Original Article

Heavy Metals Concentrations and Human Health Risk Assessment for Three Common Species of Fish from Karkheh River, Iran

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ABSTRACT

Background: The concentrations of heavy metals in the tissues and organs of fishes indicate the concentrations of heavy metals in water and their accumulation in food chains. In the present study, the concentrations of Zn, Cu, Cd, Ni, V and Pb in three common species of fish and the potential health risks to local residents via consumption of the fishes were estimated.

Methods: The concentrations of heavy metals (Zn, Cu, Cd, Ni, V and Pb) in the muscles, heart, liver, and gills of *Liza abu, Barbus grypus* and *Cyprinus carpio,* collected from Karkheh River, Southern Iran were measured. Associated human health risk was also evaluated by hazard quotient (HQ) and hazard index (HI) of muscle tissues.

Results: Bioaccumulation of heavy metals was the highest in the livers followed by gills, heart and muscle. Zn was the most accumulated metal in liver of *C. Carpio* while Cd had the lowest concentration in the muscle of *L. abu.* There were significant differences in metal concentration among different fish and different tissues (P<0.05).Zinc showed the highest concentrations in different tissues of all analyzed fish, while Cd had the lowest concentration in all tissue samples. The hazard quotients from consumption of the collected fish did not exceed the limit of 1.0.

Conclusion: The present study was a large-scale investigation of heavy metals in three common species of fish in Karkheh River. Occasional consumption of these fish is not likely to cause adverse effects. However, hazard indices for *C. carpio* and *Liza abu* were 1.751 and 1.21, respectively, which implies that continuous and excessive intake of these fish could result in chronic non-carcinogenic adverse effects.

Keywords: Chemical Water Pollutants, Fishes, Heavy Metals, Iran.

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INTRODUCTION

Heavy metals are of increasing global concern because of their persistence in the environment, effects on biogeochemical recycling, and ecological risks, their potential toxic effects and ability to bioaccumulate in aquatic ecosystems [1,2]. Heavy metals are categorized as potentially toxic agents (e.g. Cd, Pb, Ni) but some of them are essential elements for normal metabolism (e.g. Cu and Zn). Even at low concentrations, toxic metals can become harmful for human health when ingested over long periods. Essential metals can also produce toxic effects with excessive intake [3-5]. Elevated levels of heavy metals in aquatic ecosystems have raised serious public concerns around the world, due to their high potential to enter and accumulate in food chains and the correlation between heavy metals exposure and cancer in human [6]. The toxicity of these chemicals is highly influenced by geochemical factors that influence their bioavailability [7,8].

Fish is widely consumed by humans in the world due to their high protein supply and omega-3 fatty acids that help reduce the risk of certain types of cancer and cardiovascular diseases [9]. However, fish can accumulate high levels of metals through water and their food [10-12]. Fishes are widely used as bioindicators for the determination of heavy metal pollution in aquatic ecosystems [13,14]. Approximately 90% of human health risks related to fish consumption are associated with metal-contaminated fish [15]. The concentrations of heavy metals in tissues and organs of fishes could indicate their concentrations in water and their accumulation in the food chain [16].

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Recently, numerous investigations and monitoring programs have been carried out on heavy metal concentrations in different fishes [17-19]. They are largely based on measuring accumulated heavy metals in different tissues of the fish, such as liver, gills, muscle and heart. Gills and liver are mostly chosen as target organs for assessing metal accumulation. Concentrations of metals in gills reflect their water levels where the fish live, whereas concentrations in the liver represent storage of metals [20]. Increased metal concentrations in the liver may represent storage of sequestered products in this organ. Muscle is not an active tissue in accumulating heavy metals[4, 15, 20, 21].

Karkheh River originates in the Zagros Mountains and, after entering the northwest plains of Iran, crosses Ahwaz anticline near Hamidiya Village. A little further, downstream, the river turns into two parallel channels, both ending in Hawizah marshes near the Iraq-Iran border. About 10%–11% of Iran's wheat production comes from Karkheh River basin [22]. Therefore, assessing heavy metals levels in fishes for possible contamination is of utmost importance. The objective of the present study was to determine the concentrations of Zn, Cu, Cd, Ni, V and Pb in the muscles, heart, liver and gills of three common species of fish collected from Karkheh River and their potential health risk.

MATERIAL AND METHODS

Sample Collection and Preparation

The fish species were *Cyprinus carpio*, *Liza abu*, and *Barbus grypus*. Samples were collected from local anglers. In total, 120 fish samples were collected in the winter of 2014 from Karkheh River. Fish samples were put in zip-lock polyethylene bags and then were transferred to lab within 3 h. The gill, muscle, liver and heart were immediately separated in the lab, and stored at -20 ˚C until processing for analysis.

