Bioaccumulation of Hexavalent Chromium in Tissues of a Freshwater Fish

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Abstract

This study aims to investigate the bioaccumulation of hexavalent chromium in the tissues of a freshwater fish, *O. aureus* (Tilapia), and to compare the chromium concentrations in fish tissues such as muscle, skin and gill. The objective of the study was to assess the impact of different chromium concentrations on the survival and health of fish. The bioaccumulation of Cr in fish tissues was measured through a 60-day exposure period. The results showed that the accumulation of Cr in fish tissue was dose-dependent, with the highest concentration leading to the greatest bioaccumulation. The study also highlighted the importance of monitoring chromium levels in aquatic environments to ensure the health and survival of freshwater fish.

Keywords: Cr (VI); Acute and chronic effects; Aquatic pollutants; Risk assessment

Introduction

The overall toxic impact on organs like gill, kidney and liver may seriously affect the metabolic and physiologic activities and could impair the growth and behavior of fish [1]. Acute poisoning by chromium compounds causes excessive mucous secretion, damage in the gill respiratory epithelium and the fish may die with symptoms of suffocation [2]. On chronic exposure, hexavalent chromium severely affected the renal tubules causing hypertrophy of epithelial cells, reduction of tubular lumen [3], and chromium compounds also cause renal failure leading to the loss of osmoregulatory ability and respiration in fish [4]. Sub-lethal effects of chromium in fish were directly related to the inhibition of various metabolic processes [5]. The hexavalent chromium induced depletion in the profiles of liver glycogen, total protein and total lipid has been reported [6,7] studied African catfish (*Clarias gariepinus*). Concentrations used varied from 11 to 114 mg/L (K₂Cr₂O₇). They found that the main deformity reported was abnormal body axis [8]. After 45 days of exposure significant decline in the protein and carbohydrate content of gills was observed [9]. The objective of this study was to investigate the impact of different chromium (10, 15, 20, 25 and 30 μg/L Cr⁶⁺) pollutions on the survival of juvenile *Oreochromis aureus* (Tilapia) and to compare the chromium concentrations in fish tissue (muscle, skin and gill).

Material and Methods

Fish

*O. aureus* (Tilapia), a tropical freshwater and important culture fish, is a species commonly found in brackish water in estuaries all over the world [10-12]. Sixty Juvenile specimens of Tilapia were captured from ponds at Manzalla Lake and transferred to the laboratory where the experiments were conducted during April 2015. The samples were placed in aquarium for one week to allow for adaptation of the fish to the new conditions. Tap water used for the experiment had a pH value of 7.5 ± 0.3 and total hardness of 131 ± 5.3 mg CaCO₃/L. The aquaria were aerated with air stones for proper oxygen saturation (8.5 mg O₂/L). Fish were hand-fed once a day with artificial feed meal containing 22% crude lipid, 22% crude protein, 22% crude lipid, 25% ash and 12% moisture. Fish were fed at a feeding rate of 3% of body weight per day for 28 days. Any fish that showed abnormal behavior were removed immediately from the tanks.

Acclimatized fish were moved at random into six aquaria volume (50 L), five aquaria for each concentration and one aquarium for control, each containing ten fish. Each filtered sample bottle acidified to 1% HNO₃ and analyzed with Inductively Coupled Plasma Emission Spectrometry (ICP-ES). The concentrations were expressed as micrograms per gram weight (μg/ g. wt.) of tissue in organisms.

