Assessment of the essential elements and heavy metals content of the muscle of Kutum (*Rutilus frisii kutum*) from the south Caspian Sea and potential risk assessment

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Abstract

Concentrations of heavy metals were determined in muscles of Kutum (*Rutilus frisii kutum*) collected from the central part of the southern end of the Caspian Sea during February 2011. Except for silver (Ag) and nickel (Ni) which were below the limits of detection, the average levels of arsenic (As), cadmium (Cd), copper (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), lead (Pb), selenium (Se) and zinc (Zn) were 1.61, 0.025, 0.038, 0.176, 1.32, 5.83, 0.238, 0.869, 1.93 and 8.05 mg kg^{-1} wet weight, respectively. Although the maximum levels of Pb were higher than that recommended in some international guidelines (*i.e.*WHO<1.5mg kg^{-1}), the estimated daily intakes of all metals were below the acceptable daily intake set by the joint FAO/WHO expert committee on food additives, and the hazard quotient values showed that there is no risk for consumptions of Kutum in reasonable amounts for consumers.

Keywords: Trace elements, Kutum (*Rutilus frisii kutum*), Risk assessment, Caspian Sea

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Introduction

Large amounts of organic and inorganic pollutants such as heavy metals enter estuarine and coastal areas from natural and anthropogenic sources; but human activities (such as many industrial and agricultural activities) have increased the flux of heavy metals into aquatic environments (Neff, 2004). Some metals like Fe and Zn are essential for the metabolism of organisms, while others like Cd and Pb are nonessential, even in low concentrations (Clark, 2001; Canli and Atli, 2003) and potentially toxic. Aquatic organisms can absorb metals from their environment (Pourang and Amini, 2001; Copat *et al*., 2012). If levels of these metals exceed the maximum permitted concentrations, most heavy metals including the essential ones are harmful to the ecosystem, marine organisms and humans consuming seafood (Neff, 2004). Fish, among aquatic organisms, are an important source of proteins with omega-3 fatty acids, essential minerals and vitamins, and are considered a healthy food (Copat *et al*., 2012). On the other hand, the intake and accumulation of heavy metals in the human body can increase with consumption of seafood (Pourang *et al*., 2004; Pourang *et al*., 2005; Petkovšek *et al*., 2011).

The Caspian Sea is a closed basin where the amounts of pollutants (such as petroleum products, phenols, heavy metals) have increased, especially in the last decade (Pourang *et al*., 2005; UNEPA, 2008).The southern basin of the Caspian Sea is home to approximately 81 fish species, among which some species including Kutum are commercially important. Kutum has been included in the “conservation dependent organisms” list of the International Union for Conservation of Nature (IUCN) mainly because of habitat loss and decreases in population size (Naderi Jelodar and Abdoli, 2005). Reports from researches confirm the decline in Kutum stocks in the past decades (Abdoli, 1999). This decline is obviously the result of over-fishing, reduction of its spawning area as a result of economic activities, decline in Caspian Sea water levels and heavy pollutant loads (Abdolmaleki, 2006; CEP, 2011).

A few studies have been done to evaluate metal levels in bony fish, especially Kutum in the Caspian Sea (Anan *et al*., 2005; Zeynali *et al*., 2009). Therefore the present study aimed to (1) determine levels of Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se and Zn in the edible muscle tissue of Kutum captured from the southern Caspian Sea; (2) compare the results with other studies done in the Caspian Sea and other regions of the world; (3) estimate the daily intake, comparing it with acceptable daily intake (ADI) and calculating the hazard quotient to assess the human health risk from consumption of Kutum.

Materials and methods

Study area

The investigated area (35˚47´-36˚35´N, 50˚34´E) is located along the south central shoreline of the Caspian Sea (Mazandaran Province, Iran), and the sampling area stretched for about 340 km with twelve sampling sites in this area. These sites were selected according to the localization of principal sources of pollution (waste from the main urban and sewage discharge points (Hosseini *et al*., 2008) (Fig. 1).