Sample Analysis

All freeze-dried muscles, gills, heart, and liver samples were crushed, sieved, grinded, and homogenized. The tissues were dissected in sterile conditions and were used to determine the levels of Zn, Cu, Cd, Ni, V and Pb. First, 0.5 g of homogenized sample was mixed with 0.5 ml magnesium acetate and was dried at 100 ˚C for 3–4 h. The samples were extracted with 2 N nitric acid (HNO3) after being washed at 600 ˚C (8 h) and diluted to 15 ml. All containers used in the study were washed in HNO3 (10%) to prevent contamination. The Pb, Cd, Cu and Zn contents of the filtrates were analyzed using an inductively coupled plasma-optic emission spectrophotometer [23, 24].

Statistical Analysis

Statistical analysis was performed using SPSS version 16; SPSS, Chicago, IL, USA). Student's *t*-test and one-way analysis of variance (ANOVA) were used to verify significant differences in organ metal concentrations between fish species. Values are given as means ± standard deviation (SD).

Health Risk Assessment

The potential non-cancer risk for individual heavy metals is expressed as the hazard quotient (HQ) [25, 26]and can be calculated as follows:

$$HQ = ADD/ RfD \quad (1)$$

$$ADD = C \times IR/ BW \quad (2)$$

where RfD is the daily intake reference dose (μg kg^{-1} day^{-1}), ADD is the average daily intake of heavy metals (μg kg^{-1} day^{-1}), C is the mean concentrations of heavy metals in fish (μg/ kg), IR is the consumption rate of fish (63g $person^{-1}$ day^{-1}), and BW is the average adult body weight (70 kg). The RfD values were 300, 40, 20, 1, 9, and 4 μg kg^{-1} day^{-1} for zinc, copper, nickel, cadmium, vanadium and lead, respectively. If the HQ exceeds one, there might be concerns for potential non-cancer effects. As a rule, the greater the value of HQ, the higher level of concern. A hazard index (HI) approach was used to assess the overall potential non-carcinogenic health risk posed by more than one heavy metal. HI is equal to the sum of the HQs, as described in Eq. (3) [26].

$$HI = HQ_1 + HQ_2 + ...+HQ_n \quad (3)$$

RESULTS

Concentration of Heavy Metals in Fish

The concentrations of heavy metals in the three studied fish species are demonstrated in Tables 1, 2 and 3. All metals concentrations were determined on a wet weight basis. Among all metals, Zn and Pb had the highest and lowest concentrations, respectively. The hazard quotients and hazard indices of Zn, Cu, Cd, Ni, V and Pb through consuming these fishes for adults are listed in Table 4. Since muscles constitute the main part of the fish consumed by humans, we only investigated hazard quotients and hazard indices in the muscles of the specimens. Among the studied metals, Cd presented the highest HQ (0.70) and the lowest HQ was for Zn (0.038).

C.carpio showed the highest health risk among three fish species. The HI for *C. carpio* was 1.751 while it was 1.21 and 0.76 for *Liza abu* and *Barbus grypus*, respectively.

The results of one-way ANOVA are presented in Table 5. Analysis of muscles and gills tissues revealed no significant differences for Cu, Cd, Ni, V and Pb in the three fish species ($P>0.05$). However, zinc content of the livers of *L. abu* and *B. grypus* were significantly different ($P<0.05$). Heart tissues also showed significant differences for Zn, Pb and Cd ($P<0.05$) in all fish species. Metals concentrations differed significantly among liver, gills, heart, and muscles in *C. carpio, L. abu* and *B. grypus* (Table 5).

DISCUSSION

In all fishes, HQs of individual metals were below one, which means that the daily intake of these metals would be unlikely to cause adverse health effects for local residents. The results of this research showed that continuous and excessive intake of fishes studied, especially *C. carpio* and *B. grypus*, could cause adverse health effects. Generally, *C. carpio* consumption had the highest health risk for the investigated exposure groups, while *L.abu* consumption had the lowest health risk.The highest Zn concentration was observed in liver of *C.carpio*(27.11 µg/g). This result was partly similar to another report [27].The lowest Zn concentration was in muscles of *Liza abu*(12.75 µg/g).The FAO guideline for maximum Zn content is 30 mg/kg [28]. The concentrations of Zn in the present study were lower than the guideline's values. Ouranalyzed fish species had a tendency to accumulate Zn in the liver as was also reported earlier[27, 28].Zn is known to be involved in most metabolic pathways in humans, and Zn deficiency can lead to loss of appetite, growth retardation, skin changes, and immunological abnormalities. The concentrations of Zn in all the fish samples were lower than the FAO standard of 30 mg/g[29] and MAFF limit of 50 mg/g[30]. Zn, has a tendency to get bio-accumulated in fatty tissues of aquatic organisms, including the fish, and affects reproductive physiology in fishes [31].

