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ORIGINAL PAPER

Estimation of Potential Health Risks for Some Metallic Elements by Consumption of Fish

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Abstract The aim of this study was to assess the bioaccumulation of heavy metals in muscle of fish in order to determine the value daily intake of heavy metals by consumption of fish and human health risk assessment. Edible muscle tissue of *Rutilus rutilus* (Linnaeus, 1758) from the Miankaleh international wetland was analyzed for content of Cd, Ni, Pb, Cr, Fe, As, Cu, and Zn; the levels were 0.26, 0.21, 0.67, 0.08, 28, 0.31, 1.6, and 7.2 mg kg^{-1}, respectively. Provisional tolerable weekly intake (PTWI) and provisional tolerable daily intake were calculated, as well as the target hazard quotient (THQ) and target cancer risk (TR). The maximum allowable fish consumption rate (CRlim) (kg/d) was calculated to estimate health risks associated with fish consumption. Since the concentrations calculated weekly intake due to fish consumption were far below PTWI for all metals; THQ for metals were below 1 and TR for As and Ni were lower than 10^{-6}, there is no human health risk of consumption of *R. rutilus* for consumers.

Keywords Fish · Health risk · THQ · TR · PTWI · Metals

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Introduction

Metals in the aquatic environment may bioaccumulate along the food chain to levels at which they may become toxic for fish and people (Dural et al. 2007). Assessment of heavy metal concentrations in fish is important both with respect to monitoring environment and human consumption of fish.

Heavy metals can enter into the food web through direct consumption of water or organisms, or through uptake processes, and be accumulated in organs edible fish (Paquin et al. 2003). Fishes are an integral component of the aquatic ecosystems. In addition to being a source of protein to humans, they play important roles in energy flows, nutrient cycling, and maintaining community balances in these ecosystems (Chari and Abbasi 2005).

Dietary intake is the main route of exposure to heavy metals for most people. Thus information about heavy metal concentrations in food products, dietary intake, and daily intake level is very important for assessing risk to human health (Tripathi et al. 1997). Fishes are major part of the human diet because, it has high protein content, Unsaturated fatty acids in adipose and also contains omega fatty acids known to support good health, but there is concern that heavy metals accumulated in edible fish may represent a health risk, especially for populations with high fish consumption rates.

Studies on metal bioaccumulation in fish are now widespread, but in recent years, risk factor calculations for the population have become of great importance, because although sometimes the contaminants exceed the legal limits set by FAO/WHO regulations for food, not always represent a risk for human health (Copat et al. 2012). To estimate the potential risk for human health derived from ingesting contaminated fish, we have evaluated: the weekly and daily intake comparing it with the provisional tolerable weekly intake (PTWI) recommended by the FAO/WHO (2012) and