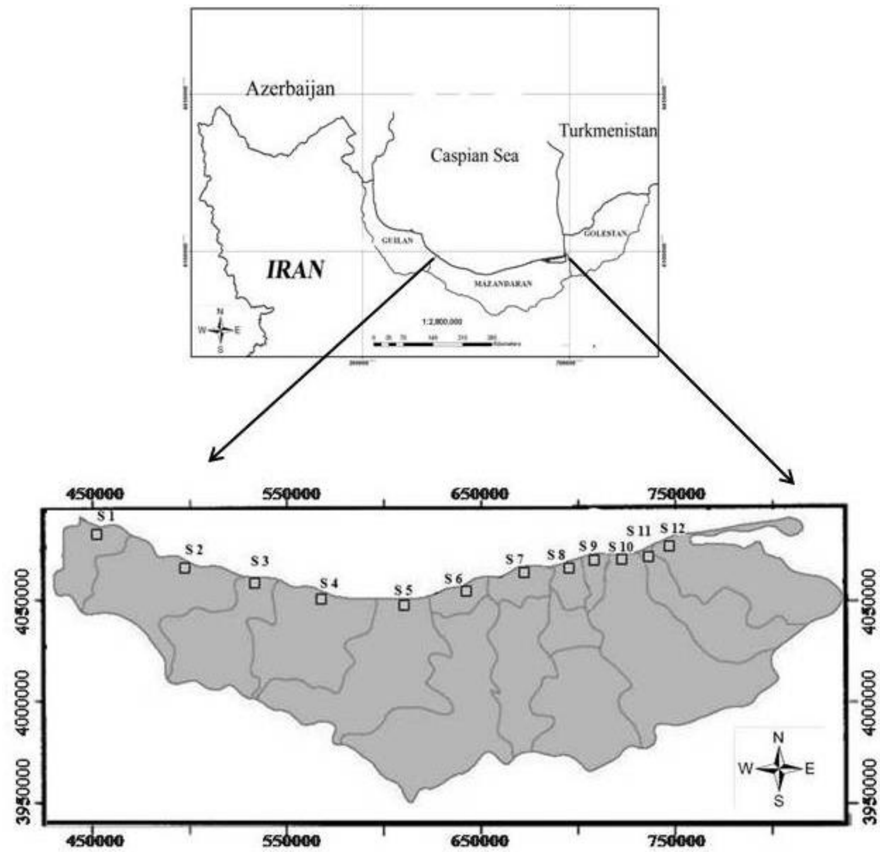

Figure 1: Sampling area and stations.

Sampling and analysis

Kutum were caught using beach seines in February, 2011. Samples of marketable size (approximately 731±26 g with 5 samples from each station (total of 60 samples)) were randomly selected from the daily catch. After purchasing, the fish were immediately transported to the laboratory in an ice-box with the fish/ice ratio of 1:3 (w/w) within approximately 5 h. Upon arrival, fish samples were washed with chilled clean water and fish muscle (the mid-dorsal muscles without skin and backbone) were obtained by dissection, washed with deionized water, packed in polyethylene bags and stored at -80 ºC for up to one week until chemical analyses. Special care was taken to prevent metal contamination of the samples by hauling and laboratory equipment. All laboratory ware was soaked in 2 M HNO_3(Merck, Darmstadt, Germany) for 24 h, and rinsed three times with distilled water, and then three times with de-ionized water prior to use.

Determination of trace elements

Each sample was analyzed three times for Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se and Zn by inductively coupled plasma-optical emission spectrophotometer (ICP-OES) (Optima 2100DV, Perkin Elmer Inc., Waltham, MA, USA).

The sample digestion procedure for determining metal concentrations has been described elsewhere (Türkmen *et al*., 2009). Before analysis, samples were thawed (in the original bag) in a refrigerator (Yakhsaran, Tehran, Iran) at 3±1°C for approximately 4.5 h and then homogenized using a meat blender (SAYA, Model: Promeat W-1800, Tehran, Iran) for 1 min. Afterward, 5 g of homogenized tissue was taken from each sample and placed in a Teflon digestion beaker. Thereafter 50 ml of ultrapure concentrated HNO_3 was slowly added. The mixture was heated on a lab digital heater (IKA®, Werke, Germany) to 100-150 ºC for about 2 h until the tissue had dissolved and the solution had evaporated to near dryness. By repeating the digestion twice more, all organic materials in each sample were completely digested. After cooling, 5 ml of 1 N HNO_3 was added to the digested residue and the samples were transferred to 25 ml volumetric flasks, and then diluted to level with deionized water. All metal concentrations were determined as mg kg^{-1} wet weight (ww) sample.

