Risk assessment of non-carcinogenic effects of heavy metals from Dez river fish

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Abstract

Background and purpose: Considering the many benefits of fish in the food basket, the purpose of this study was to examine the risk assessment effects of heavy metals from Dez river fish.

Materials and Methods: 80 samples were caught from the local species of Dez River, they were acid digested; afterwards, they were analyzed by atomic absorption spectrometry.

Results: The results showed that there was a positive correlation between accumulating metals and condition factor, especially with regard to zinc and cadmium; this correlation was more likely to be observed for the Capoeta trutta species. Also, comparing the daily and weekly intake of heavy metals with the rate limits of PTDI has shown that the absorption value of metals were considerably lower than the specified limit.

Conclusion: The high level of CRlim showed safe consumption of fish in Dez River considering the potential health risk. Zinc and cadmium played an important role in finding out the TTHQ index. The Capoeta trutta and Carasobarbus luteus species had the most and least amounts of TTHQ index. Finally, comparing the means of metal concentrations with standard ones has shown that the concentrations of zinc and cadmium were higher than the global standards.

Key words: Food safety; Dez River; Pollution; Fish; Target Hazard Quotient

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1. Introduction

In recent decades, the uncontrolled industrialization and urbanization of suburbs and coastal areas have led to the considerably increased pollution of aquatic environments (1). Heavy metals were easily absorbed and accumulated in the organisms due to high biological half-life, potential accumulation in different parts of body and biological degradability, so that they have been regarded as the potential risk for human health (2, 3). According to the statements of Environment Protection Agency (EPA), lead, cadmium, copper, zinc and arsenic are the most common heavy metals which result in the pollutions. Despite the necessity of copper, zinc and selenium for human body, high intake of them may threaten the human health from the viewpoint of toxicology. Cadmium and lead are of the most harmful effects on kidney and nervous system, respectively (4). Fish is put in the upper levels of food chain and able to accumulate high amounts of rare elements and metals; as a consequence, it is highly affected by the environmental pollutions (3). Due to high protein value, macro- and micro-elements, unsaturated fatty acids and fat-soluble vitamins, unsaturated fats and omega-3 (5, 6), the major part of human diet can be attributed to fish, and the global fish consumption has been estimated as almost 10 to 15% of total human diet (3). Therefore, despite the benefits of fish presence in the diet, the potential risk of fish exposure to chemical pollutants has to be regarded in the assessment of health quality (5). Determining the metal concentration levels in the edible part of fish which directly affects human health is of considerable importance; it is more likely to be investigated through comparing the metal concentration levels, standard limits, and maximum allowed concentrations (4). In this respect, plenty of studies have been conducted including the ones investigating heavy metals in such species as Cyprinus carpio, Barbus capito, and Chondrostoma regium in Seyhan River, Turkey (7), Yelkoma in the Northeastern Mediterranean (8), and fish in Eretva River, Bosnia Herzegovina (9). Heavy metal concentration and risk assessment in different fish tissues have been determined in Mersing Sea, the east coast of Peninsular, Malaysia (10), and the investigation of potential health risk using some heavy metals of fish consumption have been measured in Luis L. Leon storage, Northern Mexico (11). Also, heavy metal concentrations and their relationship with the health risk of the wild fish caught from Southern China sea (6), and the investigation of biological metal accumulation in the edible tissues of mullet (Mugil liza) in the tropical bay, southeastern Brazil have been determined (3). The assessment of human health and determination of metal concentrations in fish have been studied in the central part of Daya bay, southern China Sea (12). Meanwhile, lots of studies have been conducted concerning the assessment of mercury risk resulting from the consumption of white fish (Rutilus frisii kutum) in Caspian Sea, Mazandaran province (13), and the assessment of mercury risk of Otolithes ruber consumption in Mahshahr harbor, Persian Gulf (14). The risk assessment of some metals in carp fish in Zarivar wetlands has also been performed (15). risk assessment of cadmium and lead in golden mullet (Liza auratus and Liza saliens) for human health in Gorgan gulf (16), as well as the concentration of zinc, nickel, and vanadium in alosa fish (Alosa caspia), sander fish
Health Risk Assessment of fish consumption  
S. Mortazavi et al.