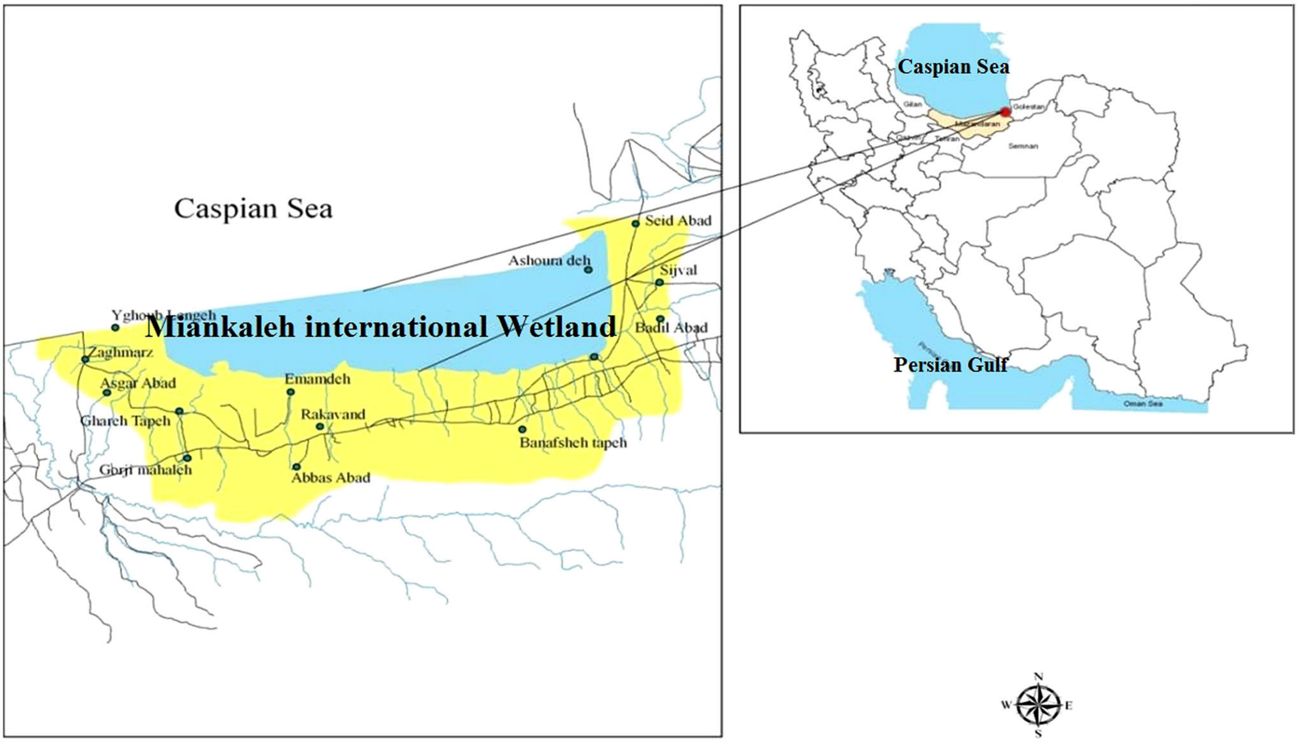

Fig. 1 Map of Miankaleh international Wetland, Iran

the target hazard quotient (THQ) and target cancer risk (TR) provided in the US EPA Region III Risk-based concentration table (USEPA 2012).

Rutilus rutilus caspicus knipowitschi (Linnaeus, 1758) (*Astrabad vobla*) to Cyprinidae is one of the most economically important and valuable telostei in south-eastern of Caspian Sea and Miankaleh international wetland. The Miankaleh international wetland is located at the south-eastern part of the Caspian Sea (Fig. 1). The Miankaleh wetland is extremely important as spawning and nursery grounds for fish, and as breeding, staging, and wintering areas for a wide variety of waterfowl.

Various studies have been carried out worldwide on the metal contamination in different edible fish species. Also, based on the THQ values, several studies on the potential risk assessment of dietary intake of heavy metals via the consumption of fish have been reported (Storelli 2008; Copat et al. 2012, 2013; Saha and Zaman 2012; Türkmen et al. 2009).

In this study, the concentrations of cadmium (Cd), Nickel (Ni), lead (Pb), chromium (Cr VI), Iron (Fe), Arsenic (As), Copper (Cu), and Zinc (Zn) were determined in edible tissue muscle of *R. rutilus* from the Miankaleh international wetland. The aim of this study was to assess the bioaccumulation of heavy metals in muscle tissue of *R. rutilus* in order to determine the value daily intake of heavy metals by consumption of fish and human health risk assessment.

Materials and Methods

Chemical Analyses

Fifteen fish samples used for this study were collected in June 2012 from the Miankaleh wetland. Fishes were randomly caught by means of beach seine and then transported to the laboratory. The fishes were washed with distilled water and the scales were removed. Of the muscle tissue samples, 1 g each was accurately weighed into 25 mL Erlenmeyer flasks, 5 mL nitric acid (65 %; from Merck, Germany) was added to each sample, and the samples were left overnight to be slowly digested (Ebrahimpour et al. 2011). Thereafter, 2.5 mL perchloric acid (72 %; from Merck, Germany) was added to each sample. Digestion was performed in a sand bath on a hot plate at 150 °C for 6 h or until solutions were clear and near to dryness. After cooling, the solutions were quantitatively transferred to 50 mL polyethylene bottles and made up to 25 mL with distilled water. Then the solution was filtered using 0.45 µm nitrocellulose membrane filters. The metal determinations were carried out using a flame atomic absorption spectrometer, (Model 97GFS, Thermo). The limits of detection were as follows: Cd (0.004), Ni (0.09), Pb (0.1), Cr (0.03), Fe (0.05), As (10), Cu (0.01), and Zn (0.005) mg kg^{-1}. The concentrations of metals in fish tissues presented in mg kg^{-1} wet weight.

