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GLOBAL POLLUTION PROBLEMS, TRENDS IN DETECTION AND PROTECTION

A comparative study of trace metals in male and female Caspian kutum (*Rutilus frisii kutum*) from the southern basin of Caspian Sea

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Abstract This study focuses on the extent of zinc (Zn), copper (Cu), cadmium (Cd), cobalt (Co), manganese (Mn), lead (Pb), mercury (Hg), and arsenic (As) bioaccumulation in edible muscles of Caspian kutum (*Rutilus frisii kutum*), in both male and female sexes at Noor and Babolsar coastal regions from the southern basin of Caspian Sea. These values were compared with the WHO and the UNFAO safety standards regarding the amount of the abovementioned heavy metals in fish tissues (mg/kg ww). Results showed that the accumulation of these elements (except for Zn) was not significantly different between sexes of male and female in Babolsar coastal regions ($P > 0.05$). In the other hand, accumulation of Hg and As at edible muscles of Caspian kutum has significant difference between two sexes of male and female in Noor coastal regions ($P < 0.05$), the female had higher concentration than the male. Furthermore, it was not significantly correlated with sex and rivers in length and weight of fish ($R^2 < 0.50$; $P > 0.05$). Based on the results, the concentration of heavy metals in the studied fish tissues proved to be significantly lower than international standards ($P < 0.05$), so its consumption is not a threat to the health of consumers.

Keywords Caspian kutum · Caspian Sea · Heavy metal bioaccumulation · Muscle · Sex

Introduction

During recent decades, the marine environments have been contaminated by a wide range of pollutants such as oil exploration, industrial, and agriculture waste water. Heavy metal contamination has been recognized as a concern in coastal environment, due to bioaccumulation by aquatic organisms through a variety of pathways, including respiration, adsorption, and ingestion (Zhou et al. 2001). Some of the metals such as iron, copper, zinc, and manganese are vital elements since they play an important role in biological systems, growth, reproduction, and energy metabolism in all organisms whereas mercury, lead, arsenic, and cadmium are nonessential metals, as they are toxic, persistent, bioaccumulative, and nonbiodegradable in nature even in traces (Cheng 2003; Cui et al. 2015; MacFarlane and Burchett 2000; Türkmen et al. 2008). The essential metals can also produce toxic effects when the metal intake is excessively raised. Fish is the major part of the human diet and it is not surprising that numerous studies have been done on metal bioaccumulation in different fish species (Agusa et al. 2004; Eslami et al. 2011; Hoseini and Tahami 2012; Raeisi et al. 2014; Saghali et al. 2014; Turkmen et al. 2008). Caspian Sea or Khazar Sea, saltwater lake in southeastern Europe and southwestern Asia, is the largest inland body of water in the world which extends about 1210 km (about 750 mile)

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in a northern and southern direction and about 210 to 436 km (about 130 to 271 mile) in an eastern and western direction. Main sources of pollution of the Caspian Sea are petroleum products, phenols, organic substances, metals, nitrogen compounds, and etc. (Monsefrad et al. 2012; Watanabe et al. 2002). They can change both aquatic species diversity and equilibrium of ecological consumers. Aquatic organisms such as fish can concentrate the heavy metals many times higher than present in water. On the other hand, Caspian kutum (*Rutilus frisii kutum*), a member of the Cyprinidae family, is the most important species of the southern part of Caspian Sea which includes more than 70 % of the fish caught by fishermen in Iran (Yousefian and Mosavi 2008). It allocated about 12,500 tons of catching bony fishes of Caspian Sea in 2015 to Caspian kutum. The population of this valuable species is collapsed due to marine pollution, and so this species is located in the "Conservation dependent organisms" list of the IUCN due to habitat limitation and decrease in population size (Esmaeili et al. 2015).

With regard to the importance of this valuable fish species in the Caspian Sea and health hazardous of heavy metals, the present study was undertaken to study the concentration levels of selected trace metals in commercially important fish species and correlate the concentration of metals with respect to their weight, length, and sex. The aim of this study was to determine the metal levels of zinc (Zn), copper (Cu), cadmium (Cd), cobalt (Co), manganese (Mn), lead (Pb), mercury (Hg), and arsenic (As) in edible muscles of Caspian kutum from Caspian Sea having different ecological characteristics, and to assess whether these fish are acceptable for human consumption.