(Sander lucioperca), and the non-carcinogenic risk assessment of its consumption in the southeast of Caspian Sea have all been measured (18). Furthermore, the estimation of daily intake and potential danger of chrome, lead and cadmium in the consumers of carp (Cyprinus carpio) and sander (Sander lucioperca) were done in Gorgan bay (17). In this regard, a large number of studies have recently been conducted concerning the accumulation of heavy metals in different fish species in Dez River; however, not sufficient information may be available on the assessment of probable risk of fish consumption. Considering the significance of this river for the residents and the adjacent cities, heavy metal concentration in four fish species mussel in this region have been investigated in order to study the effects of environmental pollutants on human health through estimating the rate limits of fish consumption, daily and weekly metal intake, comparing the achieved limits and the standard Provisional Tolerable Daily Intake (PTDI), Provisional Tolerable Weekly Intake (PTWI), and other studies effective relationships.

2. Materials and Methods

2.1. Case study and research species
Khuzestan province in the southwest of Iran is one of the most important fish breeders and consumers in Iran. Dez River originated from Lorestan province covering almost 186 km area and passing Dezful reaches Karun River and finally Persian Gulf. The studied region is perched on the eastern latitude of 48°22′24″ and the northern longitude of 32°21′44″ near Dezful. After doing the field visits and studies, the required samples were taken from the area in 2014. The studied population included 80 fish from 4 local species (20 replicates for each species) caught by the local fishermen (Table 1).

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Family</th>
<th>Local Name</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capoeta trutta (Heckel, 1843)</td>
<td>Cyprinidae</td>
<td>Botak</td>
<td>Touyeni</td>
</tr>
<tr>
<td>Luciobarbus pectoralis (Heckel, 1843)</td>
<td>Cyprinidae</td>
<td>Barzom</td>
<td>Barzom mamouli</td>
</tr>
<tr>
<td>Chondrostoma regium (Heckel, 1843)</td>
<td>Cyprinidae</td>
<td>Sheas</td>
<td>Nazok-hefie nan</td>
</tr>
<tr>
<td>Carasobarbus luteus (Heckel, 1843)</td>
<td>Cyprinidae</td>
<td>Zanbor, Zangor</td>
<td>Hemri</td>
</tr>
</tbody>
</table>

After recording and encoding the station position by GPS, the samples were carried to the laboratory by an ice-containing freezer. In the laboratory, after the primary biometry of fish (total weight and length), the samples were kept in the fridge at -20°C to finish the freezing stage. Before autopsy, the samples were first rinsed by the distilled water to remove the slimy cover and the external substances absorbing the metals from the body surface. All the muscle samples were taken from the skin and the right part of fish body. The tissue samples were also put in an oven at 65°C to be dried and reach the fixed weight (19). To digest the samples, one gram of dried tissue sample was digested by the combination of 4:1 nitric acid and perchloric acid on a...
heating block at low and high temperatures of 40°C and 140°C for 1 and 3 hours, respectively; then, the samples were distilled twice using the distilled water, and filtered by Whatman filter paper No. 1 (20). To be assured of the accuracy of digestion operation and troubleshooting which may be resulted from preparing samples while stopping the effects of used materials on the metal concentrations, one blank was regarded in each turn of digestion operation. Metal concentrations of samples were then determined by HR-CS Atomic absorption spectrometry. In this stage, the desired metal concentration was measured for the blanks and then, subtracted by the reached values of samples; the recovery value was ranged as 90-95%. LOD value (limit of detection) of the device was given as 0.2414, 1.008, 1.062 and 1.192 mg gr-1, and LOQ value (limit of quality) was computed as 0.8987, 3.797, 3.922 and 4.578 mg gr-1 for copper, lead, zinc, and cadmium, respectively. For lead and cadmium in the graphite furnace method, LOD value of the device was given as 1.008 and 1.126 ng g-1, and LOQ value was calculated as 3.797 and 3.784 ng g-1, respectively.