Statistical Analyses

Data analyses were performed using the statistical package SPSS (version 19). The Kolmogorov–Smirnov test was accomplished to analyze the normality of data distribution. Pearson's correlation coefficients were used when calculating correlations between heavy metals in muscle tissue of *R. rutilus*.

Target hazard quotient (THQ) and daily intake of metals

THQ was calculated (US EPA 2012) by the following equations:

$$\text{Target hazard quotient (THQ)} = (\text{EF} \times \text{ED} \times \text{FIR} \times \text{C}) / (\text{RfD} \times \text{WAB} \times \text{ATn}) \times 10^{-3}$$

The estimated daily intake of each heavy metal was found in the following way:

$$\text{Daily intake (mg kg}^{-1}\text{ day}^{-1}) = (\text{EF} \times \text{ED} \times \text{FIR} \times \text{C}) / (\text{WAB} \times \text{ATn}),$$

where EF is the exposure frequency (350 days year^{-1}); ED is the exposure duration (70 years for adults), equivalent to the average lifetime; FIR is the fish ingestion rate (kg person^{-1} day^{-1}), (0.02 kg person^{-1} day^{-1} for adults); C is the metal concentration in fish (mg kg^{-1}); RfD is the oral reference dose (mg kg^{-1} day^{-1}); WAB is the average body weight (kg), (65 kg for adults); and ATn is the average exposure time for noncarcinogens (365 days year^{-1} × ED)

Target Cancer Risk (TR)

The method to estimate TR was also provided in USEPA Region III Risk-Based Concentration Table (USEPA 2012). The model for estimating TR was shown as follows:

$$\text{Target cancer risk (TR)} = (\text{EF} \times \text{ED} \times \text{FIR} \times \text{C} \times \text{CSF}) / (\text{WAB} \times \text{ATc}) \times 10^{-3},$$

where EF is the exposure frequency (350 days year^{-1}); ED is the exposure duration (70 year for adults); FIR is the fish ingestion rate (kg person^{-1} day^{-1}), (0.02 kg person^{-1} day^{-1}); C is the metal concentration in fish (mg kg^{-1}); CSF is the cancer slope factor (mg/kg/day); WAB is the average body weight (kg), (65 kg for adults); and ATc is the average time for carcinogens(365 days year^{-1} × 70 years).

Calculation of Daily Consumption Limits

The following equation calculates an allowable daily consumption (CRlim) of contaminated fish, based on a contaminant's carcinogenic health effects, and is expressed in kilograms of fish per day: (US EPA 2000)

$$\text{CR\_lim} = (\text{ARL} \times \text{BW}) / (\text{CSF} \times \text{C\_m})$$

For noncarcinogenic, based on the reference dose for each of contaminants, the following equation was used:

$$\text{CR\_lim} = (\text{RfD} \times \text{BW}) / \text{C\_m},$$

where CRlim is the maximum allowable fish consumption rate (kg/d); ARL is the maximum acceptable individual lifetime risk level (10–6, unitless); BW is the consumer body weight (kg); CSF is the cancer slope factor; Cm is the metal concentration in fish (mg kg^{-1}); and RfD is the oral reference dose (mg kg^{-1} day^{-1}).