The accuracy and precision of the heavy metal determinations were assessed by using a spiking method. The recoveries of the experimental metals were determined by adding increasing known amounts of each element to samples which were then taken through the digestion procedure. The resulting solutions were analyzed for their metal concentrations. The good recoveries of the heavy metals (97.3-102%) in the spiked samples established the accuracy of the methods used.

Health risk assessment

The human health risk assessment from fish consumption for Iranians was estimated by using the ADI (Acceptable daily intake, calculated from provisional tolerance weekly intake; PTWI) and the RfD (reference dose, µg kg^{-1} bw d^{-1}) set by the United States Environmental Protection Agency (USEPA, 2011). The RfD is an estimate of a daily dose of contaminate that is likely to be without appreciable risk to deleterious effects on human health. The daily intake (µg/kg bw/d) was calculated using the following equation:

$$EDI = C_{fish} \times [dc_{fish} / bw]$$

Where C_{fish} is the mean concentration of heavy metals in fish muscle (µg/g wet weight), dc_{fish} and bw are daily per capita consumption of fish (g/day) recorded by the FAO (2011), and average body weight of 70 kg for an adult male person. The hazard quotient (HQ) was obtained by using the following equation:

$$HQ = EDI/RfD$$

When HQ is less than 1, there is no obvious risk. If it becomes more than 1, the consumption of fish might impose health hazard to the consumer, especially to susceptible people like pregnant women (Cheung *et al*., 2008).

Statistical analysis

One-way analysis of variance tests with significance levels of 5% were conducted on each metal to test for significant differences between sites. All statistical analyses were conducted using the Office Excel 2003 software package.

Results

In this study, traces of As, Cu, Fe, Mn, and Zn were found in all samples, Cr and Cd were detected in more than 50% of the samples and Co, Se and Pb were found in fewer than 50% of the fish analyzed. Also Ag and Ni were below the detection limits in muscles of all fishes (Table 1).

Table 1: The heavy metal concentrations (mg kg^{-1}) in muscle (w/w) of kutum (*Rutilus frisii kutum*) from the southern Caspian Sea.

	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	Zn
GM (geometric mean)	nd	1.61	0.025	0.038	0.176	1.32	5.83	0.238	nd	0.869	1.93	8.05
GM*	nd	6.44	0.1	0.152	0.704	5.28	23.32	0.952	nd	3.476	7.72	32.2
Guidelines												
WHO/FAO[a]			0.1							1.5		50
MAFF[b]			0.2			20				2		50
USEPA[c]		1.2[d]	0.2		8	120			1	4		120

* µgg^{-1} dry weight
[a] World Health Organization (Petkovšek *et al*., 2011)
[b]MAFF (2000)
[c]United States EPA (Mishra *et al*., 2007)
[d] Value for As are inorganic As

Discussion

The levels of As were 0.531-2.97mgkg^{-1}ww. According to Neff (2004) most of total As (usually 50 to more than 95%) in fish is in the nontoxic organic forms such as arsenobetaine. But inorganic As is very toxic and that poses the greatest threat to human safety (Fabris *et al*., 2006). Therefore the guideline values only concern inorganic form of As, and the value of 10% of total As can be used as an estimate of inorganic As (Cheung *et al*., 2008). So in this study, the levels of As in all samples were lower than the guidelines established by the USEPA (Mishra *et al*., 2007). However, the amounts were higher than the reported levels for fishes from Trans-Thane Creek area, Mumbai (Mishra *et al*., 2007), HutovoBlato (Bosnia and Herzegovina) (Has-Schön *et al*., 2008),the Fujian coastline of China (Onsanit *et al*., 2010), and ŠalekLakes, Slovenia (Petkovšek *et al*., 2011) (Table 2).