Material and methods

Sampling

There are 40 fish samples from Caspian kutum (*R. frisii kutum*) which were collected from the southern basin of the Caspian Sea in Noor (36.5736° N, 52.0139° E) and Babolsar (36.7025° N, 52.6575° E) coastal regions (Fig. 1) in February 2015. This species is a commercially important fish in Caspian Sea. The fish were transported alive to the central laboratory of Caspian Sea Ecology Research Center, and their total length (cm) and total weight (g) were measured. The samples were collected in sterile polythene bags and kept in the laboratory deep freezer (−20 °C) to prevent deterioration till further analysis.

The mean weight and length (mean ± SD) of fish in Babolsar were 935 ± 186 g and 44.8 ± 7 cm for fish and in Noor were 912 ± 166 g and 43.45 ± 5.9 cm for fish. The fish were beheaded, eviscerated, deboned, skinned, and filleted.

Analytical procedures

The procedure used for measuring trace element concentrations in fish samples has been described previously (Dadar et al. 2014). Briefly, the muscles of fish were dried in an oven at 65 °C for a period of 48 h until a constant weight was obtained and ground separately. Fish samples at 0.3 g were accurately weighed to determine the concentrations of heavy metals. Concentrations of Zn, Cu, Cd, Co, Mn, Pb, Hg, and As in edible muscles of Caspian kutum samples were determined using atomic absorption spectrometry (Thermo M5 Series AA, Germany). Sample was digested by using high-pressure decomposition vessels according to the method of our previous studies (Dadar et al. 2014). A sample was mixed with 5 mL of 68 % nitric acid (super purity quality; Romil Ltd., Cambridge, UK), 4 mL of 30 % hydrogen peroxide (suprapure quality; Merck, Darmstadt, Germany), and 1 mL concentrated perchloric acid (suprapure quality; Merck, Darmstadt, Germany). For mercury digestion, the sample was added to 45 mg V_2O_5. Then, they were diluted to 50 mL with 20 mL distilled water and $K_2Cr_2O_7$ (%2). Digestion was performed on a hotplate, at 200 °C, for at least 4 h or until clear and all particles had turned white in color. Digested samples were filtered through a 0.45-μm nitrocellulose membrane filter and diluted with high purity deionized water at a ratio of 1:5 prior to being analyzed with flame atomic absorption spectrophotometry.

Samples were analyzed in triplicate, and the results were collected on a dry weight basis using an atomic absorption spectrometer (Thermo M5 Series AA, Germany) equipped with a microcomputer-controlled acetylene flame (Dadar et al. 2014). Blank digest was also carried out in the same way. The limits of detection (LOD) for Zn, Cu, Cd, Co, Mn, Pb, Hg, and As were 0.17, 0.02, 0.006, 0.02, 0.018, 0.005, 0.001, and 0.014 mg/kg, respectively. Mean percentage of moisture content in Caspian kutum was 76.0 ± 2.2, and conversion ratio of wet weight to dry weight was 0.228. Concentrations of heavy metals in fish tissues are expressed as milligrams per kilogram of tissue on wet weight (mg/kg ww) to compare with the WHO and the UNFAO safety standards.

Statistical analysis

The data was subjected to statistical analysis using the SPSS software version no. 18 (SPSS Inc., Chicago, IL, USA). All data have been demonstrated as mean values ± standard deviation (S.D.). Analysis of variance (ANOVA) along with Duncan's method was carried out to examine mean differences among the tissues. In this