Table 1 Mean metal concentrations (mg kg^{-1}) in muscle tissue of *R. rutilus* from the Miankaleh wetland (n = 15)

Metals	Mean	SD	Range
Cd	0.26	0.04	0.19–0.33
Cr	0.08	0.03	0.01–0.15
Ni	0.21	0.1	0.1–0.44
Fe	28	11.6	11.06–47.08
As	0.31	0.19	0.09–0.81
Cu	1.6	0.6	0.95–3.11
Zn	7.2	1.5	4.97–9.32
Pb	0.67	0.23	0.24–0.99

Result

The average concentrations of Cd, Ni, Pb, Cr, Fe, As, Cu, and Zn in muscle tissue of *R. rutilus* from the Miankaleh wetland were determined as 0.26, 0.21, 0.67, 0.08, 28, 0.31, 1.6, and 7.2 mg kg^{-1} (Table 1). In general, the mean concentrations of metals followed the orders: Fe > Zn > Cu > Pb > As > Cd > Ni > Cr. Pearson correlation coefficients of metal concentrations in the muscle of *R. rutilus* are shown in Table 2. Significant positive correlations between Cd and Cr were found ($p < 0.01$). There were significant correlations between Cd and Fe ($p < 0.05$), Cr and Fe ($p < 0.01$), and Zn and Cu ($p < 0.05$).

The daily intake was estimated for economically important fish species consumed by adult people. According to FAO (2012) reports, the per capita fish consumption in Iran is 20 g person^{-1} day^{-1} for adults. This is also equivalent to 140 g per person per week. The daily intake values presented in Table 3 were estimated by assuming that a 65 kg person will consume 20 g fish per day. The daily intakes of Cd, Cr, Ni, Fe, As, Cu, Pb, and Zn and are estimated, respectively, as 7.67×10^{-5}, 2.36×10^{-5}, 6.19×10^{-5}, 8.26×10^{-3}, 9.14×10^{-5}, 4.72×10^{-4}, 1.98×10^{-4}, and 2.12×10^{-3} mg/day estimated, respectively, as for adults. The average daily intake

Table 2 Statistically significant relations (Pearson correlations) between metal concentrations of muscle tissue ($n = 15$)

	Pb	Cd	Cr	Ni	Fe	As	Cu	Zn
Pb	1	−0.063	−0.198	0.170	0.473	−0.170	0.021	−0.337
Cd		1	0.720**	−0.105	−0.633*	0.046	−0.244	0.089
Cr			1	−0.192	−0.649**	0.102	−0.168	0.146
Ni				1	0.121	−0.388	0.016	−0.013
Fe					1	0.044	0.226	−0.072
As						1	−0.082	0.056
Cu							1	0.606*
Zn								1

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level

Table 3 The estimated daily and weekly intakes for muscle tissue of *R. rutilus* consumed by adult people in Iran

Heavy metals	PTWI (mg/week/kg body weight)	PTWI (mg/week/65 kg body weight)	PTDI (mg/day/65 body weight)	EWI kg(mg/week/65 kg body weight)	EDI (mg/day/65 kg body weight)
Cd	0.007[a]	0.45	0.06	5.36×10^{-4}	7.67×10^{-5}
Cr	0.0233[b]	1.51	0.21	1.65×10^{-4}	2.36×10^{-5}
Ni	0.035[c]	2.27	0.39	4.33×10^{-4}	6.19×10^{-5}
Fe	5.6[a]	364	52	5.78×10^{-2}	8.26×10^{-3}
As	0.015[a]	0.97	0.14	6.40×10^{-4}	9.14×10^{-5}
Cu	3.5[a]	228	32.5	3.30×10^{-3}	4.72×10^{-4}
Zn	7[a]	455	65	1.48×10^{-2}	2.12×10^{-3}
Pb	0.025[a]	1.62	0.23	1.38×10^{-3}	1.98×10^{-4}

PTDI permissible tolerable daily intake, *EWI* estimated weekly intake, *EDI* estimated daily intake, *PTWI* provisional tolerable weekly intake
[a] FAO/WHO
[b] Zaidi et al. (2012) after
[c] Türkmen et al. after

of metals through fish consumption can be ordered as follows: Fe > Zn > Cu > Pb > As > Cd > Ni > Cr.