The amounts of Cd in muscles were between 0.013 and 0.071 mgkg^{-1}ww.The average value of Cd was lower than the acceptable limits set by WHO/FAO, MAFF and USEPA although mean Cd levels in the present study were higher than those previously reported for *R. frisii kutum* (Anan *et al*., 2005) and *Acipenser persicus* from the Caspian Sea (Agusa *et al*., 2004). On comparison with other sites, these results were also higher than the results reported in the fishes from HutovoBlato (Has-Schön *et al*., 2008), Trans-Thane Creek area, Mumbai (Mishra *et al*., 2007) and lower than those given for fishes from the western Indian Ocean (Kojadinovic *et al*., 2007), the Parangipettai Coast of southeast coast of India (Raja *et al*., 2009) and in a similar range to fish from the Aegean and Mediterranean Seas (Türkmen *et al*., 2009).

The concentrations of Co in fish were 0.014- 0.074 mgkg^{-1}ww. The mean value of Co in this study was higher than those reported earlier for this species (Anan *et al*., 2005) and *A. persicus* from the Caspian Sea (Agusa *et al*., 2004). The Co levels found by Raja *et al*. (2009) and Türkmen *et al*. (2009) were higher than those reported in the present study.

The levels of Cr were from 0.085 to 0.549 mgkg^{-1}ww. The content of Cr in fish muscles was lower than the limits prescribed by USEPA (Mishra *et al*., 2007). The average value of Cr in this study was lower than those reported earlier in fish species from the Caspian Sea (Agusa *et al*., 2004; Anan *et al*., 2005), and some other reports from around the world (Mishra *et al*., 2007; Raja *et al*., 2009; Türkmen *et al*., 2009).

The range of Cu was between 0.551 and 5.04 mg kg^{-1}ww.Our results showed that the levels of Cu were lower than the permissible amounts for human consumption. The content of Cu in the analyzed fish muscles were in a similar range with the results of Pourang *et al*. (2005) in muscles of five sturgeon species from the Caspian Sea, but these findings are higher than the other reported values by Anan *et al*. (2005) and Agusa *et al*. (2005) from the Caspian Sea, and Fernandes *et al*. (2008) from south Portugal and northeast of Spain, Raja *et al*. (2009) from the the south-east coast of India and Türkmen *et al*. (2009) from the Aegean and Mediterranean Seas.

The amounts of Fe in this study were 3.95- 9.16 mg kg^{-1}ww.By comparison, Fe concentrations in muscles of fish have been reported as 3.15- 17.6 mg kg^{-1}ww from the western Indian Ocean (Kojadinovic *et al*., 2007), 24.1- 50.3 mg kg^{-1}ww from the coast of southeast coast of India (Raja *et al*., 2009) and 1.52- 3.02 mg kg^{-1}ww from the coast of Fujian Province in China (Onsanit *et al*., 2010).

The concentrations of Mn ranged from 0.109 to 0.607 mgkg^{-1}ww.Our results of Mn content were higher than those reported earlier in fish muscle from the Caspian Sea (Agusa *et al*., 2004; Anan *et al*., 2005; Pourang *et al*., 2005). Also these levels were higher than those detected by Kojadinovic *et al*. (2007) from the western Indian Ocean, Onsanit *et al*. (2010) from the coast of Fujian province in China, and were in a similar range tothose reported for fishes from the Aegean and Mediterranean Seas (Türkmen *et al*., 2009).

The range of Pb was between 0.304 and 2.12 mgkg^{-1}ww. The maximum level of Pb was higher than the permissible tolerable limits set by WHO/FAO (Petkovšek *et al*., 2011) and MAFF (2000). Also the concentrations of Pb in this study were significantly higher than those in the previous studies with the different fishes of the Caspian Sea (Agusa *et al*., 2004; Anan *et al*., 2005; Pourang *et al*., 2005). The significant increase in this metal probably reflects increased pollutant inputs into the Caspian Sea, especially oil pollution, because Pb is one of the oil derivatives (Novan Magsoudi *et al*., 2007).Our results were higher than those reported in fish from different areas of the world, too (Has-Shön *et al*., 2007; Mishra *et al*., 2007; Türkmen *et al*., 2009; Petkovšek *et al*., 2011).

