Effect of Water Hardness on the Toxicity of Cobalt and Nickel to a Freshwater Fish, *Capoeta fusca*

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**Abstract**

**Objective** To determine the effects of water hardness on the toxicities of cobalt (Co) and nickel (Ni) to a freshwater fish, *Capoeta fusca*.

**Methods** Toxicity was investigated by static bioassay. Fish were exposed to cobalt (as CoCl2) and nickel (as NiCl2) for 96 h in waters with two levels of hardness (“hard” and “very hard”, nominally 130 mg/L and 350 mg/L as CaCO3, respectively).

**Results** Water hardness had a significant effect on the acute toxicity of both elements. The 96 h LC50 values for Co were 91.7 mg/L and 204.8 mg/L in hard and very hard waters, respectively, and for Ni the 96 h LC50 values were 78.0 mg/L and 127.2 mg/L, respectively.

**Conclusion** The fish were more sensitive to Co and Ni toxicity in hard water than in very hard water; very hard water protects *C. fusca* against the toxicity of Co and Ni.

**Key words:** Acute toxicity; Hard water; Lethal concentration; Mortality; Very hard water

INTRODUCTION

Qanat is a type of water management system used to provide a dependable supply of water to human settlements, particularly in arid regions. The term is derived from an ancient Semitic word meaning “to dig” and refers to a system of underground water channels consisting of vertical shafts connected at their bottom with a sloping tunnel[1]. In addition to using the water for drinking and irrigation, some native settlements also exploit fishes living in this ecosystem as a food source.

Aquatic ecosystems are directly or indirectly affected by the physical and chemical characteristics of their environment. A number of investigators have reported that the toxic effects of elements, particularly heavy metals, on freshwater organisms is related to the hardness of the water[2,5]. Cobalt (Co) is an essential element for animals because it is a necessary for the synthesis of vitamin B12[6-11]. It is widely distributed in rocks, soils, water and vegetation[12-13], and is often found in association with nickel (Ni)[12,14]. High cobalt concentrations can be found in industrial wastewater, near to cobalt-mining facilities, and in runoff of fertilizers used in agriculture[14-15]. In higher concentrations, cobalt is toxic to humans, and to terrestrial and aquatic animals and plants[12]. Its toxicity to cells results from inhibition of cellular respiration and citric acid cycle enzymes[13].

Nickel occurs naturally at low concentrations in aquatic environments and is widely used in industrial
MATERIALS AND METHODS

Sampling and Maintenance of Fish

_Capoeta fusca_ (Cyprinidae) were obtained from Qanats in Birjand, eastern Iran during June and July 2009. The means weight and total length (±SD) of fish used in experiments were 4.95±0.75 g and 6.40±0.61 cm, respectively. They were maintained in an aquarium system at 23±0.2 °C with 13 h light: 11 h darkness photoperiod, and were allowed to adjust to laboratory conditions for 7 days before experimentation. Sets of 10 fish (in triplicate) were randomly selected and exposed to cobalt (as CoCl$_2$) and nickel (as NiCl$_2$) in 50 L of water for 96 h, under the same conditions, during July. Fish were fed twice daily during the acclimation period with commercial pellets at about 2% of body weight but were not fed during the experimentation period, as recommended by Perschbacher and Wurts [26], Pandey et al. [27], and Prato et al. [28]. Fish were considered dead when movement had ceased and they exhibited no response to gentle stimulation with a glass rod. Mortalities were recorded at 24, 48, 72, and 96 h of exposure and the dead fish were removed regularly from the test solutions. No mortalities were observed in control exposures of the same duration lacking cobalt or nickel.