THQs of metals through edible muscle consumption for adults are listed in Table 4. The THQs of Cd, Cr, Ni, Fe, As, Cu, Pb, and Zn and are estimated, respectively, as 7.67×10^{-5}, 7.86×10^{-6}, 3.09×10^{-4}, 1.18×10^{-5}, 3.04×10^{-4}, 1.18×10^{-5}, 9.88×10^{-5}, and 7.08×10^{-6} for adults.

According to the results, the maximum allowable fish consumption rate (noncarcinogenic) for an adult person with mean 65 kg body weight was 0.25, 2.43, 6.19, 1.62, 0.62, 1.62, 2.7, and 0.19 kg/d based on Cd, Cr, Ni, Fe, As, Cu, Zn, and Pb concentrations, respectively. Also, the maximum allowable fish consumption rate (carcinogenic) for an adult person with mean 65 kg body weight was 1.8×10^{-4} and 1.3×10^{-4} kg/d based on nickel and arsenic concentration, respectively (Table 4).

Discussion

Daily Intake of Metals

The EWI (estimated weekly intake) and EDI (estimated daily intake) values are presented in Table 3. Also, in Table 3 compares the estimated EWI and EDI to recommended values (PTWI and PTDI). PTWI value is an estimate of the amount of a contaminant that can be consumed by human over a lifetime without appreciable risk. PTWI is established by the Joint Food and Agricultural Organization for the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (Bat et al. 2012). PTWI depends on the amount, consumption period, and contamination level of consumed food (Türkmen et al. 2008).

JECFA established PTWIs for Cd, Cr, Ni, Fe, As, Cu, Pb, and Zn is presented in Table 3, which was equivalent to 0.45, 1.51, 2.27, 364, 0.97, 228, 1.62, and 455 mg/week for a 65 kg adult, respectively. The EWI and EDI values of Cd, Cr, Ni, Fe, As, Cu, Pb, and Zn in this study were lower than to the established PTWI and PTDI.

In general, there is no health-threatening concern due to the consumption of edible muscle of *R. rutilus* from the Miankaleh wetland.

The weekly and daily intakes estimated in this study are agreement with values reported by many researchers for fish (Türkmen et al. 2008, 2009; Bat et al. 2012; Burgera and Gochfeld 2005).

Target Hazard Quotient (THQ)

The THQ value proposed by USEPA (2012) is an integrated risk index by comparing the ingestion amount of a pollutant

Table 4 Estimated target hazard quotient (THQ), target cancer risk (TR), maximum allowable fish consumption rate (CRlim) and RfD (mg/kg) of heavy metals due to consumption of *R. rutilus* in the Miankaleh wetland ($n = 15$)

Metal	*RfD (mg/kg)	THQ (mg/kg)	*CSF (mg/kg)	TR (mg/kg)	CRlim (kg/d) noncarcinogenic	CRlim(kg/d) carcinogenic
Cd	0.001	7.67×10^{-5}	–	–	0.25	–
Cr	0.003	7.86×10^{-6}	–	–	2.43	–
Ni	0.02	3.09×10^{-4}	1.7	1.05×10^{-7}	6.19	1.8×10^{-4}
Fe	0.7	1.18×10^{-5}	–	–	1.62	–
As	0.0003	3.04×10^{-4}	1.5	1.37×10^{-7}	0.62	1.3×10^{-4}
Cu	0.04	1.18×10^{-5}	–	–	1.62	–
Zn	0.3	7.08×10^{-6}	–	–	2.7	–
Pb	0.002	9.88×10^{-5}	–	–	0.19	–

* USEPA (2012)

Table 5 Metal levels (mg kg^{-1}) in muscle tissue of *R. rutilus* from the Miankaleh wetland and other selected regions