In the present investigation, Cd had the lowest concentrations of all metals in all tissues that were analyzed.The lowest concentration of Cd was in muscles of *L. abu* (0.24±0.1 µg/g) and the highest was in the liver of *C.carpio*(1.14±0.55). The bioaccumulation of Cd in liver is in accordance with previous studies[32, 33].Maximum Cd concentration detected in the liver of *C. carpio*was 0.75 mg/kg [27].Cd can accumulate in human body and may cause kidney dysfunction, skeletal damage, and reproductive impairment [5]. The concentrations of Cd in all fish samples were far below the WesternAustralian authorities' proposed level of 5.5 µg/g[34], and FAO standard of2.0 µg/g [28]. Cd occurs naturallyin the environment in low levels. It is also used inbatteries, pigments, and metal coatings [35].

The concentrations of Cu in the samples analyzed ranged from 3.22±1.45 to 8.89±1.57 µg/g, with the highest concentration of 8.89±1.57 µg/g, in the gills of *C.carpio*. However, the lowest concentration of 3.22±1.45 µg/g was in the muscle of *L. abu*, which was far below the FAO guideline of 30 mg/kg [28]. Thus, the concentrations of Cu in the fish samples analyzed were all below the recommended limits [28]. Ahmed et al.[35] reported the highest concentrationof 575.34±61.86 mg/kg, in prawn and the lowest concentration of 4.39±0.49 mg/kg in *Gagatayoussoufi*. Cu is an essential trace element, but in high concentrations can cause adverse health problems such as liver and kidney damage [36].According to UK Food Standards Committee Report, Cu concentration in food should not exceed 20 mg/kg of wet weight[37,38]. Spanish legislation also proposed 20 mg/kg of wet weight as the permissible level [15], but Australian Food StandardCode accepted 10 mg/kg wet weight as the maximum safe concentration of Cu [10].

Major sources of Ni in humans are processed food and uptake from natural resources[37,38]. The concentrations of Ni in the samples ranged from 2.31±1.52 to 6.91±1.34 mg/kg. Maximum Ni levels were in the livers (6.91±1.34) of *C. carpio*and the minimum was in the muscles of *L. abu*(2.31±1.52 µg/g). Ni is normally found at very low concentrations in the environment, and at high levels can result in a variety of pulmonary adverse effects, such as lung inflammation, fibrosis, emphysema, and tumors [39]. WHO recommends 100–300 µgNi for daily intake [40].The concentration of V was higher in the livers of *C.carpio*(6.84±2.21). The lowest V accumulation was in the musclesof*L. Abu*(2.54±1.38).The highest concentration of V was detected in the gills of big-head carps (167.83 ± 38.11) [41].

Ahmed et al.[35] reported the highest concentration of Vwas 1.84±0.11 mg/kg in *M. pancalus* and the lowest Vaccumulation (0.17±0.04 mg/kg) was in *M. rosenbergii*. V is an essential element for normal cell growth and some enzymes, particularly the Vnitrogenase used by some nitrogen-fixing microorganisms. V complexes can reduce growth of cancer cells and improve human diabetes mellitus but can be toxic when presented at higher concentrations [42]. V content in the literature has been reported in the range of 0.047–1.310 mg/kg in dietary fish [43,44].

In our study, maximum concentration of Pb observed was 1.52±0.37 µg/g in the livers of *Barbusgrypus* and the lowest was 0.37±0.1µg/g in the muscles of *L.*The highest Pb concentration was detected in *R. decussates* [20]. Ahmed etal.[35] stated the highest concentration of Pbwas in *Indoplanorbisexustus*, and the lowest was measured in *A.coila*. Gu et al.[2] reported the maximum concentration of Pb observed was 27.31 ng/g in *Siganusoramin*. Petkovšek et al.[27] found the maximum concentration of Pbinthe gills of *A. alburnusalburnus* (0.88 mg/kg) and *C. auratiusgibelio*(0.48 mg/kg). Pb poisoning can cause reduced cognitive development and intellectual performance in children, increased blood pressureand cardiovascular diseases in adults [45].Pb is a non-essential element and it is well documented that Pb can cause neurotoxicity, nephrotoxicity, and many others adverse health effects [46]. The maximum permitted lead level is 2.0 µg/g [30, 47]. Lead levels in our investigated fish tissue samples were lower than the standard.

Table 1. Mean heavy metals concentrations(µg/kg, wet weight) in the muscle, heart, liver and gills of *Liza abu* from Karkheh River.