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The concentrations of Cr measured in the water aquariums throughout the experiment were ±10% of the nominal concentrations (Table 1). Therefore, nominal concentrations from now on will take this value as the basis. The general agreement of Cr\(^{6+}\) with the Cr remained in the Cr\(^{6+}\) form throughout the experiment [14]. Tables 1 and 2 summarize the measured Chromium concentration in water (Cr\(^{6+}\) mg/L) and the survival of the fish during a 4-week-exposure period for juvenile specimens. Survival of control fish was nearly 100% throughout the experiment. Chromium concentrations (25 and 30 mg/L Cr\(^{6+}\)) reduced fish survival beginning from the first week of exposure to Cr\(^{6+}\) doses of 10 mg/L to 30 mg/L Cr\(^{6+}\) mediums were 38 to 45% mortality at the end of experiments. Nearly no mortality was recorded in the experiment controls. The Cr concentrations (μg/g.wt.) of Cr in the organs fish increased when they were exposed to Cr (Figures 1a-1e). Chromium 1.25 to 12.25 μg/g wt., while concentrations in the gill were within the range of 3.06 - 44.83 μg/g wt. (Figures 1a-1e). As can be seen in Figure 1a, maximum level of Cr\(^{6+}\) was 12.25 μg/gwt. on fish muscle having lived 28 days at 10 μg/L Cr\(^{6+}\) initial concentrations. On the other hand, higher concentration of Cr\(^{6+}\) (12.25 μg/g wt.) was observed on fish muscle after 28 days at 30 μg/L Cr\(^{6+}\) (Figure 1e). Maximum levels of Cr\(^{6+}\) concentrations on the muscle tissues at 15 (after 28 days) in Figure 1b, 20 (after 28 days) in Figure 1c and 25 (after 28 days) μg/L Cr\(^{6+}\) treatments in Figure 1d were 2.43, 9.03 and 10.81 μg/g wt., respectively. The concentrations of Cr in gills were almost twice, three and four times as high as in the muscle at 20, 25 and 30 μg/L Cr\(^{6+}\) mediums, respectively. The Cr accumulation on the skin of Tilapia for all experimental concentrations was nearly twice as much as in the muscle of samples at the end of experiment.

### Table 1: Mean of measured chromium concentration in different Tissues of juvenile Tilapia (O. aureus) exposed to Cr\(^{6+}\) as K\(_2\)Cr\(_2\)O\(_7\) during 28 days.

| Chromium conc. (Cr\(^{6+}\) mg/L) in water | Mean of measured Chromium conc. in water (Cr\(^{6+}\) mg/L) (SD) | Initial number of Tilapia per aquarium | Percent survival in exposure days (d) |
|------------------------------------------|------------------------------------------------|----------------------------------------|-------------------------------------|
| Control (0)                              | 1 (0.1)                                           | 10                                     | 100\(^{a}\) 100\(^{a}\) 96.4\(^{a}\) 96.4\(^{a}\) 96.4\(^{a}\) 95.8\(^{a}\) |
| 10                                       | 9.4 (0.6)                                         | 10                                     | 96.4\(^{a}\) 96.2\(^{a}\) 96.3\(^{a}\) 92.3\(^{a}\) |
| 15                                       | 12.8 (0.5)                                        | 10                                     | 96.4\(^{a}\) 95.1\(^{a}\) 85.4\(^{a}\) 81.6\(^{a}\) |
| 20                                       | 18.7 (0.6)                                        | 10                                     | 93.6\(^{a}\) 94.2\(^{a}\) 88.7\(^{a}\) 82.3\(^{a}\) |
| 25                                       | 23.4 (0.8)                                        | 10                                     | 88.5\(^{a}\) 81.8\(^{a}\) 81.4\(^{a}\) 62.4\(^{a}\) |
| 30                                       | 27.8 (1.1)                                        | 10                                     | 86.3\(^{a}\) 75.9\(^{a}\) 72.1\(^{a}\) 52.7\(^{a}\) |

Different superscript letters (a, b and c) designates difference at p < 0.05 within a same day.

**Statistical evaluation**

For the survival tests, Statistical Analysis of data was carried out with SPSS statistical package program. A value of p < 0.05 was considered to be significant for the accumulation tests.

### Results

The concentrations of Cr measured in the water aquariums throughout the experiment were ±10% of the nominal concentrations (Table 1). Therefore, nominal concentrations from now on will take this value as the basis. The general agreement of Cr\(^{6+}\) with the Cr remained in the Cr\(^{6+}\) form throughout the experiment [14]. Tables 1 and 2 summarize the measured Chromium concentration in water (Cr\(^{6+}\) mg/L) and the survival of the fish during a 4-week-exposure period for juvenile specimens. Survival of control fish was nearly 100% throughout the experiment. Chromium concentrations (25 and 30 mg/L Cr\(^{6+}\)) reduced fish survival beginning from the first week of experiment, where high mortality rate of Cr-exposed fish occurred within 14 to 21 days. Survival rate decreased to 52.7% in fish exposed to 30 mg/L Cr\(^{6+}\) dose from days 21 to 28 (Table 2). The behavioral changes of the control group and Tilapia exposed to various doses of K\(_2\)Cr\(_2\)O\(_7\) were compared with each other during the experiments. The control group displayed normal behavior during the test period. The lowest concentrations (10 and 15 mg/L Cr\(^{6+}\)) had similar behavior to that of the control group. From the dose 20 mg/L Cr\(^{6+}\) the fish started to show behavioral disorders such as loss of equilibrium, sudden startling and respiratory difficulties. 25 mg/L Cr\(^{6+}\) and at the highest concentration (30 mg/L Cr\(^{6+}\)) onwards, there was shivering, rather high respiratory disorder and swimming in capsized position. The accumulations of chromium (μg/g.wt.) in the gill, skin and muscle tissues of Tilapia during the exposure period are shown in Figures 1a-1e. The initial Cr\(^{6+}\) concentrations were, 10 mg/L, 15 mg/L, 20 mg/L, 25 mg/L, 30 mg/L. The chromium accumulation on the skin of Tilapia for all experimental concentrations was nearly twice as much as in the muscle of samples at the end of experiment. Concentrations in the tissues of control fish (<0.007 μg/L Cr\(^{6+}\)) throughout the experiments.