Description of Experiments

Stock solutions (1 000 mg/L) were prepared by dissolving analytical-grade CoCl$_2$ or NiCl$_2$ (Merck) in distilled water. To design a suitable range of test concentrations, preliminary trials were conducted to estimate the minimum lethal and maximum nonlethal concentrations of Co and Ni. The initial concentrations of Co and Ni in the test solutions are shown in Table 1. Experimental waters with two different hardness levels were used in the investigation: “hard water” with nominal total hardness 130 mg/L CaCO$_3$, and “very hard water” at 350 mg/L CaCO$_3$ (water <75 mg/L CaCO$_3$ is considered “soft”); 75-120 mg/L CaCO$_3$ is “moderately hard”; 120 and 200 mg/L CaCO$_3$ is “hard”; and >200 mg/L CaCO$_3$ is considered “very hard” [29]). For the very hard water was Birjand tap water; a mixture of distilled water and tap water was used for the hard water. Dissolved oxygen (mg/L), temperature (°C) and pH were recorded individually in each test aquarium during the exposure periods. Total hardness, magnesium and nitrite concentrations (mg/L) were determined before commencing the experiments, using a Palintest 8 000 photometer (Table 2).
Table 1. Concentrations of Co and Ni Used in Toxicity Tests on C. fusca

<table>
<thead>
<tr>
<th>Element</th>
<th>Hard Water</th>
<th>Very Hard Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>60, 80, 100, 120, and 140 mg/L</td>
<td>100, 150, 200, 250, and 300 mg/L</td>
</tr>
<tr>
<td>Ni</td>
<td>30, 60, 90, 120, and 150 mg/L</td>
<td>40, 80, 120, 160, and 200 mg/L</td>
</tr>
</tbody>
</table>

Table 2. Physical and Chemical Characteristics of the Qanat Water and Test Waters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Qanat Water</th>
<th>Very Hard Test Water</th>
<th>Hard Test Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hardness (as CaCO(_3), mg/L)</td>
<td>460</td>
<td>350±3.1</td>
<td>130±2.7</td>
</tr>
<tr>
<td>pH</td>
<td>8.3±0.1</td>
<td>7.8±0.2</td>
<td>7.8±0.2</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>21</td>
<td>23±0.2</td>
<td>23±0.2</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>6.6±0.2</td>
<td>6.3±0.2</td>
<td>6.3±0.2</td>
</tr>
<tr>
<td>Mg (mg/L)</td>
<td>24±3</td>
<td>33±2</td>
<td>15±2</td>
</tr>
<tr>
<td>Nitrite (mg/L N)</td>
<td>0.001</td>
<td>0.007</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Statistical Analysis

Median lethal concentration (LC\(_{50}\)) values and 95% confidence limits were calculated from the data obtained in acute toxicity bioassays using the EPA probit analysis computer program (Version 1.5).

Table 3. Lethal Concentration (LC\(_{50}\) with 95% confidence limits in parentheses) of Cobalt and Nickel at Two Different Water Hardness Levels, Estimated Using the EPA Probit Analysis Computer Program

<table>
<thead>
<tr>
<th>Exposure Duration</th>
<th>LC(_{50}) Values (mg/L) and 95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Cobalt</td>
<td></td>
</tr>
<tr>
<td>Hard Water</td>
<td>123.9 (118.9-129.4)</td>
</tr>
<tr>
<td>Very Hard Water</td>
<td>255.9 (241.0-274.0)</td>
</tr>
<tr>
<td>Nickel</td>
<td></td>
</tr>
<tr>
<td>Hard</td>
<td>121.3 (66.9-227.0)</td>
</tr>
<tr>
<td>Very Hard</td>
<td>215.0 (214.0-479.4)</td>
</tr>
</tbody>
</table>

RESULTS

Relationship between Water Hardness and Mortality of C. fusca

Qanat water was harder (460 mg/L CaCO\(_3\)) than either of the test waters (130 mg/L and 350 mg/L CaCO\(_3\)).

In preliminary tests, in which minimum lethal and maximum nonlethal were accurately calculated, in the hard water, the probability of cobalt mortality after 96 h of exposure was 100%, whereas in the very hard water the probability of cobalt mortality was only about 35%. For nickel, in the hard water, the probability of mortality of after 96 h of exposure was 100% but, in the very hard water, was only about 80%.