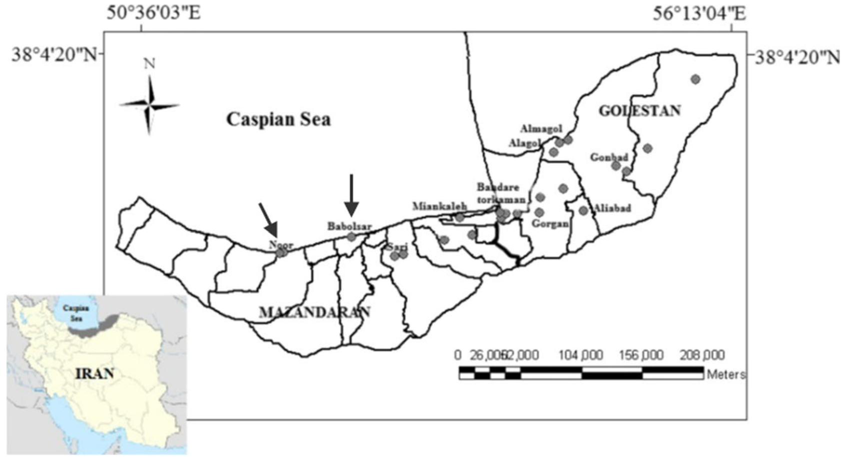

Fig. 1 Sampling sites (Noor and Babolsar coastal regions, show with *black arrow*) in Mazandaran province, north of Iran

study, *P* values of ≤0.05 were used to determine significant differences. In addition, heavy metal concentrations in fish tissues were compared with the WHO and the UNFAO safety standards using one-sample *t* test.

Results

The fish's biometric characteristics of the specimens are shown in Table 1. The mean concentrations of trace elements in edible tissues of Caspian kutum from Noor and Babolsar locations are presented in Table 2. Moreover, mean concentrations of trace elements in different sexes of Caspian kutum in different coastal regions are shown in Fig. 2. The results indicated that metal concentrations were significantly different among muscles of Caspian kutum (Table 2). Zn concentrations were highest among the trace elements examined, while concentrations of Hg were generally the lowest in the analyzed tissues (Table 2). In general, different trace elements showed different bioaccumulations. The accumulation of these elements (except for Zn) was not significantly different between sexes male and female in Babolsar coastal regions ($P > 0.05$). In the other hand, accumulation of Hg and As at edible muscles of Caspian kutum was significantly different between the two sexes of male and female in Noor coastal regions ($P < 0.05$), the female had higher concentration than the male (Table 2). The distribution patterns of trace element concentration follow the order Zn > Cu > Cd > Co > Mn > Pb, As > Hg. In the present study, mean concentrations of Pb for muscle of Caspian kutum varied from 0.044 ± 0.007 to 0.041 ± 0.006 mg/kg ww in different locations. Mean concentration of Cd, As, and Hg varied from 0.182 ± 0.019 to 0.177 ± 0.014, from 0.042 ± 0.006 to 0.038 ± 0.006, and from 0.0012 ± 0.0001 to 0.0010 ± 0.0001 mg/kg ww, respectively. Results showed that the accumulation of these elements was significantly different between river population in Caspian kutum for Zn, Mn, and As ($P < 0.05$) and they were not significantly different for others ($P > 0.05$). Furthermore, the Pearson correlations revealed that Zn, Cu, Cd, Co, Mn, Pb, Hg, and As in edible muscles of Caspian kutum were not significantly correlated with sex and rivers in length and weight ($R^2 < 0.50$; $P > 0.05$) (data not shown).

Table 1 Biometry data of Caspian kutum from the Babolsar and Noor coastal regions of the southern basin of Caspian Sea ($n = 50$)

Coastal region	Sex	Length (cm)	Weight (g)
Babolsar	Male	44.2 ± 7.1[a]	904.1 ± 195.8[a]
	Female	45.4 ± 2.3[a]	966 ± 181.9[a]
	Statistical test	*p* = 0.716	*p* = 0.473
Noor	Male	44.7 ± 5.6[a]	966.3 ± 178.8[a]
	Female	42.2 ± 6.2[a]	858.8 ± 142.6[a]
	Statistical test	*p* = 0.363	*p* = 0.153

The statistical test used is the analysis of variance (ANOVA); statistical test data as mean ± SD. The values for each parameter of biometry with same letters are no significantly different among the regions and both of sexes of male and female ($P > 0.05$).

