Barbusgrypus as A Bioindicator of Heavy Metal Pollution in Downstream Karoon and Dez Rivers in Khouzestan, Iran

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ABSTRACT
Levels of contaminants in fish are of particular interest because of the potential risk to humans who consume them and in this research attention has focused on self-caught fish from downstream Karoon and Dez Rivers. The aim of this research was to evaluate the potential of Barbusgrypus which is abenthopelagic species as a bioindicator of heavy metal pollution in downstream Karoon and Dez Rivers. This study was carried out to investigate contamination of heavy metals (Cd, Pb, Ni and Hg) in gill, liver and muscle of Barbusgrypusin downstream of Karoon (Shekariyeh 3 village) and Dez (Ali abad village) Rivers of Khouzestan, Iran. Heavy metal levels in fish samples were analyzed by Perkin Elmer AS-800 autosampler and Perkin Elmer Analyst 700 model AAS equipped with MHS 15 CVAAS system was used for mercury determination. The results were shown the mean concentrations of heavy metals (Cd, Pb, Ni and Hg) in gill, liver and muscle of Barbusgrypusin Dez River were higher than in Barbusgrypusin Karoon River (P < 0.05), except for concentration of Pb that in Barbusgrypusin Karoon River was higher than in Barbusgrypusin Dez River (P < 0.05). In Barbusgrypusin Karoon and Dez Rivers, concentrations of heavy metals (Cd, Pb, Ni and Hg) were in the sequence gill > liver > muscle, except for concentration of Hg that in Barbusgrypusin Dez River was in the sequence liver > gill > muscle. Heavy metals were contamination in gill, liver and muscle of Barbusgrypus. Significant variations in metal values were evaluated using student’s t test at p < 0.05. In Barbusgrypus high levels of cadmium, lead, nickel and mercury were measured in gill (1.49, 2.25, 1.02 and 0.89 mgkg\(^{-1}\)dw) except for mercury (1.06mgkg\(^{-1}\)dw) in Barbusgrypus in Dez River was high in liver. Among heavy metals (Cd, Pb, Ni and Hg), Pb was the highest accumulating metal in Barbusgrypus in Karoon and Dez Rivers (P < 0.05). The results indicated that the muscles of Barbusgrypus were highly contaminated by heavy metals and exceeded WHO and FAO legal limits.

Key words: Heavy metal; Barbusgrypus; bioindicator; standard.

Introduction
The contamination of fresh waters with a wide range of pollutants has become a matter of great concern over the last few decades [8]. The river systems may be excessively contaminated with heavy metals released from domestic, industrial, mining and agricultural effluents [30,29]. Contamination of rivers with heavy metals may have devastating effects on the ecological balance of the aquatic environment, and the diversity of aquatic organisms becomes limited with the extent of contamination [41]. In fresh water systems, fish is one of aquatic products humans consume, and also provide a good indicator of trace element pollution [38]. Fish are the major part of human diet and it is not surprising that numerous studies have been carried out on metal accumulation in different species [31]. The commercial and edible species have been investigated in order to check for those hazardous to human health. Metals can be taken up by fish from water, food, sediment and suspended particulate material [1]. Biomonitoring of trace metal pollution has been gaining attention, since various organisms have the ability to accumulate these substances and transfer them in a large concentration to animals and human beings when consumed [23]. Heavy metals are major components of the industrial waters, which along with other products from industrial operations are discharged into the aquatic environment. These substances are toxic to aquatic life [18, 22]. Heavy metals like cadmium, lead, mercury and nickel are among the most metallic pollutants. Nickel is essential metal since its play an important role in biological systems, whereas cadmium, lead and mercury are non-essential metals, as they are toxic, even in trace amounts [17]. For the normal metabolism of the fish, the essential metals must be taken up from water, food or sediment [7]. These
essential metals can also produce toxic effects when the metal intake is excessively elevated [43]. Bioaccumulation of these metals is known to adversely affect liver, muscle, kidney, gill and other tissues of fish, disturb metabolism and hamper development and growth of fish[12]. Knowledge of heavy metal concentrations in fish is important both with respect to nature management and human consumption of fish.Karoon-Dez River basin, (10 48˚- 30 52 E, 20 30˚- 05 34’N), is located in southern part of Iran. Karoon River is one of the largest river in Iran and has an area of 60500 Km² and an averageannual discharge of 18700 million m³, also Dez River is one of the watery river in Iran and has an area of 21100 Km² and an average annual discharge of 7396 million m³. They play an important role in water and fish supply. Barbusgrypus has high market value and is one of the main fish product in Karoon and Dez Rivers. Being the complexity of heavy metal bioaccumulation of fishes, it was important to study the heavy metal accumulation in different commercial fishes such as Barbusxanthopterus in Karoon and Dez Rivers for the food safety. The main objective of this study was to determine the contents of heavy metals (Cd, Pb, Ni and Hg) in the gill, liver and muscle of Barbusxanthopterus which is abenthopelagic species as abioindicator of heavy metal pollution in downstream Karoon and Dez Rivers; This could help us understand the enrichment behavior of heavy metals in downstream Karoon and Dez Rivers and emphasize the need to discard the most polluted tissues of the fish.