### Table 2: Mean of measured chromium concentration in different Tissues of juvenile Tilapia (O. aureus) exposed to Cr\(^{6+}\) as K\(_2\)Cr\(_2\)O\(_7\) during 28 days.

| Chromium Conc. (Cr\(^{6+}\) mg/L) in water | Mean of measured Chromium concentration in different Tissues in exposure days (d) |
|------------------------------------------|--------------------------------------------------------------------------------|
| G = gill, S = skin and M = muscle        | 7\(^{th}\) day | 14\(^{th}\) day | 21\(^{st}\) day | 28\(^{th}\) day |
| Control (0)                              | 0.001 | 0.002 | 0.001 | 0.004 | 0.003 | 0.002 | 0.004 | 0.003 | 0.002 | 0.007 | 0.006 | 0.005 |
| 10                                       | 1.15 | 0.91 | 0.4 | 3.20 | 1.38 | 0.75 | 4.20 | 2.25 | 0.95 | 3.06 | 2.72 | 1.25 |
| 15                                       | 3.71 | 1.6 | 0.9 | 6.50 | 2.85 | 1.45 | 6.54 | 3.25 | 1.75 | 7.95 | 4.91 | 2.43 |
| 20                                       | 4.77 | 2.1 | 1.2 | 9.35 | 4.45 | 2.20 | 33.45 | 18.20 | 8.64 | 37.41 | 19.1 | 9.03 |
| 25                                       | 9.85 | 4.2 | 2.5 | 15.95 | 9.10 | 4.31 | 37.85 | 18.75 | 9.55 | 40.61 | 22.5 | 10.81 |
| 30                                       | 11.25 | 6.4 | 3.29 | 23.75 | 13.25 | 6.45 | 41.56 | 22.10 | 10.31 | 44.83 | 25.3 | 12.25 |

G = gill, S = skin and M = muscle

**Discussion**

It is widely known that metal toxicity is more accurately measured in fresh water than in sea water, because metal appear to a great extent as complex compounds in sea water and this reduces the toxicity of metal ions. In the present study, the mortality increased with an increase in Cr\(^{6+}\) concentration and also the duration of the exposure to Cr\(^{6+}\). This may be rather significant, because smaller fish being more generally more active than larger ones, metal uptake and elimination of metal could also occur in higher rates in these smaller ones [15,16]. A comparison of toxicity values for 10 μg/L Cr\(^{6+}\) to 30 μg/L Cr\(^{6+}\) experiments demonstrated a decline in survival rate following longer exposure periods. Even though the magnitude of this decline varied between the different chromium concentrations, lower (10 to 20 μg/L Cr\(^{6+}\)) mediums were about 10 to 20% mortality while higher (25 and 30 μg/L Cr\(^{6+}\)) mediums were 38 to 45% mortality at the end of experiments. Nearly no mortality was recorded in the experiment controls. The Cr concentrations (10 to 30 μg/L ppb) Cr (VI) used in the present study has been proved not to be sub lethal for juvenile Tilapia and have been considered as representatives of environmental exposure, fish showed externally abdominal distention and hemorrhagic (reddening) and-
The damage was noted increasing at the exposure of 30 mg/L onwards. In the lowest concentration through the effect was basement membrane and sub mucosa. The gill filaments and its constituents were damaged gradually which reached to the total collapsed level at the highest concentration (40 mg/L). At the higher concentration, the gill rakers start hypertrophy and that was follow by the hypertrophy in gill filaments by their hypertrophy. All these changes caused the fish to avoid taking food and intake of oxygen. These patterns of changes have also been reported by many workers [24,25]. It was also observed that the heavy metals in muscle tissue were at low levels compared with other organs [26]. The results also show that chromium is more accumulated in the samples of gill than in the skin or muscles [23].

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