To perform the statistical analyses, the data normality was investigated using SPSS Software, version 21 and Kolmogorov-Smirnov test. Also, one-sample T-test and Pearson correlation were used to compare and specify the relationships of metal concentration in muscles and available standards. The graphs related to the study results were also drawn by means of Microsoft Excel, version 2010.

2.2. Condition Factor (CF)
Condition factor as one of the total health factors was presented by Schreck and Moyle in 1990 (21) to survey the general conditions of used fish. It indicates the ability against the environmental stresses and the total contamination effects on fish. Its low costs and simplicity have led it to be widely applied as a tool or value for studying the general conditions of fish (22). This factor is computed by equation (1).

\[ CF = \frac{BW}{L^3} \times 100 \]  
(1)

2.3. Estimated Daily and Weekly Intake of Heavy Metals
Estimated daily and weekly intakes of heavy metals were calculated using their respective average concentration in samples by the weight of food items consumed by an individual, which can be considered as the equation (2) and (3), respectively (Shaheen et al., 2016):

\[ EDI = \frac{(FIRD \times C)}{BW} \]  
(2)

\[ EWI = \frac{(FIRW \times C)}{BW} \]  
(3)

Where Estimated Daily Intake (EDI) is estimated daily intakes of heavy metals in body, Estimated Weekly Intake (EWI) is estimated weekly intakes of heavy metals in body; FIRD is the food ingestion rate (g/person/day) (For fish about 20 g/day (24)); FIRW is the food ingestion rate (g/person/week) (For fish about 140 g/week (24)); C is the metal concentration in food samples (mg/kg); and BW is the body weight (body weight 70 kg for an adult in Iran (13)).
2.4. Health Risk Assessment from Fish Consumption

Health hazard from mussels fish Potential non-carcinogenic effects were evaluated by calculating a goal risk quotient (THQ). For one compound, the target hazard quotient (THQ) is the ratio of the Chronic Daily Intake (CDI) to a reference dose (RFD) (5):

\[ \text{THQ} = \frac{\text{CDI}}{\text{RfD}} \]  

(4)

where:
- CDI is chronical daily intake (mg kg\(^{-1}\) day\(_{-1}\))
- Rfd is reference dose.

The intake or dose for ingesting fish muscles is intended based on the equation (25):

\[ \text{CDI} = C \times \text{IR} \times \text{EF} \times \text{ED} \times \text{BW} \times \text{AT} \times 10^{-3} \]  

(5)

Where:
- C = concentrations (mg/kg) of the investigated chemical pollutants in muscle tissues;
- Rf = reduction factors (unitless);
- IR = ingestion rate (g day\(_{-1}\)) (20 g/Day) (24);
- Cf = conversion factor (10\(_{-3}\) kg/g);
- ABS = ingestion absorption factor (fraction absorbed);
- EF = exposure frequency (days year\(_{-1}\)) (365 Day/year);
- ED = exposure duration (years) (Year);
- BW = body weight (kg) (70 Kg);
- AT = average time of exposure (days) (70×365 Day).

The reduction factor is a number between 0 and 1 that describes the part of the contaminants firstly that remains after the mussels was cooked. As this reduction value was not known for the two investigated mussel species, a factor of 1 was used as a conservative assumption. The absorption factor (ABS) is the fraction of pollutants absorbed during ingestion and is specific. Commonly, it is assumed to be 100%, which is conservative, and thus 100% ABS was used in the present study. All the other parameters used in the equation were default values got from the USEPA documents (25, 26).