Material and Methods

The concentration of heavy metals cadmium, lead, nickel and mercury were measured in the muscle, gills and liver of Barbusgrypus and Barbusxanthopterus caught by gillnet from downstream of Karoon (Shekariyeh3 village) and Dez (Ali abad village) Rivers of Khouzestan in summer 2009. The number of samples were 48 B.grypus and 48 B.xanthopterus in each river. After capture, fish were placed in plastic bags and transported to the laboratory in freezer bags with ice. The mean length and weight were measured which ranged from minimum and maximum value as 287.5 and 425 mm for length and 223.8 and 585 g for weight of B.grypus and 248.5 and 377.5 mm for length and 173 and 561.3 g for weight of B.xanthopterus in Karoon and Dez Rivers. Total body length (mm) and weight (g) were recorded for fishes. After biometry,fish were immediately frozen at -20°C. All reagents were of analytical reagent grade unless otherwise stated. Double deionized water (Milli-Q Millipore 18.2 MΩ cm -1 resistivity) was used for all dilutions. HNO 3, H 2 O 2 and HCl were of suprapur quality (E. Merck, Darmstadt, Germany). All the plastic and glassware were cleaned by soaking in dilute HNO 3 (1/9, v/v) and were rinsed with distilled water prior to use. The element standard solutions used for calibration were produced by diluting a stock solution of 1000 mg/l of the given element supplied by Sigma Chem. Co. St. Louis, USA. A Perkin Elmer AAnalyzer 700 (Norwalk, CT, USA) atomic absorption spectrometer equipped with HGA graphite furnace and with deuterium background corrector was used. Lead, cadmium and nickel were determined in graphite furnace. For graphite furnace measurements, argon was used as inert gas. Pyrolytic coated graphite tubes (Perkin Elmer Part No. B3 001264) with a platform were used. Samples were injected into the graphite furnace using Perkin Elmer AS-800 autosampler. The atomic absorption signal was measured as a peak height mode against an analytical curve. Perkin Elmer Analyst 700 model AAS equipped with MHS 15 CVAAS system was used for mercury determination. A hallow cathode lamp operating at 6 mA was used and a spectral bandwidth of 0.7 nm was selected to isolate the 253.7 nm mercury line. NaBH 4 (1.5%) (w/v) in NaOH (0.5%) (w/v) was used as reducing agent. The analytical measurement was based on peak height. Reading time and argon flow rate was selected as 10 s and 50 ml min -1. Milestone Ethos D microwave (Sorisole-Bg, Italy) closed system (maximum pressure 1450 psi, maximum temperature 300 °C) was used. One gram of sample was digested with 6 ml of concentrated HNO 3 (65%) (Suprapure, Merck, Darmstadt, Germany) and 2 ml of concentrated H 2 O 2 (30%) (Suprapure, Merck, Darmstadt, Germany) in microwave digestion system and diluted to 10 ml with double deionized water (Milli-Q Millipore 18.2 M cm -1 resistivity). A blank digest was carried out in the same way (digestion conditions for microwave system were applied as 2 min for 250 W, 2 min for 0 W, 6 min for 250W, 5 min for 400 W, 8 min for 550 W, vent: 8 min, respectively) [44]. The whole data were subjected to a statistical analysis and correlation matrices were produced to examine the inter-relationships between the investigated trace metal concentrations of the samples. Data statistics were performed using SPSS 17 software. Paired samplesT-Test was used to compare differences between samples. A P-value less of 0.05 was considered statistically significant.

