



## Estimation of target hazard quotients for heavy metals intake through the consumption of fish from Sirvan River in Kermanshah Province, Iran

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### Original Article

#### Abstract

The aim of this research was to investigate concentrations of cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu), and zinc (Zn) in the muscle, gill, and liver of common carp (*Cyprinus carpio*), tuwini (*Capoeta trutta*), and Grass carp (*Ctenopharyngodon idella*) from Sirvan River, Kermanshah Province, Iran, during November to December 2014. This investigation was conducted in order to determine the potential health risk of the intake of these metals through the consumption of the edible parts of fish and also to assess the safe dietary intake levels of these metals. The results of the present study indicated that the highest and lowest accumulated metal concentrations were related to Zn and Cd, respectively. Moreover, the metal concentrations in the gill and liver were higher than in the muscles of the three fish species. The target hazard quotients (THQs) for an adult with mean body weight of 71.5 kg were below 1 based on Cd, Pb, Cr, Cu, and Zn levels. In conclusion, the obtained results indicated that the levels of metals in the edible muscle of fish species in this study were below the level of concern for human consumption.

**KEYWORDS:** Gills, Liver, Metals, Carp, Body Weight

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#### Introduction

Over the past decade, pollution of freshwater ecosystems by heavy metals has become a problem on local, regional, and global scales. Heavy metals are toxicants, and highly persistent and nonbiodegradable contaminants, and may be accumulated in the human food chain.<sup>1,2</sup> Moreover, heavy metals are divided into two important groups; essential metals and nonessential metals. Metals such as copper (Cu) and zinc

(Zn) are essential elements for growth and development of the body, while other metals such as cadmium (Cd), lead (Pb), and chromium (Cr) are nonessential elements for the body and have no known role in biological and physiological mechanisms.<sup>3,4</sup> Several studies have indicated that Cd and Pb are toxic heavy metals for humans and other organisms and affect a number of organs and systems such as the kidney, nerve tissue, circulatory system, reproductive system, and immune systems.<sup>5,6</sup> Thus, an important step in monitoring freshwater ecosystems is knowledge of the metal levels

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in the organs of aquatic organisms regarding both aquatic ecosystems management and human consumption of aquatic organisms.

Numerous researches have assessed metal levels in aquatic ecosystems using different types of aquatic organisms such as seaweeds and filter-feeding molluscs,<sup>7,8</sup> shrimp and crab,<sup>9,10</sup> mussels and oysters,<sup>11,12</sup> and fish.<sup>1,13</sup> Fish is a source of minerals, protein, and omega-3 polyunsaturated fatty acids for human consumption and has a positive impact on cardiovascular disease, inflammatory conditions, and brain and immune system health.<sup>14,15</sup> Fish are good bioindicators of heavy metals in aquatic ecosystems, because they are at the top of the aquatic food chain and accumulate high levels of metals from surrounding waters.<sup>16,17</sup> Therefore, the purpose of the present research was to investigate the distribution of the selected metals of Cd, Pb, Cr, Cu, and Zn in the muscle, gill, and liver of three fish species from the Sirvan River, Kermanshah Province, Iran. This study was conducted in order to assess the potential health risk of the intake of these heavy metals through the consumption of the edible parts of fish and also to determine the safe dietary intake level of these metals.

## Materials and Methods

Fish samples were collected from random catches in Sirvan River during November to December, 2014. Fish species were carried to the laboratory in a thermos flask containing ice. In this study, the three fish species of common carp (*Cyprinus carpio*) ( $n = 20$ ), tuwini (*Capoeta trutta*) ( $n = 20$ ), and Grass carp (*Ctenopharyngodon idella*) ( $n = 20$ ) were investigated in terms of metal levels (Cd, Pb, Cr, Cu, and Zn) in the edible muscle, gill, and liver. In the laboratory, they were immediately dissected using a stainless steel dissection instrument and clean plastic gloves. Samples of muscle were separated from under the dorsal fin of fish without skin.<sup>13</sup>

