



Evaluation of trace elements contaminations in muscles of *Rutilus kutum* (Pisces: Cyprinidae) from the Southern shores of the Caspian Sea

Masoud Sattari^{1,2*}, Mehdi Bibak¹, Mohammad Forouhar Vajargah¹

¹Fisheries Department, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Iran

²Department of Marine Sciences, Caspian Sea Basin Research Center, University of Guilan, Rasht, Iran

Abstract

Background: There is little information about the trace elements (TEs) concentrations in the muscle tissue of Caspian kutum (*Rutilus kutum*) in the South Caspian Sea.

Methods: A total of 51 *R. kutum* specimens were caught at five fishing stations (Gorgan, Sari, Kiashahr, Anzali, and Astara) in the South Caspian Sea from September 2018 to January 2019. The inductively coupled plasma-optical emission spectrometry (ICP-OES) was employed to measure the TEs concentrations in the fish muscles.

Results: The maximum concentrations of Al (7.2 ppm), Pb (0.07 ppm), and Ni (0.02) were reported in Astara, and the highest concentrations of As (0.2 ppm), Cu (0.49 ppm), Cr (0.12 ppm), and Zn (1.56 ppm) were reported in Sari and Gorgan. The TEs concentrations measured in the fish muscles (except Mg, Zn, As, Al, Na, and S) had no significant differences among the sampling areas. The TEs concentrations were higher in the eastern areas (Sari and Gorgan) than in the western areas (Astara, Anzali, and Kiashahr) excluding As and Al. The concentrations of Pb, Zn, Mn, Cu, Sn, Sb, Al, Cr, and Cd in muscles of *R. kutum* were found to be significantly lower ($P < 0.05$) than the maximum permitted levels according to the WHO/FAO standards, while As concentrations were comparable to these standards.

Conclusion: The TEs concentrations in different fishing stations of Kiashahr, Anzali, and Astara, located in the southwestern areas were respectively compared with those obtained from Sari and Gorgan, located in the middle and southeastern shore of the Caspian Sea. According to the results, the TEs concentrations obtained from the fish muscles cannot pose a threat to human health.

Keywords: Caspian Sea, *Rutilus kutum*, Elements, Human health

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*Correspondence to:

Masoud Sattari

Email: msattari647@gmail.com

Introduction

The trace elements (TEs) contamination in the marine habitats has long been known as a serious environmental concern (1-3). Marine organisms can uptake TEs via a variety of ways, including adsorption, respiration, and ingestion (4). The TEs contamination has been found as a serious problem in shoreline areas, owing to disposal of industrial, agricultural, and urban sources wastewater (5-7). The TEs concentrations are increased in marine ecosystems by human activities (8). Fish is considered as a good indicator for the long-term research about TEs contamination in the aquatic habitats (9) and many studies have been conducted on the TEs concentrations in different fish species (10,11).

The Caspian Sea is the largest lake in the world (12,13). It is under pressure from environmental threats such as

discharge of industrial and agricultural effluents and growing urbanization in most riparian countries of the Caspian Sea (14,15).

The TEs concentrations in the fish muscles, as the main consumable part for human consumption, must be treated with utmost concern, although their liver and skin are also consumed to some extents.

Rutilus kutum (Kamensky, 1901) is a benthopelagic fish inhabited the Caspian Sea (16). It is also a favorable and highly-steamed fish for fishermen in the North Iran, but few local reports are available about TEs concentrations in its tissues (16). Therefore, the present study was conducted to evaluate the concentrations of some TEs in muscle tissue of *R. kutum* caught from the South Caspian Sea, to compare their concentrations in fish obtained from different fishing stations, and also to assess the human



health risk of the elements.

Materials and Methods

The present study was performed at five fishing stations namely Torkaman port (36° 89' 28" N, 54° 04' 64" E), Sari (36° 78' 39" N, 53° 03' 99" E), Kiashahr (37° 42' 20" N, 49° 94' 95" E), Anzali (37° 46' 39" N, 49° 47' 99" E), and Astara (38° 42' 25" N, 48° 86' 87" E), along the South Caspian Sea (Figure 1).

Sampling was performed from September 2018 to January 2019 and 51 pieces of *R. kutum* were collected. The specimens were caught using a beach seine. The collected specimens were transferred to the Fish Disease Laboratory, University of Guilan, Iran, by a styrofoam box at 4°C. Fish were carefully washed by distilled water. The pieces of muscle were then placed in an oven for drying at 80°C for 18 hours (13), and then, the morphometric characteristics of the fish were determined (Table 1).