Results:

In this research concentrations of Cd, Pb, Ni and Hg were determined in gill, liver and muscle of B. grypus which is abenthopelagic species as abioindicator of heavy metal pollution in downstream Karoon and Dez Rivers. All metal concentrations were determined on a dry weight basis. In B. grypus in Karoon River heavy metal concentrations in gill were found 1.2728 mgkg -1 for Cd, 2.2550 mgkg -1 for Pb, 0.8091 mgkg -1 for Ni, 0.8991 mgkg -1 for Hg and in liver were found 1.0753 mgkg -1 for Cd, 2.1094 mgkg -1 for Pb, 0.6247 mgkg -1 for
1for Ni, 0.7919 mgkg⁻¹ for Hg and in muscle were found 0.8481 mgkg⁻¹ for Cd, 1.7578 mgkg⁻¹ for Pb, 0.4614 mgkg⁻¹ for Ni, 0.7350 mgkg⁻¹ for Hg. Also, in *B. grypus* in Dez River heavy metal concentrations in gill were found 1.4969 mgkg⁻¹ for Cd, 1.7016 mgkg⁻¹ for Pb, 1.0259 mgkg⁻¹ for Ni, 0.944 mgkg⁻¹ for Hg and in liver were found 1.3497 mgkg⁻¹ for Cd, 1.5525 mgkg⁻¹ for Pb, 0.9572 mgkg⁻¹ for Ni, 1.0616 mgkg⁻¹ for Hg and in muscle were found 1.0997 mgkg⁻¹ for Cd, 1.2944 mgkg⁻¹ for Pb, 0.8531 mgkg⁻¹ for Ni, 0.9050 mgkg⁻¹ for Hg and the concentrations of heavy metals differed significantly in each tissue between Karoon and Dez Rivers (P < 0.05) but non-significantly for Hg in the gill of *B. grypus* between two rivers (Table 1).

Heavy metal concentrations in gill, liver and muscle of *B. grypus* in Karoon and Dez Rivers decreased in the sequence as Pb > Cd > Hg > Ni, and only heavy metal concentrations in the gill of *B. grypus* in Dez River decreased in the sequence as Pb > Cd > Ni > Hg and the distribution patterns of Cd, Pb, Ni and Hg in tissues of *B. grypus* in Karoon and Dez Rivers follows the order: gill > liver > muscle except for Hg level in the *B. grypus* in Dez River that follow the order: liver > gill > muscle. The comparison of heavy metal levels (Cd, Pb, Ni, Hg) in various tissues of *B. grypus* between Karoon and Dez Rivers was showed that heavy metal levels in *B. grypus* in Dez River were higher than in *B. grypus* in Karoon River (P < 0.05), except for level of Pb that in *B. grypus* in Karoon River was higher than in *B. grypus* in Dez River (P < 0.05) (Fig. 1, 2, 3).

Discussion:

Pollution is considered the most important environmental problems [30, 51, 52, and 53]. In this study among various tissues, the gill of *B. grypus* in Karoon and Dez Rivers showed significantly higher (P < 0.05), except for the liver of *B. grypus* in Dez River that the level of Hg showed significantly higher (P < 0.05). Also, in *B. grypus* two rivers, metal levels in the muscle were lower than gill and liver. In this study the highest heavy metal concentrations were found in the gill and liver (P < 0.05), while the muscle tended to accumulate less metal. The metal concentration in muscle is important for the edible parts of the fish. Fish generally accumulate contaminants from aquatic environments, have been largely used in food safety studies. The mean concentrations of heavy metals (Cd, Pb, Ni, Hg) in the muscle of *Barbus grypus* Karoon and Dez Rivers were higher than the maximum permitted concentrations proposed by WHO [48, 49, 11, 16]. The concentrations of heavy metals have significant differences (P < 0.05) in muscle of *B. grypus* with WHO and FAO standards in Karoon and Dez Rivers (Table 2).

Water pollution leads to fish contaminated with toxic metals, from many sources, e.g. industrial and domestic waste water, natural runoff and contributory rivers [42, 5, 6]. In this research the concentrations of heavy metals differed significantly in each tissue between Karoon and Dez Rivers (P < 0.05) but non-significantly for Hg in the gill of *B. grypus* between two rivers (Table 1). In the literature, heavy metal concentrations in the tissues of fresh water fish vary considerably among different studies [10, 37].