So the equation CDI was changed to equation (6):

\[ \text{CDI} = \frac{C \times \text{IR} \times \text{EF} \times \text{ED}}{\text{BW} \times \text{AT}} \times 10^{-3} \]  

(6)

2.5. Consumption Rate Limit of Fish (CR\(_{lim} \))

To calculate the maximum allowable fish consumption rate for a non-carcinogen (27) the following formula is used:

\[ \text{CR}_{lim} = \left(\frac{\text{RfD}}{\text{BW}}\right) \times \frac{\text{BW} \times \text{AT}}{C_m} \]  

(7)

where:
- \(\text{CR}_{lim}\) = maximum allowable fish consumption rate (kg/day)
- BW = Mean body weight of the general population or sub-population of concern (kg).
- Cm = measured concentration of chemical contaminant in a given species of fish (mg/kg).

Assumptions used in calculating consumption rates are:
- An average adult weighs 70 kg and a child (1 - 6 years of age) weighs 16 kg.

Finally, THQ was used based on the equation (2) (5) and the value of Rfd was accounted as 4×10\(^{-2}\), 4×10\(^{-3}\), 3×10\(^{-1}\) and 1×10\(^{-3}\) mg kg\(^{-1}\) for copper, lead, zinc, and cadmium according to American Environmental Protection Agency, respectively (6, 28).

\[ \text{THQ} = \frac{C \times \text{IR} \times \text{EF} \times \text{ED}}{\text{BW} \times \text{AT} \times \text{RfD}} \times 10^{-3} \]  

(8)

Since being exposed to two or several pollutants may lead to higher mutual effects, the total THQ (TTHQ\(_{1}\)) which is

\(^{1}\) Total Target Hazard Quotient

Iran J Health Sci 2017; 5(4): 14
about the studied combinations is applied as the equation (3) (5):
\[ TTHQ = THQ (Cu) + THQ (Pb) + THQ (Zn) + THQ (Cd) \] (9)

3. Results
The average length, weight, and values of CF are presented in Table (2). This factor which is reached by the link between the length and weight of each species can show the environmental conditions, sensitivity, and affectability of species. As it was observed, the values of CF for the studied species had a decreasing trend.

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Mean length (cm)</th>
<th>Mean weight (g)</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capoeta trutta</td>
<td>20.8±0.8</td>
<td>85.107±0.05</td>
<td>0.945</td>
</tr>
<tr>
<td>Luciobarbus pectoralis</td>
<td>22.5±0.9</td>
<td>100.109±0.09</td>
<td>0.878</td>
</tr>
<tr>
<td>Chondrostoma regium</td>
<td>20.9±0.7</td>
<td>79.35±0.04</td>
<td>0.869</td>
</tr>
<tr>
<td>Carasobarbus luteus</td>
<td>16.1±0.5</td>
<td>62/52±0.01</td>
<td>1.498</td>
</tr>
</tbody>
</table>

The results regarding the relationships between the metal concentrations in various species and CF are shown in Table 3.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capoeta trutta</td>
<td>0.044</td>
<td>0.504</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>*0.646</td>
<td>0.240</td>
<td>**0.959</td>
<td>**0.892</td>
</tr>
<tr>
<td>Luciobarbus pectoralis</td>
<td>0.529</td>
<td>0.839</td>
<td>0.000</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>0.227</td>
<td>-0.075</td>
<td>**0.957</td>
<td>*0.744</td>
</tr>
<tr>
<td>Chondrostoma regium</td>
<td>0.246</td>
<td>0.767</td>
<td>0.000</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>0.391</td>
<td>-0.108</td>
<td>**0.965</td>
<td>**0.828</td>
</tr>
<tr>
<td>Carasobarbus luteus</td>
<td>0.000</td>
<td>0.421</td>
<td>0.000</td>
<td>0.476</td>
</tr>
<tr>
<td></td>
<td>**0.951</td>
<td>0.287</td>
<td>**0.973</td>
<td>0.256</td>
</tr>
</tbody>
</table>

*99% and 95% significance levels

Figure 1 shows the daily metal intake of zinc and figure 2 shows the metal intake of copper, lead and cadmium. Therefore, the maximum intakes of zinc, copper, lead, and cadmium were recorded for Chondrostoma regium, Carasobarbus luteus, Luciobarbus pectoralis and Capoeta trutta, respectively.
Based on the drawn graphs, the weekly intake of zinc and those of copper, lead, and cadmium are presented in Figures 3 and 4, respectively. The maximum weekly intake was of a similar trend to the daily one.