To extract the TEs, 0.5 g of muscle tissue was digested in 10 mL 65% nitric acid in a microwave oven, passed through a Whatman filter paper No. 40, and then, diluted with distilled water to obtain the exact volume.

Statistical analysis

An inductively coupled plasma-optical emission spectrometry (ICP-OES, Zarazma Co. Tehran, Iran) was employed to determine the TEs concentrations in the samples. The instrument detection limit for TEs is 0.02

mg kg⁻¹, and also for major elements (Al, Fe, Ca, K, Mn, Mg, Na, and Si) is 0.1 mg kg⁻¹.

The TEs concentrations were expressed as the metal selectivity index (MSI) for fish tissues, according to the Eq. (1).

$$MSI = \frac{A}{T} \times 100 \tag{1}$$

Where *A* is the absolute level of a metal in a tissue and *T* is the total level of all TEs in that tissue.

The estimated dietary exposure limits for meals according to the USEPA guideline were employed the parameters set for adults and children are as follows (17).

- Adults Body weight: 70 kg
- Children Body weight: 16 kg
- Consumption per meal for adults: 227 g
- Consumption per meal for children: 114 g

Estimates of consumption of metals in per meal were calculated based on the following equation (18).

Presumably, the consumed dose is considered to be equal to the absorbed dose, and also, cooking does not affect the pollutant concentrations.

$$EDI = \frac{C \times MS}{BW} \tag{2}$$

Where *C* is element concentration (mg kg⁻¹), *MS* is meal size (g), and *BW* is body weight (kg). The above-mentioned formula was used to calculate permissible

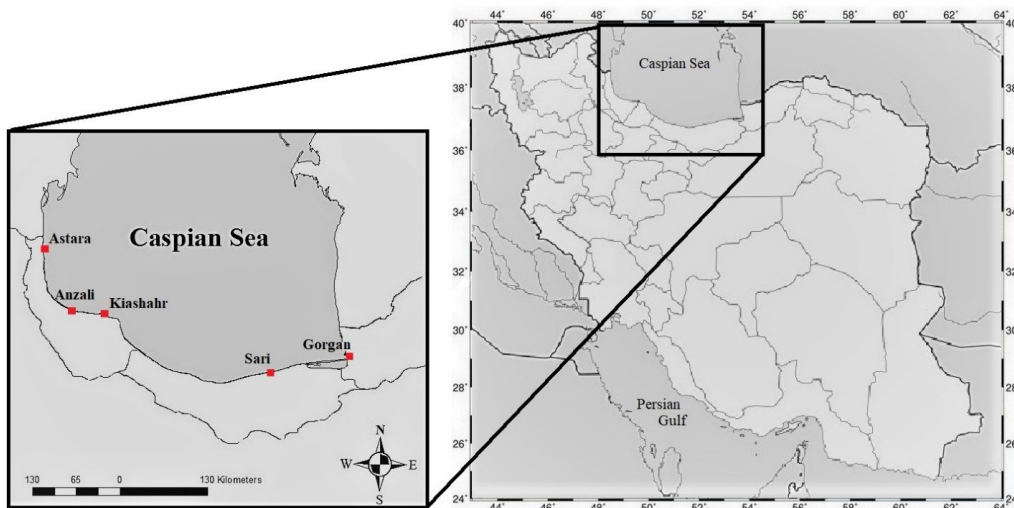


Figure 1. Location of the sampling stations in the South Caspian Sea.

Table 1. Morphometric characteristics of *Rutilus kutum* from the five study areas in the South Caspian Sea

Values	Total weight (g)	Total length (cm)	Head length (cm)	Snout length (cm)	Eye diameter (cm)	Body height (cm)	Body width (cm)
Average	702.36	40.54	14.4	4.50	2.3	15.6	8.0
Max	1156.66	47.33	18.10	10.28	5.10	19.10	9.00
Min	51.66	18	5.70	1.50	0.90	6.50	4.00
SD	254.1	6.24	0.63	0.32	0.13	0.96	0.54

daily consumption. The results showed the amount of fish consumption (kg) per day, assuming that there was no other source of these elements in the daily diet.

The daily consumption of fish without any risk for human health is calculated by the following formula (17,18).

$$CR_{lim} = \frac{RFD \times BW}{C} \quad (3)$$

Where *RFD* is reference dose ($\text{mg kg}^{-1} \text{d}^{-1}$), *C* is concentration of metals in fish muscles (mg kg^{-1}), and *BW* is body weight (kg).