In Sir Dam Lake in Turkey, mean Hg concentration in the muscle of *Cyprinus carpio* and *Silurus glanis* was reported as: 0.07 µgg⁻¹ and 0.26 µgg⁻¹ respectively [14], values recorded for Cd and Pb in the muscles of *Petrocephalus bane*, *Pomadasys jubelini*, *Elops alcatract*, *Pseudolothususelangatus*, *Mugil cephalus*, *Eutropiopsnioticus*, *Caranxhipus*, *ChryscALTHYNNATODIGITALIS* and *PseuTiussebae* in Calabar River of Nigeria, were 0.02, 0.02 µgg⁻¹; < 0.01, 0.02 µgg⁻¹; 0.01, 0.02 µgg⁻¹; 0.02, 0.02 µgg⁻¹; < 0.01, < 0.01 µgg⁻¹; 0.01, 0.03 µgg⁻¹; 0.03, 0.01 µgg⁻¹; < 0.01, 0.01 µgg⁻¹; 0.03, 0.01 µgg⁻¹ respectively [13], the mean of Ni and Pb concentrations in the muscles of *Barbus saxanthopteras* and *Barbus jrajanorum* in Ünlü Ataturk Dam Lake of Turkey, were reported as: 0.08, 0.68 µgg⁻¹; 0.04, 0.66 µgg⁻¹ respectively [2] and in Sunderban mangrove wetland of northeast India, mean Hg concentration in the muscle, liver and gill of *L. parsia* was reported as: 0.12, 0.65 and 0.54 µgg⁻¹ respectively [40], a comparison of these concentrations with our data clearly shows higher values in *B. grypus* in Karoon and Dez Rivers. Heavy metal levels in different tissues of fish depend on feeding habits [4, 39, 35, 47], age, size and length of the fish [32, 3] and their habitats [7]. Heavy metal concentrations varied significantly depending upon the type of fish tissues and locations.

In Dez River, heavy metal concentrations in gill, liver and muscle tissues of *B. grypus* were higher than those observed in Karoon River (P < 0.05), except for Pb that in *B. grypus* Karoon River was higher than in *B. grypus* in Dez River (P < 0.05) (Fig. 1, 2, 3).

The target organ for Hg accumulation in fish from heavily contaminated localities was the liver [19]. The liver has the ability to accumulate large quantities of pollutants from the external environment and also plays an important role in storage, redistribution, detoxification and transformation of pollutants [15]. Studies carried out with various fish species have shown that heavy metals accumulate mainly in metabolic organs such as liver that stores metals to detoxicate by producing metallothioneins [9, 21, 26].
Metal concentration in the gills could be due to the element complexing with the mucus, which is impossible to remove completely from between the lamellae, before tissue is prepared for analysis. The absorption of metals on to the gill surface, as the first target for pollutants in water, could also be an important influence in the total metal levels of the gill [20]. The relatively high concentrations of heavy metals in liver and gill were also found in different species of fish in Tigris River and Atatürk Dam Lake [45,25].

Table 1: The concentrations of heavy metals (mg kg⁻¹ dry weight) in various tissues of Barbusgrypus in Karoon and Dez Rivers, Khouzestan, Iran.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>River</th>
<th>Cd</th>
<th>Pb</th>
<th>Ni</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill</td>
<td>Karoon</td>
<td>1.27 ± 0.07</td>
<td>2.55 ± 0.17</td>
<td>0.80 ± 0.04</td>
<td>0.89 ± 0.07</td>
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<tr>
<td></td>
<td>Dez</td>
<td>1.49 ± 0.01</td>
<td>1.70 ± 0.02</td>
<td>1.02 ± 0.03</td>
<td>0.94 ± 0.30</td>
</tr>
<tr>
<td>Liver</td>
<td>Karoon</td>
<td>1.07 ± 0.09</td>
<td>2.10 ± 0.13</td>
<td>0.62 ± 0.08</td>
<td>0.79 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Dez</td>
<td>1.34 ± 0.01</td>
<td>1.55 ± 0.01</td>
<td>0.95 ± 0.06</td>
<td>1.06 ± 0.06</td>
</tr>
<tr>
<td>Muscle</td>
<td>Karoon</td>
<td>0.84 ± 0.08</td>
<td>1.75 ± 0.10</td>
<td>0.46 ± 0.05</td>
<td>0.73 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Dez</td>
<td>1.09 ± 0.01</td>
<td>1.29 ± 0.06</td>
<td>0.85 ± 0.05</td>
<td>0.90 ± 0.03</td>
</tr>
</tbody>
</table>

Data are presented as means ± S.E.: a, b: P<0.05, significantly different in each tissue between two rivers.

Table 2: Comparison of heavy metals (mg kg⁻¹ dry weight) in muscle of Barbusgrypus with standards in Karoon and Dez Rivers, Khouzestan, Iran.