Approximately 1 g wet weight of gill,

liver, and muscle were dissected from each sample and washed with distilled water, and accurately weighed into 150-ml Erlenmeyer flasks. To each sample, 10 ml nitric acid (65%) was added. Samples were left overnight in order to digest slowly. Afterward, 5 ml perchloric acid (70%) was added to each sample.<sup>1,13</sup> Digestion was performed on a hot plate (sand bath) at 150 °C before dilution with 25 ml deionized water. The concentrations of Cd, Pb, Cr, Cu, Ni, Zn, and Fe were measured using inductively coupled plasma atomic emission spectroscopy (ICP-OES) (AMETEK Materials Analysis Division, Germany). The detection limits for Cd, Pb, Cr, Cu, and Zn, were, respectively, 0.01, 0.2, 0.07, 0.3, and 0.75 µg/g. Moreover, the mean recovery for Cd, Pb, Cr, Cu, and Zn were 98.7, 98, 97.4, 96.7, 94.5, 96.6, and 99.4 percent, respectively.

Statistical analysis was performed using SPSS Software (version 16, SPSS Inc., Chicago, IL, USA). The one-way analysis of variance (ANOVA) was used to verify significant differences in organ metal concentrations among the three fish species. The metal concentrations in organs were expressed as microgram per gram wet weight (ww). Values are given in mean ± standard deviation (SD).

Daily consumption limits of metal contaminated fish were calculated according to the following equation.<sup>18-20</sup>

$$CR_{lim} = (RfD \times BW) / C_m$$

Where  $CR_{lim}$  is maximum allowable fish consumption rate (kg/d), RfD is reference dose (10 for Cd, none set for Pb, 3 for Cr, 300 for Zn, 20 for Cu, and 360 for Fe µg/kg/day), BW is the consumer's body weight (kg), and  $C_m$  is measured concentration of chemical contaminant m in a given species of fish (mg/kg).<sup>20</sup>

The consumption limit is determined in part by the size of the meal consumed. A 0.227 kg average fish meal size was assumed.<sup>18</sup> The following equation was used to convert daily consumption limits to the

number of allowable meals per month.<sup>20</sup>

$$CR_{mm} = (CR_{lim} \times T_{ap})/MS$$

Where  $CR_{mm}$  is the maximum allowable fish consumption rate (meals per month),  $CR_{lim}$  is the maximum allowable fish consumption rate (kg/d),  $MS$  is the average fish meal size (0.227 kg fish/meal), and  $T_{ap}$  is the time averaging period (365.25 days/12 months = 30.44 days/month).

Hazard quotient (HQ) is the ratio of the estimated exposure dose of a contaminant to its RfD. The HQ can be calculated with the following equation:<sup>19-20</sup>

$$HQ = [(MCC \times CR)/BW]/RfD$$

Where HQ is the health risks through consumption of fish by the local inhabitants, RfD is reference dose (10 for Cd, none set for Pb, 3 for Cr, 300 for Zn, and 20 for Cu  $\mu\text{g}/\text{kg}/\text{day}$ ), BW is the consumer's body weight (kg), MCC is measured concentration of chemical contaminant  $m$  in a given species of fish (mg/kg), and CR is the average consumption rate (0.003 kg fish/meal). A target hazard quotient (THQ) below 1 means the exposure population is unlikely to experience evident adverse effects.<sup>20</sup> Acceptable daily and weekly uptake of heavy metals were calculated with the following equation:<sup>20</sup>

$$DI = C_m \times IR$$

where DI is the daily intake,  $C_m$  is the concentration of metal  $m$  in fish ( $\mu\text{g}/\text{g}$ ) and IR is the intake rate.

## Results and Discussion

The heavy metals concentrations in the muscle, gill, and liver of *C. carpio*, *C. trutta*, and *C. idella* are presented in figures 1 to 6. The results of the present research indicated that the metal levels in the liver and gill were higher than the muscle. Moreover, the results of statistical analysis showed that the levels of metals in organs of the three fish species were not significant ( $P > 0.05$ ) (Table 1). The THQs in the three species of fish were below 1 (Table 2).