Thirty-six major and TEs were statistically tested excluding Ag, Ba, Co, Cd, and Mo, which were not found by the ICP-OES (Tables 3 and 4). After testing the normality of the data and also homogeneity of variances, the one-way analysis of variances (ANOVA) was performed considering the fish age as a covariate (ANCOVA) to assess variability of the TEs concentrations (Table 3). In some cases, when homogeneity of variances was not confirmed, the Kruskal-Wallis test was used (Table 4) (19).

The concentrations of Pb, Zn, Mn, Cu, Sn, Sb, Al, Cr, As, and Cd in the fish muscles were compared with the WHO/FAO maximum permitted levels using single student t-test (19). Statistical analyses were performed by SPSS software (SPSS Inc., Chicago, IL) and statistical significant level was considered at $\alpha = 0.05$.

Results

In this study, 51 pieces of *R. kutum* were collected and their muscles were assessed to detect 35 TEs. Finally, 23 TEs including arsenic (As), aluminum (Al), beryllium (Be), bismuth (Bi), calcium (Ca), chromium (Cr), cesium (Cs), copper (Cu), iron (Fe), potassium (K), lithium (Li), lanthanum (La), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb), rubidium (Rb), sulfur (S), antimony (Sb), silicon (Si),

scandium (Sc), tin (Sn), strontium (Sr), titanium (Ti), thorium (Th), thallium (Tl), vanadium (V), yttrium (Y), tungsten (W), and zinc (Zn) were statistically analyzed (Tables 3 and 4).

According to Tables 3 and 4, the TEs concentrations measured in the muscles (excluding Mg, Zn, As, Al, Na, and S) did not exhibit any significant differences between the five study areas. The TEs concentrations were relatively higher in the eastern areas (Sari and Gorgan) than in the western areas (Astara, Anzali, and Kiashahr), even though there were no significant differences between these two areas except for As and Al. The maximum concentrations of Pb and Al were reported in Astara and Kiyashahr, respectively, and the highest concentration of Zn was reported in Sari and Gorgan.

The concentrations of Pb, Zn, Mn, Cu, Sn, Sb, Al, Cr, and Cd in *R. kutum* muscles were significantly lower ($P < 0.05$) than the WHO/FAO maximum permitted levels presented in Table 2 (Table 4), while the As concentrations were in the range of permitted levels ($P = 0.05$, $df = 12$, $t = -0.71$, Table 4).

The maximum concentrations of Al (7.2 ppm), Pb (0.07 ppm), Rb (0.72 ppm), and Ni (0.02) were reported in Astara while the maximum concentration of Th (0.07 ppm) was reported in Astara and Anzali, also the highest of concentrations of As (0.2 ppm), Cu (0.49 ppm), Cr (0.12 ppm), Mg (55.95 ppm), Fe (1.73 ppm), Sb (0.08 ppm), Si (0.45 ppm), Sr (2.96 ppm), and Zn (1.56 ppm) were reported in Sari and Gorgan and the maximum concentrations of Sn (0.12 ppm) and Ti (0.02 ppm) were reported in Kiyashahr and (Astara and Kiyashahr), respectively (Tables 3 and 4).

According to Figure 2, the highest MSI levels for Al, Pb, and Cu were observed in Astara. In terms of the human health, muscle is the main tissue for determining the accumulation of TEs. The MSI index of most elements

Table 2. Maximum permitted levels in parts per million (ppm) suggested by the Codex Alimentarius Commission (FAO/WHO, amended in 2018), FDA (2011)

Elements	Pb	As	Cr	Al	Sb	Sn	Cu	Mn	Zn
Maximum permitted concentration (PPT)	0.5	0.1	2	100	1	230	10	0.5	100

Table 3. Trace elements concentrations in the fish muscles at five fishing stations in the South Caspian Sea

Elemental Variables (ppm)	Mean \pm SE					P value
	Astara	Anzali	Kiashahr	Sari	Gorgan	
Mn	0.30 \pm 0.06	0.35 \pm 0.03	0.33 \pm 0.03	0.00 \pm 0.00	0.00 \pm 0.00	1.00
Pb	0.07 \pm 0.01	0.03 \pm 0.01	0.03 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00	0.40
Rb	0.73 \pm 0.08	0.69 \pm 0.07	0.62 \pm 0.14	0.00 \pm 0.00	0.00 \pm 0.00	0.44
Th	0.07 \pm 0.01	0.07 \pm 0.01	0.05 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00	0.42
Ti	0.02 \pm 0.00	0.01 \pm 0.00	0.02 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.81
Zn	0.53 \pm 0.08	0.52 \pm 0.02	0.52 \pm 0.09	1.56 \pm 0.21	1.56 \pm 0.30	0.00*

*Statistical significant levels were considered at $P < 0.05$ (ANCOVA).