<table>
<thead>
<tr>
<th>River</th>
<th>Cd</th>
<th>Pb</th>
<th>Ni</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karoon</td>
<td>0.84 ± 0.08</td>
<td>1.75 ± 0.10</td>
<td>0.46 ± 0.05</td>
<td>0.73 ± 0.06</td>
</tr>
<tr>
<td>Dez</td>
<td>1.09 ± 0.01</td>
<td>1.29 ± 0.06</td>
<td>0.85 ± 0.05</td>
<td>0.90 ± 0.03</td>
</tr>
<tr>
<td>WHO</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>FAO</td>
<td>0.5</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a, b, c, d: P<0.05, significantly different in muscle of Barbusgrypus and Barbusxanthopterus with WHO(World Health Organization) standard.

References: [48,49,11,16].

Fig. 1: Concentration of heavy metals in gill.

Fig. 2: Concentration of heavy metals in liver.
Fig. 3: Concentration of heavy metals in muscle.

The levels of heavy metals (Cd, Pb, Ni, Hg) in the muscle determined by the present study were lower than those determined in gill and liver. Similar results were reported from a number of fish species which shows that muscle is not an active tissue in accumulating heavy metals [9,28,26,46,24]. [8] determined the highest levels of Cd, Pb and Ni in gill and liver than muscle of *Cyprinus carpio*, *Barbus capito* and *Chondrostoma reigium* in the Seyhan River, Turkey and [2] reported that in *Barbus xanthopterus* and *Barbus rajani* in Atatürk Dam Lake, Turkey, heavy metal concentrations in gill and liver were maximum, while these concentrations were least in muscle. Thus, these results were alike our results in this research. [36] has reported the concentrations of Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in gill, liver, muscle and kidney of *B. grypus* in Atatürk Dam Lake, Turkey and [33] determined the concentrations of Pb, Cd, Zn, Ni, Cu, Cr and Hg in gill, liver and muscle of *Labeorohita* and *Ctenopharyngodon idella* in lake of Bhopal, India; They reported that the liver and gill showed higher metal concentrations than muscle. So, the results of our study were similar to the above studies. The results from this research indicated that metal accumulation depended on the tissues probably as a consequence of metabolic needs, physiochemical properties, and detoxification processes specific for each element.

The results of this study showed that Pb was the highest accumulating metal compared with other metals (P < 0.05), that could be traced to urban and industrial located near the Karoon and Dez Rivers and their wastes that discharged in to these rivers. The Pb finds its way in rivers through the discharge of industrial waste waters, such as from painting, dyeing, battery manufacturing units and oil refineries etc. Pb also enters the rivers both from terrestrial sources and atmosphere and the atmospheric input of Pb aerosols can be substantial [34]. The high levels of Pb in the Karoon and Dez Rivers have toxic effects on fish metabolism.

In this research we demonstrate that *B. grypus* is also a good bioindicator of environmental exposure to this metal in Karoon and Dez Rivers and it is important to consider the biological effects of contamination on fish health in Karoon and Dez Rivers. So, Waste management in urban and industrial centers in Khouzestan, especially around the cities of Karoon and Dez Rivers, have remained very unsatisfactory to data and it can be concluded that this poses a health hazard to both aquatic life and humans alike.

Conclusion:

This study was carried out to provide information on heavy metal concentrations in *B. grypus* Karoon and Dez Rivers in Khouzestan, Iran. In this research we demonstrate that *B. grypus* is also a good bioindicator of environmental exposure to this metal in Karoon and Dez Rivers and it is important to consider the biological effects of contamination on fish health in Karoon and Dez Rivers. All results were high the limits for fish proposed by WHO and FAO standards. High levels of heavy metals were found in the gill and liver of *B. grypus* Karoon and Dez Rivers while the lowest levels were found in the muscle. Mean metal concentrations were found to be higher in *B. grypus* Dez River than in *B. grypus* in Karoon River, except for level of Pb that in *B. grypus* in Karoon River was higher than in *B. grypus* in Dez River (P < 0.05). The results of this study showed that Pb was the highest accumulating metal compared with other metals (P < 0.05), that could be traced to urban and industrial located near the Karoon and Dez Rivers and their wastes that discharged in to these rivers. Waste management in urban and industrial centers in Khouzestan, especially around the cities of Karoon and Dez Rivers, have remained very unsatisfactory to data and it can be concluded that this poses a health hazard to both aquatic life and humans alike.
References


