and Hg toxicity for Caspian roach fry. However, chronic toxicity tests would provide valuable data about the long-term effects of sub-lethal Cu and Hg concentrations on Caspian roach.

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