Regions of study	Cd	Cr	Ni	Fe	As	Cu	Zn	Pb	References
WHO	1	50	0.5	100	–	30	100	2	WHO (1989)
FAO	0.5					10	100	0.5	FAO (1983)
Anzali Wetland, Iran	0.29	0.7	–	–	–	7.4	19.4	1.3	Ebrahimpour et al. (2011)
Qeshm Island, Iran	0.13	0.33	0.48	–	–	–	–	0.10	Norouzi et al. (2012)
Hara Biosphere, Iran	0.16	0.5	1.52	–	–	–	–	0.32	Mohammadnabizadeh et al. (2012)
NE Coast, India	0.33	–	–	49.2	0.64	3.9	19.9	–	Kumar et al. (2012)
Mediterranean Seas, Turkey	0.05	0.38	0.75	37	–	7.05	14	0.34	Türkmen et al. (2009)
Gulf of Cambay, India	0.2	0.77	–	94.4	1.7	2.4	38.2	1.09	Reddy et al. (2007)
Miankaleh Wetland	0.26	0.08	0.21	28.42	0.31	1.62	7.15	0.67	In this study

with a standard reference dose and has been widely used in the risk assessment of metals in contaminated foods. The THQ value has been recognized as one of the reasonable parameters for the risk assessment of metals associated with the consumption of contaminated fish (Li et al. 2012).

A THQ below 1 means the exposed population is unlikely to experience obvious adverse effects, whereas a THQ above 1 means that there is a chance of noncarcinogenic effects, with an increasing probability as the value increases (Saha and Zaman 2012).

In the present study, the THQ values for all the metals are below 1, which indicate that the intakes of metals by consuming these fish do not result in an appreciable hazard risk on human body.

Copat et al. (2012) estimated the THQs for Cr, Hg, Pb, and Cd in fish from the Sicily, Mediterranean Sea. THQs results indicated that there is no carcinogenic risk. Storelli (2008) measured the Hg, Cd, and Pb concentrations in fish from the Adriatic Sea, reported that the THQs of Cd (0.01–0.04) and Pb (0.002–0.18) from consumption of fish being less than 1, suggested that health risk was insignificant. Conversely, mercury THQs values, ranging from 0.08 to 1.87, were of concern.

Copat et al. (2013) estimated the target hazard quotient of metals consumed in fish and shellfish from eastern Mediterranean Sea, reported that the THQ values for Cd, Cr, Mn, Ni, V, and Zn were all below 1. That means that there is no risk for developing chronic systemic effects due to the intake of the above named metals, but Arsenic THQ values were above 1.

Target Cancer Risk (TR)

Target cancer risk (TR) of arsenic and nickel is summarized in Table 4. The value of TR for arsenic and nickel was 1.37×10^{-7} and 1.05×10^{-7}, respectively. The cancer risk estimated for consumption of arsenic and nickel in muscle of *R. rutilus* was lower than 10^{-6}, which is the risk of cancer benchmark often used by USEPA (2012). TR results indicate that there is no carcinogenic risk for arsenic and nickel exposure through muscle of *R. rutilus* consumption.

Exposure to compounds of five metals has been shown in epidemiological studies to correlate with increased incidences of cancer in humans. These metals are nickel, chromium, arsenic, cadmium, and beryllium (Nordberg et al. 2007).

International Agency for Research on Cancer (IARC) concluded that there was sufficient evidence in humans for the carcinogenicity of chromium(VI) compounds, nickel and Cadmium; thus, they belong to group 1 of the IARC classification system (WHO 2010; IARC 2014).

Nickel normally occurs at very low levels in the environment and it can cause variety of pulmonary adverse health effects, such as lung inflammation, fibrosis, emphysema, and tumors (Forti et al. 2011). Cadmium is deemed as an element capable of producing chronic toxicity even when it is present at the concentration of 1 mg/kg (Rahman et al. 2012). Exposure to cadmium can be potentially lethal for both wildlife and humans, and the metal has been known to cause damage to the kidneys and intestinal tract, in addition to other life-threatening conditions such as cancer.

Based on the sufficient evidence in humans that arsenic in drinking water causes cancers of the urinary bladder, lung, and skin, arsenic in drinking water has been classified as a group 1 carcinogen to humans (IARC 2014). Arsenic is widespread in the environment due to both anthropogenic and natural processes. It is a ubiquitous, but potentially a toxic, trace element (Rahman et al. 2012).

There is some data indicating that lead exposure in the general population is associated with cancer risk (Lustberg and Silbergeld 2002). Lead is a non-essential element and it is well documented that Pb can cause neurotoxicity, nephrotoxicity, and many other adverse health effects (Rahman et al. 2012).