Discussion

Fish consumption is one of the main routes of metal accumulation and contamination among human beings; therefore, studies on metal contamination in fish are in fact addressing their health for human consumption (Agusa et al. 2004). On the other hand, the *R. frisii kutum* is a species of cyprinid forms which represents more than 70 % of the fish caught by fishermen in Iran (Yousefian and Mosavi 2008). So, it is important to study about the health hazardous of toxic elements in this species to protect human beings from bioconcentration of those metals. For the first time, this study demonstrated that metal concentrations in muscles of examined species were generally not significant in muscles of male

Table 2 Mean concentration (±SD) of heavy metals (mg/kg ww) in Caspian kutum from Babolsar and Noor coastal regions of the southern basin of Caspian Sea

Trace elements	Sex/Babolsar	Concentrations/Babolsar	Sex/Noor	Concentrations/Noor
Zn	Male	12.87 ± 0.19	Male	12.77 ± 0.23
	Female	13.10 ± 0.25	Female	12.93 ± 0.15
	Statistical test	$P = 0.04$	Statistical test	$P = 0.10$
Cu	Male	1.38 ± 0.07	Male	1.36 ± 0.02
	Female	1.39 ± 0.09	Female	1.35 ± 0.09
	Statistical test	$P = 0.86$	Statistical test	$P = 0.72$
Cd	Male	0.177 ± 0.015	Male	0.175 ± 0.012
	Female	0.185 ± 0.021	Female	0.180 ± 0.015
	Statistical test	$P = 0.36$	Statistical test	$P = 0.59$
Co	Male	0.067 ± 0.010	Male	0.065 ± 0.006
	Female	0.069 ± 0.009	Female	0.066 ± 0.008
	Statistical test	$P = 0.46$	Statistical test	$P = 0.86$
Mn	Male	0.050 ± 0.008	Male	0.044 ± 0.005
	Female	0.051 ± 0.011	Female	0.045 ± 0.005
	Statistical test	$P = 0.99$	Statistical test	$P = 0.78$
Pb	Male	0.043 ± 0.007	Male	0.039 ± 0.004
	Female	0.045 ± 0.008	Female	0.043 ± 0.007
	Statistical test	$P = 0.48$	Statistical test	$P = 0.21$
Hg	Male	0.001 ± 0.0001	Male	0.001 ± 0.0001
	Female	0.001 ± 0.0001	Female	0.001 ± 0.0001
	Statistical test	$P = 0.44$	Statistical test	$P = 0.03$
As	Male	0.040 ± 0.005	Male	0.034 ± 0.004
	Female	0.044 ± 0.007	Female	0.041 ± 0.006
	Statistical test	$P = 0.25$	Statistical test	$P = 0.01$

and female, except for Zn in Babolsar and Zn, Hg, and As in Noor coastal region which exhibited the significant difference between two sexes ($P < 0.05$) which the female had higher concentration than the male. The data for this study was grouped together for the statistical analysis of fish length vs. metal concentration. Significant correlations were not shown between the length of the specimens and the trace element concentrations in the muscles.

Zinc was the highest for the analyzed species in both of the coastal regions and followed by Cu. On the other hand, mercury, arsenic, and lead were generally the lowest. Similar situations were reported by many researchers (Anan et al. 2005; Monsefrad et al. 2012), but many factors which influence fish metabolism can alter this pattern (Ruelas-Inzunza et al. 2008). Zn concentrations in all the muscle of fish were extremely high compared to the concentrations of other trace elements and toxic metals that were considered in this study. The maximum concentration of Zn recorded in the muscles was 11.22–11.56 mg/kg ww. Concentrations of Zn in all the fish samples were lower than the Food and Agricultural Organization (FAO) maximum guideline of 30 mg/kg ww of Zn for safe human consumption. Concentrations of Cu in the fish muscles were in the range of 1.18–1.23 mg/kg ww. The limit for Cu in fish is determined 20.0 mg/kg ww for human health risk concerns (Akoto et al. 2014). The concentrations of Cu in muscles of Caspian kutum were far below this value; therefore, regular consumption of fish with such low amounts of Cu could not lead to any serious health risks so far as Cu is concerned.

Our study demonstrated that the maximum concentration of Cd in the fish muscles was 0.032 mg/kg ww and a minimum value was recorded 0.152 mg/kg ww. Variation in the concentrations of Cd in the fish muscles was not significant. According to the European Commission (EC), the permissible limit for Cd in fish for human consumption is 0.2 mg/kg ww (Akoto et al. 2014; Somers 1974). So, the concentrations of Cd in all the samples were not exceeding the stipulated limit.