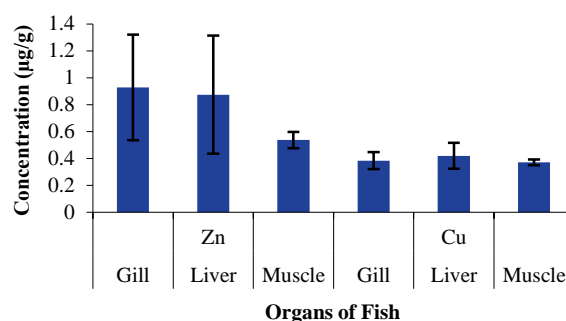


Figure 1. Cu and Zn concentrations ( $\mu\text{g}/\text{g}$ ) in organs of *Cyprinus carpio*

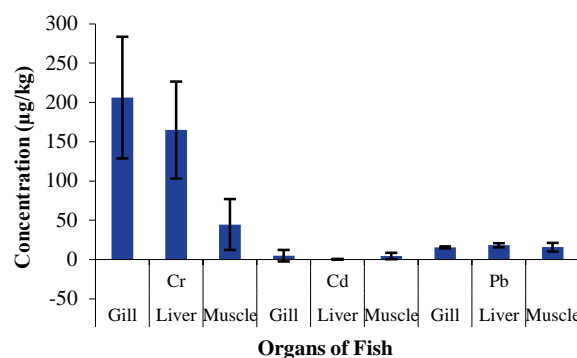


Figure 2. Cd, Pb, and Cr concentrations ( $\mu\text{g}/\text{kg}$ ) in organs of *Cyprinus carpio*

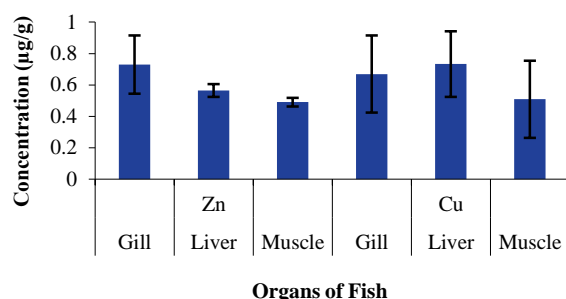


Figure 3. Cu and Zn concentrations ( $\mu\text{g}/\text{g}$ ) in organs of *Capoeta trutta*

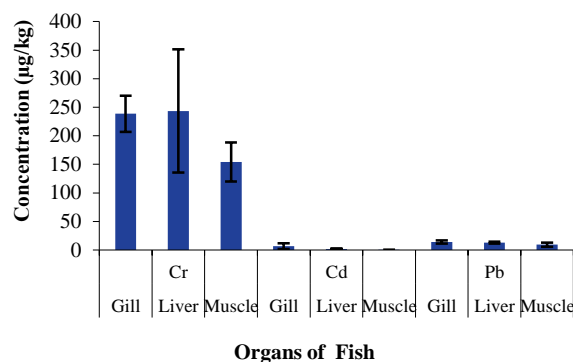


Figure 4. Cd, Pb, and Cr concentrations ( $\mu\text{g}/\text{kg}$ ) in organs of *Capoeta trutta*

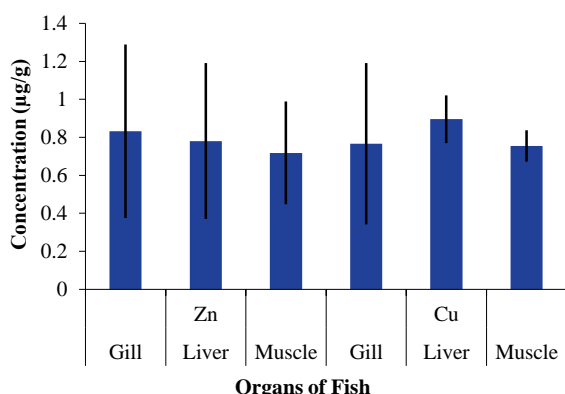


Figure 5. Cu and Zn concentrations ( $\mu\text{g/g}$ ) in organs of *Ctenopharyngodon idella*