The estimated values of potential risk of copper, lead, and cadmium in four species of Dez River are also shown in Figures 5 and 6, respectively. TTHQ values are distinguished for each species.

Figure 1. Daily intake of zinc for the used fish species

Figure 2. Daily intake of Cu, Pb and Cd for the used fish species

Figure 3. Weekly intake of zinc for the used fish species

Figure 4. Weekly intake of Cu, Pb and Cd of used fishes

Figure 5. Potential non-carcinogenic risk of Zn

Figure 6. Potential non-carcinogenic risk of Cu, Pb and Cd
According to Table 4, the values of TTHQ for each species and THQ share percent was computed.

**Table 4. TTHQ values and THQ share percent for each species**

<table>
<thead>
<tr>
<th>Species</th>
<th>%THQ Cu</th>
<th>%THQ Pb</th>
<th>%THQ Zn</th>
<th>%THQ Cd</th>
<th>TTHQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capoeta trutta</td>
<td>1.16</td>
<td>0.17</td>
<td>98.27</td>
<td>0.40</td>
<td>0.41</td>
</tr>
<tr>
<td>Luciobarbus pectoralis</td>
<td>1.78</td>
<td>0.11</td>
<td>97.81</td>
<td>0.30</td>
<td>0.38</td>
</tr>
<tr>
<td>Chondrostoma regium</td>
<td>1.03</td>
<td>0.16</td>
<td>98.72</td>
<td>0.09</td>
<td>0.48</td>
</tr>
<tr>
<td>Carasobarbus luteus</td>
<td>1.29</td>
<td>0.26</td>
<td>98.43</td>
<td>0.03</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Based on Table 5, the consumption rate limit of fish about copper, lead, zinc, and cadmium was estimated for four studied species for adults and children.

**Table 5. Consumption rate limit of fish (kg per day) for adults and children**

<table>
<thead>
<tr>
<th>CRlim</th>
<th>Cu children</th>
<th>Pb children</th>
<th>Zn children</th>
<th>Cd children</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capoeta trutta</td>
<td>4.21</td>
<td>2.92</td>
<td>0.37</td>
<td>0.30</td>
<td>2.78</td>
</tr>
<tr>
<td>Luciobarbus pectoralis</td>
<td>2.98</td>
<td>4.83</td>
<td>0.41</td>
<td>0.44</td>
<td>4.05</td>
</tr>
<tr>
<td>Chondrostoma regium</td>
<td>3.99</td>
<td>2.55</td>
<td>0.31</td>
<td>1.19</td>
<td>10.85</td>
</tr>
<tr>
<td>Carasobarbus luteus</td>
<td>3.60</td>
<td>1.81</td>
<td>0.35</td>
<td>4.12</td>
<td>37.65</td>
</tr>
</tbody>
</table>

In order to compare the average metal concentrations in the muscle tissues, a variety of standards have been presented in Table (6).