Lead was detected in all investigated samples in amounts ranging from 0.034 to 0.046 mg/kg ww. The estimated maximum guidelines by the FAO, WHO, EC and other regulatory bodies of various countries have determined the maximum tolerable limit (MTL) of Pb in fish meat as 2 mg/kg ww (Chary et al. 2008; Huang 2003; Xue et al. 2012). The Pb concentrations in the muscles of fish were lower than the maximum permitted limit. Previous studies have also reported similar levels of Pb in fish muscles (Türkmen et al. 2008; Monsefrad et al. 2012). The heavy metals such as Cd and Pb

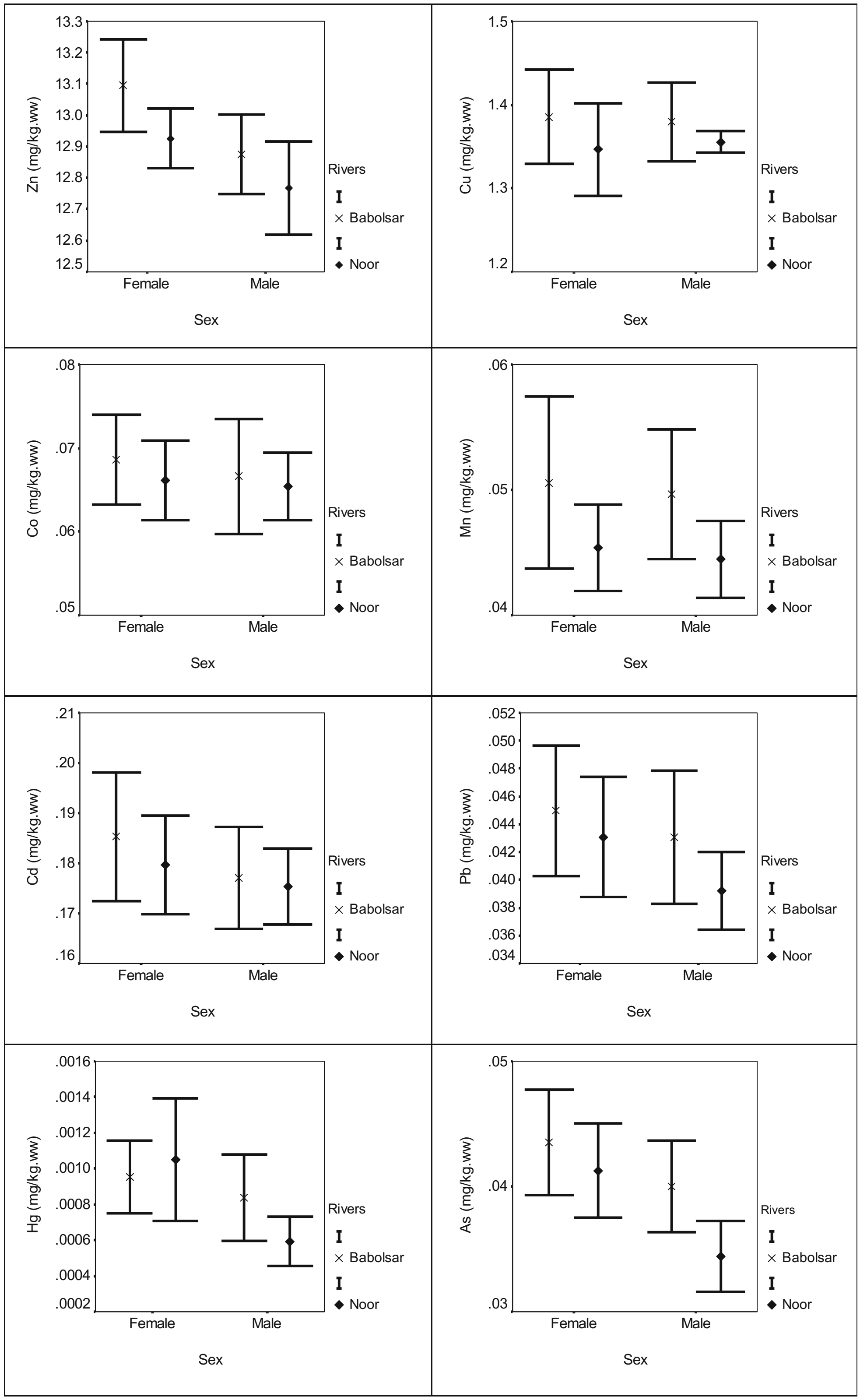

Fig 2 Mean concentrations (mg/kg ww) of Zn, Cu, Cd, Co, Mn, As, Hg, Pb, and Mn in female and male Caspian kutum from the Babolsar and Noor coastal regions of the southern basin of Caspian Sea

recognized as toxic elements do not play any metabolic functions but can be serious systemic health problems in humans, even at low concentrations, when ingested over a long period of time (Adeosun et al. 2015; Carvalho et al. 2005).