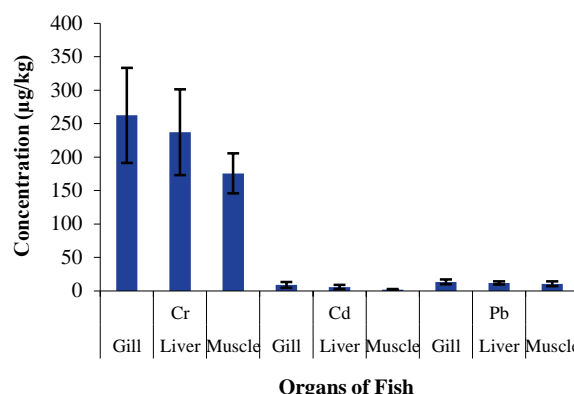


Figure 6. Cd, Pb, and Cr concentrations ( $\mu\text{g/kg}$ ) in organs of *Ctenopharyngodon idella*

Table 1. Statistical analysis of metal levels in organs of the three fish species

| Area/Fish species | Cd            |    | Pb            |    | Cr            |        | Cu            |    | Zn            |    |
|-------------------|---------------|----|---------------|----|---------------|--------|---------------|----|---------------|----|
|                   | One-way ANOVA |    | One-way ANOVA |    | One-way ANOVA |        | One-way ANOVA |    | One-way ANOVA |    |
|                   | F-value       | P  | F-value       | P  | F-value       | P      | F-value       | P  | F-value       | P  |
| C. carpio         | 0.74          | NS | 0.52          | NS | 5.85          | < 0.05 | 0.42          | NS | 1.15          | NS |
| C. trutta         | 3.23          | NS | 2.38          | NS | 1.64          | NS     | 0.73          | NS | 3.69          | NS |
| C. idella         | 3.97          | NS | 0.52          | NS | 1.77          | NS     | 0.27          | NS | 0.06          | NS |

P = significance level; NS = not significant

In this study, Cd, Pb, Cr, Cu, and Zn levels were higher in the gill and liver of the three fish species than the muscles. One reason for high concentrations of heavy metals in the gills and liver is the presence of large amounts of metallothionein (MT) protein and metal retention in these organs.<sup>21</sup> MTs, the multipurpose proteins, are low-molecular-weight proteins with many sulfhydryl groups which bind a variety of metals, such as Cu, Zn, and Cd, and show a strong affinity toward certain essential and non-essential metals.<sup>22</sup> In contrast, in muscle tissue, due to low metabolic activity, the accumulation of metals is low. Therefore, heavy metals uptake from aquatic environment via the muscle is much less important than the gills and liver. The liver also has an important role in contaminant storage, redistribution, and detoxification or transformation and acts as an active site of pathological effects induced by contaminants.<sup>23</sup> The liver tissue is highly active in the uptake and storage of heavy metals. Fish respond to heavy metal exposure by producing MT, particularly in the liver.<sup>21</sup> Thus, the liver tissues in fish are more often

recommended as an environmental indicator of water pollution than any other fish organs.

Table 2. Hazard quotient, daily intake, and consumption rate indexes for the three species of fish in this study

| Species   | HQ      | DI ( $\mu\text{g/g}$ ) | CR (g) |
|-----------|---------|------------------------|--------|
| C. carpio |         |                        |        |
| Zn        | 0.0007  | 10.90                  | 39.1   |
| Cu        | 0.0070  | 7.50                   | 3.7    |
| Cr        | 0.0060  | 0.80                   | 4.7    |
| Cd        | 0.0001  | 0.08                   | 175.0  |
| Pb        | 0.0006  | 0.30                   | 46.6   |
| C. trutta |         |                        |        |
| Zn        | 0.0007  | 10.10                  | 42.8   |
| Cu        | 0.0108  | 10.30                  | 2.7    |
| Cr        | 0.0220  | 3.10                   | 1.3    |
| Cd        | 0.00005 | 0.01                   | 1400.0 |
| Pb        | 0.0003  | 0.18                   | 77.7   |
| C. idella |         |                        |        |
| Zn        | 0.0006  | 14.60                  | 29.2   |
| Cu        | 0.0161  | 15.30                  | 1.8    |
| Cr        | 0.0375  | 3.50                   | 8.0    |
| Cd        | 0.0004  | 0.02                   | 800.0  |
| Pb        | 0.0004  | 0.20                   | 70.0   |