Element	Muscle	Heart	Liver	Gill
Zn (µg/kg)	12.75±2.25c	15.91±2.28c	21.13±2.5d	20.87±3.7d
Cu (µg/kg)	3.22±1.45b	3.24±1.22b	4.35±1.70c	4.29±1.7c
Cd (µg/kg)	0.24±0.1a	0.31±0.1a	0.43±0.21a	0.37±0.19a
Ni (µg/kg)	2.31±1.52b	4.43±1.37b	5.55±1.65c	5.51±0.25c
V (µg/kg)	2.54±1.38b	3.02±1.33b	3.76±1.25c	3.22±0.25c
Pb (µg/kg)	0.37±0.1a	0.58±0.21	1.21±0.28b	0.95±0.25c

Values within the same row with different letters are significantly different ($p<0.05$)

Table 2. Mean heavy metal concentrations (µg/kg, wet weight) in the muscle, heart, liver and gills of *Barbus grypus* from Karkheh River.

Element	Muscle	Heart	Liver	Gill
Zn	15.32±2.5c	19.58±2.67d	22.21±2.25d	20.51±2.4d
Cu	4.48±1.55	4.56±0.64c	5.69±2.2c	5.51±1.4c
Cd	0.42±0.2a	0.63±0.1a	0.88±0.18a	0.71±0.11a
Ni	3.69±0.87b	3.75±1.19c	4.23±1.22c	3.79±0.99c
V	3.68±1.84	4.85±1.64c	5.96±2.25c	5.12±0.37c
Pb	0.68±0.2a	1.10±0.18b	1.52±0.37b	1.35±0.25b

Values within the same row with different letters are significantly different ($p<0.05$)

Table 3. Mean heavy metal concentrations (µg/kg, wet weight) in the muscle, heart, liver and gills issues of *Cyprinus Carpio* from Karkheh River.

Element	Muscle	Heart	Liver	Gill
Zn	22.85±4.69c	24.51±3.69c	27.11±3.96c	25.89±2.5c
Cu	6.55±1.67b	7.65±0.69b	8.23±2.02b	8.89±1.57b
Cd	0.78±1.5a	0.87±0.11a	1.14±0.55a	0.93±0.1a
Ni	5.53±1.28b	6.83±1.57b	6.91±1.34b	6.18±1.1b
V	4.17±1.37b	5.21±1.67b	6.84±2.21b	6.53±0.55b
Pb	0.75±0.2a	0.85±0.1a	1.50±0.62a	1.01±0.25a

Values within the same row with different letters are significantly different ($p<0.05$)

Table 4. Hazard quotient (HQ) and hazard index (HI) of heavy metals from consumption of three fish species collected from the Karkheh River.

Heavy metals	**Hazard quotient (HQ)**		
	Liza abu	*Barbus grypus*	***Cyprinus Carpio***
Zn	0.038	0.045	0.068
Cu	0.072	0.100	0.147
Cd	0.216	0.378	0.703
Ni	0.103	0.166	0.248
V	0.254	0.368	0.417
Pb	0.083	0.153	0.168
Hazard index (HI)	0.766	1.21	1.751

Table 5. Statistical Analysis of metal concentrations in the muscle, heart, liver, and gills of *Liza abu*, *Barbus grypus* and *Cyprinus carpio*.

Fish species	**Zn**		**Cu**		**Cd**		**Ni**		**V**		**Pb**	
	One-way	ANOVA	One-way	ANOVA	One-way	ANOVA	One-way	ANOVA	One-way	ANOVA	One-way	ANOVA
	Fvalue	Pvalue	Fvalue	Pvalue	Fvalue	Pvalue	Fvalue	Pvalue	Fvalue	Pvalue	Fvalue	Pvalue
L. abu	126.25	<0.001	12.27	<0.001	14.88	<0.001	123.46	<0.002	37.14	<0.001	299.79	<0.001
B.grypus	99.58	<0.001	452.48	<0.002	49.69	<0.001	13.44	<0.002	240.11	<0.001	63.74	<0.001
C. Carpio	41.80	<0.001	212.85	<0.001	54.77	<0.001	26.88	<0.001	280.14	<0.001	65.52	<0.001

CONCLUSION

The present study was a large-scale investigation of heavy metals (Zn, Cu, Cd, Ni, V and Pb) in three common species of fish in Karkheh River in South of Iran. Occasional consumption of these fish is not likely to cause adverse effects. However, hazard indices for *C. carpio*and *Liza abu* were 1.751 and 1.21, respectively. This indicated that continuous and excessive intake of these fish could result in chronic non-carcinogenic adverse effects.

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