Manganese as an essential micronutrient does not occur naturally as a metal in aquatic ecosystems but is found in various minerals and salts (Nussey et al. 2000). It has an important role in biochemical processes and causes improvements of impaired glucose tolerance. Also, it has an indirect role in the management of diabetes mellitus (Choudhury et al. 2007). The distribution pattern of manganese in all samples showed similar trends as Pb and ranged between 0.038 and 0.046 mg/kg ww. The daily requirement of Mn concentrations for an adult man (a 70-kg person) is 2.8 mg, and this study showed that the concentration of Mn was below the limited range. Manganese in the literature has been reported in the range of 0.001–1.84 mg/kg ww in muscles of fish from coastal waters of Caspian Sea (Anan et al. 2005), 0.16 mg/kg ww in muscles of *Pagrus pagrus* (Miniadis-Meimaroglou et al. 2007), 0.14–3.36 mg/kg ww in muscles of fish from Indian markets (Sivaperumal et al. 2007), 0.07–0.45 mg/kg ww in muscles of seafood from Mediterranean seas (Türkmen et al. 2008), and 0.08–1.12 mg/kg ww for muscles of fish from coastal waters of Turkey (Dural et al. 2007). Our Mn concentrations were generally in agreement with the literatures.

The minimum and maximum cobalt concentrations were obtained 0.05 mg/kg ww in male and 0.06 mg/kg ww in female for muscles in Caspian Sea, respectively. Cobalt concentrations in the literature have been reported in the range of 0.006–0.244 mg/kg ww for muscles of fish from the coastal waters of the Caspian Sea (Anan et al. 2005), 0.02–0.67 mg/kg ww for muscles of fish from internal markets of India (Sivaperumal et al. 2007), and 0.003–0.015 mg/kg ww for livers of fish from Mediterranean Sea region, Turkey (Türkmen et al. 2008). Cobalt levels determined in this work were higher than the literature values. Maximum permissible cobalt limit in fish tissues is 0.4–0.5 mg/kg ww for muscles (Staniskiene et al., 2006). The Co concentrations in the muscles of fish were lower the maximum permitted limit.

Mercury has high toxicity and most governments established toxicological standards for mercury in seafood which is of great concern for international trade. The most commonly applied standard for Hg is 0.5 mg/kg ww. In this study, it demonstrated that Hg concentration was maximum range of 0.004 mg/kg ww in female and minimum range of 0.002 mg/kg ww in male. These concentrations were below the permissible limits for Hg.

The minimum concentrations of arsenic in our study were 0.034 mg/kg ww in male and 0.042 mg/kg ww in female and maximum concentrations were 0.030 mg/kg ww in male and 0.036 mg/kg ww in female for muscles in Caspian kutum. Maximum permissible arsenic limit in fish tissues is 0.06–0.23 mg/kg ww for muscles (Staniskiene et al., 2006). This study showed that these concentrations were in the range of permissible limits by WHO. The reason for presenting Pb, Cd, and Hg in *R. frisii kutum* tissues is probably due to the increase in agricultural and industrial activities as well as in the pollution of the Caspian Sea. It was also reported by other researchers in Caspian kutum in Caspian Sea (Eslami et al. 2011; Monsefrad et al. 2012; Pourang et al. 2005).

Conclusion

The actual toxic element concentrations present in Caspian kutum must be considered like a risk not only for this species but also for the organizations that develop in the region, including humans. The results presented in this study clearly depicted that the Caspian Sea coastal water may faced metal pollution (xenobiotic metals) in particular areas for Cd, Hg, As, and Pb whereas the levels obtained for these elements are in the range of permissible limits. Contaminant information on a broad range of metals in commercial fish is generally not available to the public. Thus, we suggest that there is a need for more information on contaminant levels in Caspian kutum from Caspian Sea and that the public should be provided with the information on exact species identification, collection location, and the concentration of more trace elements.

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