Table 4. Trace elements concentrations in the fish muscles at five fishing stations in the Caspian Sea

Elemental Variables (ppm)	Median ± SD					Z	P value
	Astara	Anzali	Kiashahr	Sari	Gorgan		
Al	7.20 ± 0.38	4.20 ± 0.56	6.20 ± 1.39	2.61 ± 1.29	2.61 ± 1.29	9.86	0.04*
As	0.04 ± 0.01	0.07 ± 0.00	0.05 ± 0.01	0.20 ± 0.06	0.20 ± 0.06	10.99	0.03*
Ca	130.70 ± 35.64	130.50 ± 17.25	140.30 ± 25.65	429.05 ± 151.39	429.05 ± 151.39	5.91	0.21
Cr	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	8.79	0.07
Cu	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.49 ± 0.45	0.49 ± 0.45	8.66	0.07
Fe	0.80 ± 0.13	0.67 ± 0.22	0.70 ± 0.20	1.73 ± 0.25	1.73 ± 0.25	8.55	0.07
K	212.40 ± 23.13	295.00 ± 42.34	250.90 ± 80.01	457.10 ± 10.04	457.10 ± 10.05	6.79	0.15
Mg	17.70 ± 1.65	21.80 ± 2.87	23.30 ± 2.43	55.95 ± 18.31	55.95 ± 18.31	9.93	0.04*
Na	35.20 ± 5.74	28.30 ± 2.52	42.90 ± 8.71	85.40 ± 21.92	85.40 ± 21.92	10.29	0.04*
Ni	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	8.14	0.09
P	175.40 ± 35.58	214.50 ± 14.87	238.40 ± 25.65	648.95 ± 222.53	648.95 ± 222.53	9.36	0.05
S	84.48 ± 8.29	87.85 ± 3.58	113.13 ± 7.68	373.05 ± 119.29	373.05 ± 119.29	10.55	0.03*
Sb	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.08 ± 0.01	0.08 ± 0.01	9.09	0.06
Si	0.39 ± 0.06	0.42 ± 0.06	0.23 ± 0.07	0.45 ± 0.03	0.45 ± 0.03	7.95	0.09
Sn	0.04 ± 0.03	0.06 ± 0.01	0.12 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	9.24	0.06
Sr	0.29 ± 0.07	0.37 ± 0.02	0.40 ± 0.13	2.96 ± 1.17	2.96 ± 1.17	8.15	0.09

*Statistical significant levels were considered at $P < 0.05$ (Kruskal-Wallis test).

was higher in Astara than other areas.

Since Ni, Cu, Pb, and Zn are important elements for human health, EDI for consumers (adults and children) in all fishing areas were calculated, indicating that consumption of *R. kutum* poses no health risk to consumers (Table 5) (17).

The maximum safe daily consumption rate (CRLim) is reported in Table 6. The CRLim was calculated for only three metals since there was no recommended reference dose (RFD) for Pb (20).

Discussion

Numerous reports have been published on the TEs

contamination in aquatic habitats of Iran, some of which have been conducted on the TE concentrations in *R. kutum* muscles caught from the Southern parts of Caspian Sea. Few elements (Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb, and Zn) have been measured in these reports mostly using atomic absorption spectrophotometry.

According to some studies, fish species may differ considering the TEs concentrations in tissues, which is probably due to diet, metabolic activities, growth rates, feeding habits and habitats (21). It was claimed that species with higher exposure to the TEs-enriched sediments and their interactions with benthic animals lead to the accumulation of higher concentrations of TEs (22).