The levels of Se were 0.805-3.41 mgkg^{-1}ww. Our results were higher than those in the studies done by Kojadinovic *et al*. (2007) and Onsanit *et al*. (2010) in the western Indian Ocean and the coast of Fujian Province in China, respectively.

The amounts of Zn were between 3.94 and 12.8 mgkg^{-1}ww in this study. These levels were significantly lower than the prescribed limits by WHO/FAO (Petkovšek *et al*., 2011), MAFF (2000) and USEPA (Mishra *et al*., 2007).By comparison, the levels of Zn in our study were higher than those reported in the Caspian Sea with Kutum (Anan *et al*., 2005) and sturgeons (Agusa *et al*., 2004; Pourang *et al*., 2005). Also our results were higher than those reported by Fernandes *et al*. (2008), Onsanit *et al*. (2010) and in similar range tothose of Türkmen *et al*. (2009) and Petkovšek *et al*. (2011).

Table 2: Average of metals (μgg^{-1} wet weight) in fish muscle tissues in different ecosystems.

Species	Ag	As	Cd	Co	Cr	Cu
Acipenser persicus (Caspian Sea)[a]*	<0.001	-	0.002	0.004	0.37	1.74
Rutilus frisii kutum(Caspian Sea)[b]*	<0.001	-	0.001±0.001	0.009±0.003	0.33±0.08	1.01±0.25
Sturgeons (Caspian Sea)[c]*	0.001-0.002	-	0.001-0.006	0.002-0.009	0.314-0.401	1.23-1.91
Cyprinus carpio(Hutovo Blato;Bosnia)[d]	-	0.081-0.104	0.007-0.015	-	-	-
Fish(Mumbai, India)[e]	-	0.0198±0.006	0.02±0.02	-	0.78±0.48	0.31±0.16
Pelagic fishes(Western Indian Ocean)[f]	-	-	0.03-0.26	-	-	0.16-0.50
Dicentrarchus labrax(South Portugal and Northeast of Spain)[g]	-	-	0.003-0.008	-	-	0.25-1
Marine fishes(Parangipettai Coast,India)[h]	-	-	0.18-0.54	0.05-0.28	0.65-0.92	0.12-0.31
Marine fishes(Aegean and Mediterranean seas)[i]	-	-	<0.01-0.39	<0.01-0.45	0.07-1.48	0.51-7.05
Marine fish in cages (Fujian coastline, China)[j]	<0.03-0.07	1.54-4.48	0.01-0.04	0.01-0.04	-	0.06-0.16
Fish(Šalek Lakes, Slovenia)[k]	-	0.02-0.08	<0.01	-	-	-

	Fe	Mn	Ni	Pb	Se	Zn
Acipenser persicus(Caspian Sea)[a]*	-	0.511	-	0.006	-	21.70
Rutilus frisii kutum(Caspian Sea)[b]*	-	0.450±0.191	-	0.008±0.006	2.0±0.3	17.2±3.0
Strugeons(Caspian Sea)[c]	-	0.323-0.566	-	0.004-0.037	-	17.95-24.47
Cyprinus carpio(Hutovo Blato)[d]	-	-	-	0.007-0.019	-	-
Fish(Mumbai, India)[e]	-	-	0.68±0.55	0.08±0.06	-	8.36±5.79
Pelagic fishes(Western Indian Ocean)[f]	3.15-17.6	0.05-0.09	-	-	0.40-3.95	10.4-40
Dicentrarchus labrax(South Portugal and Northeast of Spain)[g]	-	-	-	-	-	5.3-7

Table 2 continued:

Marine fishes(Parangipettai Coast,India)[h]	24.1-50.3	0.31-1.20	0.38-1.54	-	-	14.1-33.5
Marine fishes(Aegean and Mediterranean seas)i	9.18-136	0.18-2.78	0.03-1.72	0.21-1.28	-	3.51-53.5
Marine fish in cage(Fujian coastline, China)[j]	1.52-3.02	0.04-0.11	-	-	0.18-0.87	2.39-4.49
Fish(Šalek Lakes, Slovenia)[k]	-	-	-	0.01-0.04	-	6.71-16.5

* $\mu g g^{-1}$ dry weight; [a]Agusa *et al*., 2004; [b]Anan *et al.*, 2005; [c]Pourang *et al*., 2005;[d] Has-Shön *et al*., 2007; [e]Mishra *et al.*, 2007; [f]Kojadinovic *et al.*, 2007; [g] Fernandes *et al.*, 2008; [h] Raja *et al.*, 2009; [i] Türkmen *et al.*, 2009; [j]Onsanit *et al.*, 2010; [k]Petkovšek *et al.*, 2011

Average metal concentrations were used to estimate the human health risk through consumption of Kutum. Evaluation of fish consumption in Iran indicates that the rate of fish consumption is 6.9 kg per year (FAO, 2011) which is equal to 18.9 $g day^{-1}$. Based on this data the estimated daily intake (EDI) of metals was calculated for an adult person with a mean weight of 70 kg (Table 3) assuming their daily consumption of fish was always Kutum from the Caspian Sea.The calculations of the EDI showed that metal absorption through the consumption of the fish analyzed in this report were much lower than the established ADI and RfD guidelines set by the USEPA (2011). According to our result, even though the levels Pb in the examined muscles of fish were above the permissible tolerable limits set by WHO/FAO (Petkovšek *et al*., 2011), there is no concern for human health from consuming this fish. However fish contamination levels should be monitored on a regular basis, to detect changes that could become a risk to human safety, and provide solutions to reduce and control the pollution inputs to the Caspian Sea, because of the increasing amounts of the mentioned metals.

Table 3: The estimated daily intakes of metals through Kutum (*Rutilus frisii kutum*) in Iran.

Metal	Average conc. ($\mu g g^{-1}$)	EDI[1] ($\mu g kg^{-1}$ bwd^{-1})	ADI[2] ($\mu g kg^{-1}$ bwd^{-1})	RfD[3] ($\mu g\ kg^{-1} bwd^{-1}$)	Hazard quotient
Ag	nd	-	5[b]	5	-
As(inorganic)[a]	0.161	0.043	2.15[b]	0.3	0.145
Cd	0.025	0.007	1[b]	1	0.007
Co	0.038	0.010	20[b]	0.3	0.034
Cr	0.176	0.048	2.17[c]	3	0.016
Cu	1.32	0.356	500[b]	40	0.009
Fe	5.83	1.574	800[b]	700	0.002
Mn	0.238	0.064	140[b]	140	0.000
Ni	nd	-	11.7[c]	20	-
Pb	0.869	0.235	3.57[d]	4	0.059
Se	1.93	0.521	5[b]	5	0.104
Zn	8.05	2.174	133[c]	300	0.007

[1] EDI: Estimated Daily Intake
[2] ADI: Acceptable daily intake, calculated from provisional tolerance weekly intake; PTWI(ADI= PTWI/ 7)
[3] Reference doses for metals set by U.S. EPA (2011)
[a] Mean level of inorganic As was calculated by using a value of 10% of total As(Agusa *et al.*,2008)
[b] (Onsanit *et al*. 2010)
[c] (Cheung *et al*., 2008)
[d] (FAO/WHO, 2004)

In conclusion this study was carried out to provide information on heavy metal concentrations in Kutum from the Caspian Sea. Accumulation of heavy metals in the body of Kutum not only has detrimental effects on seafood consumers, but also has an effect on fish survival and reproduction. Results obtained from present study showed that although the maximum levels of some elements, like as Pb were higher than that recommended in some international guidelines (*i. e.,* WHO<1.5 mg kg^{-1}), the estimated daily intakes of all metals were below the acceptable daily intake set by the joint FAO/WHO expert committee on food additives. According to our results, the examined fish were not associated with enhanced metal content in their muscle and were safe within the limits for human consumption.

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