The 96 h percentage mortalities of C. fusca increased as the concentrations of Co and Ni in the test solutions were increased (Figures 1 and 2). Mortalities were higher in the hard water than in the very hard water treatments, at all concentrations of both elements. LC\(_{50}\) values for Co and Ni to C. fusca were generated from these mortality data (Table 3). The 95% confidence limits indicated that the 96 h LC\(_{50}\) values for both cobalt and nickel were significantly lower in the harder water.

Figure 1. Effect of varying the cobalt concentration on the percentage mortality of C. fusca after 96 h exposures to the metal at two different levels of water hardness.

Figure 2. Effect of varying the nickel concentration on the percentage mortality of C. fusca after 96 h exposures to the metal at two different levels of water hardness.
**Comparison of Co and Ni Toxicities**

*C. fusca* was more sensitive to both Co and Ni in the hard water than in very hard water treatment, and their toxicities increased with the duration of the exposure (Table 3). In the hard water, the LC50 values for Co decreased from 123.9 mg/L after 24 h exposure to 91.7 mg/L after 96 h, while in the very hard water LC50 values decreased from 255.9 mg/L at 24 h to 204.8 mg/L at 96 h. The acute toxicities of Ni to *C. fusca* after 24 h exposure were approximately similar to those of Co but Ni toxicity was higher in the longer exposures. Thus, in the hard water, the LC50 values for Ni decreased from 121.3 mg/L at 24 h to 78.0 mg/L at 96 h and, in very hard water, decreased from 215.0 mg/L to 127.2 mg/L.

**DISCUSSION**

This study examined a basic toxicological concept, the “dose-response” or “concentration-response” in which the response of an organism is proportional to the dose or concentration of the test substance at the target site. In many cases, the target site is unknown, or measurement of the substance at the site is not possible. Instead, surrogate measures of the target site concentration have been used. There are many scientific fields that use fish as models in research, including respiratory and cardiovascular research, cell culture, ecotoxicology, aging, pharmacological, and genetic studies. However, to understand the behavior of individual toxicants requires knowledge of the associated physical and biochemical conditions.

Both Co and Ni were more toxic to *C. fusca* in hard water than in the very hard water. A 2.7-fold increase in water hardness (from 130 mg/L to 350 mg/L CaCO3) reduced the 96 h acute toxicity of Co about 2.5-fold, and of Ni about 1.5-fold. Javid et al. (29) showed that the 96 h LC50 and lethal concentrations of Ni were significantly different among three fish species (*Catla catla, Labeo rohita,* and *Cirrhina mirgala*). *C. catla* was least sensitive to nickel, with LC50 value of 45.0 mg/L, followed by that of *Labeo rohita* and *Cirrhina mirgala*, with LC50 values of 30.1 mg/L and 20.4 mg/L, respectively. Ebrahimpour et al. (31) reported that a 9.5-fold increase in water hardness (from 40 mg/L to 380 mg/L CaCO3) substantially reduced the toxicity of Cu (up to 6.5-fold) and Zn (up to 7.5-fold) to *Capoeta fusca* at 96 hours exposure. They also showed that in water of hardness 150 mg/L CaCO3, the LC50 for Cu at 96 h exposure was 5.4 mg/L, compared with 7.5 mg/L in water of hardness 350 mg/L CaCO3. Similarly, the 96 h LC50 values for Zn 74.4 mg/L and 102.0 mg/L, respectively. Clearly, heavy metal toxicities vary greatly among individual fish species and are highly dependent on the chemical characteristics of the environment.

While high levels of water hardness may limit the growth of fish, softer waters increase the sensitivity of fish to toxic metals. That is, higher hardness is beneficial by reducing metal toxicity to fish. Previous studies have shown that the toxic effects of heavy metals on other aquatic organisms are also dependent on the water hardness. Heavy metal toxicity is lower in hard water because of competition between the contaminant metal ions and Ca2+ and Mg2+ ions for uptake sites on the body surface of organisms. Thus, water hardness reduces metal toxicity by saturating gill surface binding sites with Ca2+ and Mg2+ ions to the exclusion of toxic metal cations [4].

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