There is inadequate evidence in humans for the carcinogenicity of chromium(III) compounds, iron, copper, and zinc (Nordberg et al. 2007). Copper is an essential part of several enzymes and is necessary for the synthesis of hemoglobin. However, high intake of Cu has been recognized to cause adverse health problem (Rahman et al. 2012).

Zinc being a heavy metal, has a tendency to get bioaccumulated in the fatty tissues of aquatic organisms, including fish and is known to affect reproductive physiology in fishes (Ghosh et al. 1985). Some authors reported that chronic exposure to Cu and Zn is associated with Parkinson's disease (Gorell et al. 1997) and these elements might act alone or together over time to induce the disease (Prasad 1983).

Acute iron overload resulting from unintentional or intentional overdose is potentially life threatening. Chronic iron overload leads to slowly developing (and, in extremis, lethal) damage to organs such as the heart and liver. However, the nature of the accumulated damage that eventuates in such organ failure is not yet fully known (Nordberg et al. 2007).

Allowable Daily Consumption (CRlim)

CRlim represents the maximum lifetime daily consumption rate (in kilograms of fish) that would not be expected to cause adverse noncarcinogenic health effects. Also, the calculated daily consumption limit (CRlim) represents the amount of fish (in kilograms) expected to generate a risk no greater than the maximum ARL used, based on a lifetime of daily consumption at that consumption limit (USEPA, 2000). Therefore, consumption limits, expressed as a month intake rate that can be safely consumed, were determined for some metals of potential concern for human health (Taweel et al. 2013).

Comparison of Metal Levels in Fish with Concentrations Taken from the Other Regions

The concentrations of the metals detected in the muscle of *R. rutilus* of this study were compared with the other reported values (see Table 5) as an effort to determine the degree of contamination in the study area. Reported results in the literatures showed that metal contents in the fish muscles varied widely depending on where and which species were caught (Rahman et al. 2012).

The values of Cr, Ni, Fe, As, Cu, and Zn were lower than to other studies (Ebrahimpour et al. 2011; Norouzi et al. 2012; Mohammadnabizadeh et al. 2012; Kumar et al. 2012; Türkmen et al. 2009; Reddy et al. 2007).

The Cd concentration was approximately similar from Anzali Wetland, Iran (Ebrahimpour et al. 2011) and Gulf of Cambay, India (Reddy et al. 2007), but higher than reported by Kumar et al. (2012) from North East Coast of India, However, lower than reported by Norouzi et al. (2012) from Qeshm Island, Iran and Mohammadnabizadeh et al. (2012) from Hara biosphere, Iran.

The reported Pb in this study was higher than from Mediterranean seas, Turkey (Türkmen et al. 2009), Hara biosphere, Iran (Mohammadnabizadeh et al. 2012) and Qeshm Island, Iran (Norouzi et al. 2012), but lower than fishes from Anzali Wetland, Iran (Ebrahimpour et al. 2011) and Gulf of Cambay, India (Reddy et al. 2007).

The metal concentrations in the muscle of *R. rutilus* from the Miankaleh wetland are below levels of concern for human consumption as defined by the FAO (1983) and WHO (1989).

Conclusion

Food consumption had been identified as the major pathway of human exposure to heavy metals, and consuming foodstuff threatens the health of the population. The levels of studied metals in the edible part of the *R. rutilus* were generally lower than the maximum permitted concentrations recommended by FAO (1983) and the WHO (1989). Considering the average values, all the THQ of the eight metals were far below 1 in the edible part of the *R. rutilus*. In addition, a calculated weekly intake due to fish consumption was far below PTWI for all metals. Also, the cancer risk estimated for consumption of arsenic and nickel in muscle of *R. rutilus* was lower than 10^{-6}; therefore, we can conclude that these metals should not pose any health threat to the consumers resulting from the consumption of studied fish.

We suggest that more specific recommendations regarding human consumption are done according to the data concerning levels of environmental pollutants in the most consumed fish.

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