**Table 6. Comparison of metal concentrations and international standards in fish (Mg g⁻¹ of dry weight)**

<table>
<thead>
<tr>
<th>Standard</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
<th>Cd</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAO</td>
<td>20</td>
<td>2</td>
<td>50</td>
<td>0.3</td>
<td>29</td>
</tr>
<tr>
<td>WHO²</td>
<td>10</td>
<td>0.3</td>
<td>1000</td>
<td>0.2</td>
<td>30</td>
</tr>
<tr>
<td>UK(MAFF)</td>
<td>20</td>
<td>2</td>
<td>50</td>
<td>0.2</td>
<td>31, 32</td>
</tr>
<tr>
<td>NHMRC</td>
<td>10</td>
<td>1.5</td>
<td>150</td>
<td>0.05</td>
<td>31</td>
</tr>
<tr>
<td>New Zealand</td>
<td>30</td>
<td>-</td>
<td>40</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>Australian</td>
<td>10-70</td>
<td>-</td>
<td>40-1000</td>
<td>0.2-5.5</td>
<td>34</td>
</tr>
<tr>
<td>Turkish guideline</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>0.5</td>
<td>35</td>
</tr>
<tr>
<td>EC</td>
<td>-</td>
<td>0.2</td>
<td>-</td>
<td>0.05</td>
<td>36</td>
</tr>
<tr>
<td>FDA</td>
<td>5</td>
<td>35</td>
<td>2</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>FAO/WHO limits</td>
<td>30</td>
<td>0.5</td>
<td>40</td>
<td>0.5</td>
<td>37</td>
</tr>
<tr>
<td>ISIRI³</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
<td>0.3</td>
<td>38</td>
</tr>
<tr>
<td>Germany present study</td>
<td>0.091±0.771</td>
<td>0.046±0.105</td>
<td>16.1±58.53</td>
<td>0.065±0.116</td>
<td></td>
</tr>
</tbody>
</table>

² World Health Organization
³ Institute of Standards and Industrial Research of Iran
In Table 7, the comparisons of research results and other values reported by previous studies have been presented.

Table 7. Comparisons of research results and other values on fish muscle in Dez River

<table>
<thead>
<tr>
<th>Species</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
<th>Cd</th>
<th>Site</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbus grypus</td>
<td></td>
<td>-</td>
<td>1.2944</td>
<td>-</td>
<td>Dez River</td>
<td>40</td>
</tr>
<tr>
<td>Liza abu</td>
<td>0.7</td>
<td>-</td>
<td>0.33</td>
<td>-</td>
<td>Dez River</td>
<td>41</td>
</tr>
<tr>
<td>Liza abu</td>
<td>0.902</td>
<td>-</td>
<td>-</td>
<td>0.348</td>
<td>Dez River</td>
<td>42</td>
</tr>
<tr>
<td>Barbus grypus</td>
<td>-</td>
<td>0.236</td>
<td>-</td>
<td>0.111</td>
<td>Dez River</td>
<td>43</td>
</tr>
<tr>
<td>Barbus grypus</td>
<td>-</td>
<td>0.227</td>
<td>-</td>
<td>0.146</td>
<td>Dez River</td>
<td>44</td>
</tr>
<tr>
<td>Capoeta trutta</td>
<td>1.42</td>
<td>3</td>
<td>1.22</td>
<td>1.42</td>
<td>Dez River</td>
<td>45</td>
</tr>
<tr>
<td>Barbus xanthopterus</td>
<td>-</td>
<td>0.955</td>
<td>-</td>
<td>0.799</td>
<td>Dez River</td>
<td>46</td>
</tr>
<tr>
<td>Capoeta trutta</td>
<td>0.665</td>
<td>0.096</td>
<td>56.17</td>
<td>0.23</td>
<td>Dez River</td>
<td>present study</td>
</tr>
<tr>
<td>Luciobarbus pectoralis</td>
<td>0.94</td>
<td>0.058</td>
<td>51.61</td>
<td>0.158</td>
<td>Dez River</td>
<td>present study</td>
</tr>
<tr>
<td>Chondrostoma regium</td>
<td>0.701</td>
<td>0.11</td>
<td>66.97</td>
<td>0.059</td>
<td>Dez River</td>
<td>present study</td>
</tr>
<tr>
<td>Carasobarbus luteus</td>
<td>0.778</td>
<td>0.155</td>
<td>59.37</td>
<td>0.017</td>
<td>Dez River</td>
<td>present study</td>
</tr>
</tbody>
</table>