HQ: Hazard quotient; DI: Daily intake; CR: Consumption rate

Cd, Pb, and Cr are toxic and non-essential metals and have no well-known role in the biological systems. They have been

recognized as environmental contaminants mainly due to their high toxicity at low concentrations.<sup>1</sup> Long term exposure to Pb and Cd may result in kidney and liver failure, and circulatory system and nerve tissue deficiency. Hanaa et al. have reported that exposure to high Pb concentrations may cause death or deterioration of the central nervous system, brain, and kidney.<sup>5</sup> Reported Pb levels in edible muscle of *C. carpio* (0.018 µg/g), *C. trutta* (0.012 µg/g), and *C. idella* (0.011 µg/g) were lower than the maximum acceptable concentrations (2.0 µg/g fresh weight of fish as seafood) reported by the European Commission (EC),<sup>24</sup> the UK Food Standards Agency,<sup>25</sup> Australian National Health and Medical Research Council (ANHMRC),<sup>26</sup> and Spanish legislation.<sup>27</sup> The concentrations of Cd obtained in *C. carpio* (0.004 µg/g), *C. trutta*, (0.001 µg/g), and *C. idella* (0.001 µg/g) edible muscles were lower than the maximum acceptable concentrations established by the EC (2001) (0.05 µg/g). Moreover, we found the Cd levels to be lower than the standards of the ANHMRC (2.0 µg/g), Western Australian authorities (5.5 µg/g),<sup>28</sup> and Spanish legislation (1.0 µg/g).<sup>27</sup>

The Food and Agriculture Organization of the United Nations (FAO)<sup>29</sup> has established a maximum permissible concentration of 30 µg/g for Cu (in this study, 9.8 µg/g for *C. carpio*, 12.8 µg/g for *C. idella*), and 30 µg/g for Zn (in this study, 19.5 µg/g for *C. carpio* and 15.4 µg/g for *C. idella*). The Cu and Zn concentrations in the edible muscle of both fish from Sirvan River were below the levels of concern for human consumption of toxic compounds. The Zn and Cu concentrations in the liver of *C. carpio*, *C. trutta*, and *C. idella* in the present study were lower than those in *Hypophthalmichthys molitrix*, *Ctenopharyngodon idellus*, and *Megalobrama amblycephala* in China,<sup>2</sup> and *Labeo calbasu*, *Cirrhinus reba*, and *Rita rita* in Chenab River, Pakistan.<sup>30</sup>

Hajeb et al. reported that the daily intake of heavy metals through food consumption is dependent on several factors such as the metal

concentrations in food and amount of food consumed.<sup>31</sup> Health risk estimate levels measured for Cd, Pb, Cr, Cu, and Zn in all three fish species (Sirvan River) were below the international criteria in edible fish muscle for human protection.<sup>32</sup> In this study, to estimate the human health risk from consuming metal-contaminated fish, the estimated exposure doses were calculated for five metals. The maximum HQs determined for Cd, Pb, Cr, Cu, and Zn in all three fish species were 0.0004, 0.0004, 0.02, 0.01, and 0.0007, respectively. According to these results, THQ was lower than 1, and a THQ below 1 means the exposure population is unlikely to experience noticeable adverse effects.<sup>20</sup>

## Conclusion

The health risks posed by exposure to heavy metals of Cd, Pb, Cr, Cu, and Zn, in local inhabitants of Kermanshah Province through the consumption of contaminated fish were investigated based on THQs. The results of the present study indicated that the THQ values are less than 1 for adults by consuming fish. The metal concentrations in the gill and liver were higher than in the muscles of the three species of fish. According to the results of this study, the levels of metals in the edible muscle of fish species in this study were below levels of concern for human consumption.

## Conflict of Interests

Authors have no conflict of interests.

## Acknowledgements

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