4. Discussion

CF values reached for various fish species showed that these values (2.9 - 4.8) were not ranged as the introduced standard ones for fish in high seas and were not of suitable conditions for the health and environmental stress tolerance (48). Thus, the conditions of desired region in Dez River was found to be not suitable for fish as compared to the bed of high seas, which can be as a result of the effects of environmental factors. Investigating the relationship between the metal concentration and CF has indicated a positive correlation between the metal accumulation and the achieved values of CF in almost all of the studied species; the correlation was highly observed about zinc and cadmium. So, it was claimed that the metal accumulation in various species was related to the effects of environmental conditions, body condition, age, element features, ability to transfer, and nutrition of species. In addition, the estimated daily and weekly intakes (EDI & EWI) of metals were compared to the provisional tolerable daily and weekly intakes (PTDI & PTWI). PTWI is the maximum pollutant value to which a person can be exposed during a week without any risk (49, 15). Also, the daily consumption dose of one specific metal contributes to estimate the maximum nutrient which can be used safely with no harmful effects. In this respect, PTDI was presented as the provisional tolerable daily intake (3). Table 6 presents PTDI for 1 kg of body weight determined by JFCFA, FAO and WHO. Comparing the results and the related rate limits in this study showed that the metal intake for four desired species was considerably lower than the standard one; thus, consuming fish caught from Dez River will not be a concern for human health. This finding is in accordance with those related to some species in Catania.
Gulf in the east of Mediterranean Sea reported by Copat et al. (2), a study conducted in Slovenia by Al Sayegh Petkovsek et al. on 10 fish species in a gulf (50), an investigation about Psetta maxima in Black sea done by Bat et al. (51), and a study on Cyprinus carpio and Sander lucioperca by Banagar et al. (17), as well as a research conducted by Banagar et al. on Liza auratus and Liza saliens in Gorgan bay (16).

Table 8. Provisional tolerable daily and weekly intake of heavy metals in the used fish (mg per day/week for a 70 kg man)

<table>
<thead>
<tr>
<th></th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
<th>Cd</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTDI</td>
<td>35000</td>
<td>250</td>
<td>70000</td>
<td>70</td>
<td>(52, 11)</td>
</tr>
<tr>
<td>PTWI</td>
<td>245000</td>
<td>1750</td>
<td>490000</td>
<td>490</td>
<td>(52, 53, 1)</td>
</tr>
</tbody>
</table>

The assessment of potential health risk of heavy metals concentration in mussel fish is shown in the adult populations in Table 8. It is observed that the health risk of nutrients which is the ratio of determined pollutant dose to the reference one is more likely to be obtained in the sea contaminated foods based on THQ (23). The results reported the most and least THQ values for zinc and copper, respectively. If THQ is bigger than one, the people is probably exposed to the harmful effects (54). In this paper, none of the metals exceeded one individually; therefore, it can be declared that these exposures should not lead to damages to a human being (55). The decreasing trend of THQ values for different metals is as follows:

THQ: zinc > cadmium > lead > copper

On the other hand, the cumulative health risk of fish consumption is given by health hazards of four desired metals (55). So, TTHQ values are the consequences of combined non-carcinogenic effects of several elements (23). To interpret this index, it should be noted that the values lower than one show lack of observable risk in consuming fish, and those bigger than one show the probability of non-carcinogenic effects (12). Harmful effects resulting from consuming fish with TTHQ which is higher than one depend on the consumption rate, pollutant concentration and type, physical conditions, and the age of consumers (56). In current research, the TTHQ values lower than one showed that the non-existence of cumulative health risk and the highest and lowest values was attributed to the Capoeta trutta, and Carasobarbus luteus species, respectively. As it was shown in Table 4, zinc and cadmium was the main parts of TTHQ, and copper and lead had a small share as compared to the above-mentioned metals. The TTHQ values more than one revealed the potential harmful effects of these metals on human health have to be studied at higher levels (55). The results of potential health risk assessment reached in this study corresponded to those reported by Sadeghi Bajgiran et al. (18) on nickle and vanadium in the Sander lucioperca and Alosa caspia species TTHQ for heavy metals in Rutilus frisii kutum in the southern beaches of Caspian Sea and Clupeonella cultriventris caspia about chrome, nickle, zinc and copper. The findings of the current research was also in line with the findings of a study conducted by Banagar et al. (16) on Liza auratus and Liza saliens on cadmium and
lead in Gorgan bay, in addition to an investigation on *Rutilus rutilus* and about heavy metals in Miankale wetlands done by Alipour et al. (57). The results were also in agreement with some other studies, such as the study of Banagar et al. (17) on *Cyprinus carpio* and *Sander lucioperca* in Gorgan bay, a research on *Oreochromis niloticus* concerning heavy metals performed by Taweel et al. (58). However, the findings of the present study was found to be not in line with the results of the research of Idriss and Ahmad (59) on 13 fish species in a river in Malaysia with respect to zinc and copper, since they estimated the limits of cadmium and lead to be more than one, which is contrary to the results of this study. Since the daily consumption rate limit (CRlim) is actually the maximum one with no harmful non-carcinogenic effects (60, 18), thus, it can be stated that as the concentration of heavy metals in the studied species became lower, the consumption rate limit turned to be higher. In this research, the most consumption rate limit for the children and adults were observed in *Capoeta trutta*, *Luciobarbus pectoralis*, and *Carasobarbus luteus* species in related to copper, lead, zinc, and cadmium, respectively; therefore, it can be concluded that the *Capoeta trutta* species is of better conditions as compared to the other species, whereas the *Chondrostoma regium* species is put in the lowest place. The comparison of results with standards table (6) with one-sample T-test have shown that there existed a significance difference between the metal concentrations and the defined standards (p<0.05); the means comparison also indicated that the concentrations of copper and lead were lower than the standard ones, but the concentrations of zinc and cadmium were higher than the limits established by the Ministry of Agriculture Fisheries and Food, Food and Agriculture Organization, as well as Australian National Health and Medical Research Council, and European Commission. The results of the current research were also found to be in accordance with the findings of Askary Sary et al. in Karun River, Dez River and Bahmanshir concerning camium (42), Velayatzade and Tabibzade, as they documented that the high concentration in Karun River was related to lead and cadmium (61), while Kheirou and Dadalhi Sohrab claimed it was related to cadmium and copper (62). At the same time, Bandani et al. (63) and Zalaghi et al. found that the high concentration of heavy metals in Karun River was concerning lead (64). In table (7) the average concentration achieved in the most cases except zinc was also found to be lower than those reported by the other studies. Variations of absorption and accumulation trends were then more likely caused by such elements as element type, fish species, sex, weight, age, food habits, physiological properties of fish, ecologic features, environmental conditions, biological enlargement of element, and physical and chemical properties of environment including hardness, temperature and nutrients (62). In other words, the access to metals can be influenced by biological and non-biological factors which control one specific metal and its biological accumulation (46). Based on the findings of the current study, periodic monitoring and risk assessment programs were observed to be necessary to survey the extent of human effects on the ecologic balance and human health. In this research, the concentration of copper, lead, zinc, and cadmium and human health risk resulting from the consumption of fish were
assessed in Dez River. The findings of this study could then be generalized and applied in a global range, local data were considered in order to simplify the decision making, and improve the heavy metals management strategies. Although the results indicated low concentrations of mentioned metals in the desired species, the periodic monitoring of metal pollution was observed to be essential to avoid the dangers caused by human activities concerning the aquatic ecosystems and food safety. Thus, the identification of pollutant concentrations in nutrients and the estimation of consumption rate limit are of high importance from the viewpoint of the benefits and hazards assessment. On the other hand, in spite of the relatively low fish consumption rate in Iran as compared to the universal average limit, the rate has had lots of variations in the country; in the northern and southern provinces, the consumption rate is even higher than the world per capita, but in some other cities, the rate is just once a year. Thus, it can be suggested that the health risk of other nutrients, such as vegetables, fruits, rice, and grains be also estimated in the region.

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Conflict of Interests
The Authors have no conflict of interest.

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Health Risk Assessment of fish consumption

S. Mortazavi et al.


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