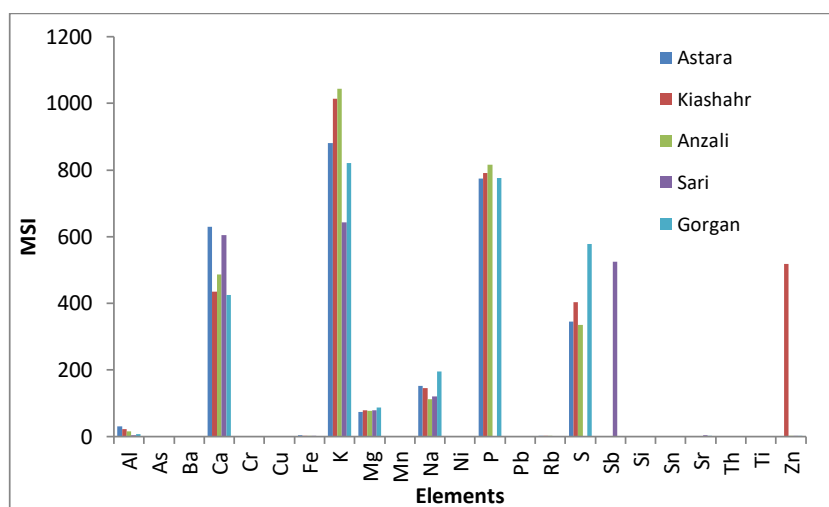


Figure 2. Metal selectivity index (MSI) in the muscles of *Rutilus kutum* at five fishing stations in the South Caspian Sea.

Table 5. EDI in adults (A) and children (C) for four TEs found in *Rutilus kutum* muscles at different fishing stations in the south Caspian Sea

TI	Metals	Astara		Anzali		Kiashahr		Gorgan		Sari	
		EDI A	EDI C	EDI A	EDI C	EDI A	EDI C	EDI A	EDI C	EDI A	EDI C
500	Cu	0.13	0.29	0.12	0.26	0.11	0.24	1.57	3.46	1.64	3.60
-	Ni	0.05	0.12	0.00	0.00	0.03	0.07	0.00	0.00	0.13	0.42
3.75	Pb	0.22	0.48	0.11	0.24	0.09	0.19	0.00	0.00	0.00	0.00
300-1000	Zn	1.73	3.80	1.69	3.71	1.68	3.68	5.06	11.12	4.17	13.51

Table 6. CR_{lim} in adults (A) and children (C) for three TEs found in *Rutilus kutum* muscles at different fishing stations in the south Caspian Sea

Metals	Astara		Anzali		Kiashahr		Gorgan		Sari	
	CR _{lim} .A	CR _{lim} .C	CR _{lim} .A	CR _{lim} .C	CR _{lim} .A	CR _{lim} .C	CR _{lim} .A	CR _{lim} .C	CR _{lim} .A	CR _{lim} .C
Cu	7.00	1.600	76.36	17.45	84.00	19.20	5.77	1.32	5.54	0.51
Ni	8.4	1.9	0.00	0.00	140.00	32.00	0.00	0.00	35.00	0.04
Zn	3.9	0.9	40.38	9.23	40.65	9.29	13.46	3.08	16.34	1.29

Krishnamurti and Nair reported the maximum concentrations of metals (Cu, Cd, Ni, Pb, and Zn) in benthic feeder's species compared to planktivores and pelagic carnivores (23) (Table 7).

Various biological and environmental parameters, food sources, and seasonal alterations affect the TEs bioaccumulation (29). Anan et al (34) reported that the metabolic rate and also dilution of TEs during growth period can be responsible for the aforementioned relationship. So that, smaller fish maintain higher levels of metabolic rate and accumulate TEs concentrations via consumption of food and water much faster than larger ones.

The effects of the trace elements on the body

As is a highly toxic agent for human. Single high doses of about 0.6 mg/kg/d or higher, which are consumed orally, have led to death. Acute oral exposure to lower doses of arsenic has affected gastrointestinal tract (vomiting, nausea, and diarrhea), nervous system (delirium, headaches, lethargy, and weakness), cardiovascular system (hypotension, sinus tachycardia, and shock), as well as liver, kidney, and blood (anemia and leukopenia). The oral LD₅₀ amounts for arsenic are 15-112 mg/kg (39,40). The As concentrations were reported by some researchers (29,32) (Table 7). The As concentrations in the present study were 0.04-0.2 mg kg⁻¹ DW in the Caspian kutum muscles, which are higher than the FAO/WHO permitted levels (amended in 2018) (41).

Pb is detectable in all phases of the inert habitats and in all biological ecosystems (37). The FAO/WHO suggested the provisional tolerable weekly intake (PTWI) of 0.025 mg/kg body weight per week for Pb, while the EU suggested the maximum permitted Pb levels in seafood as 0.2 mg kg⁻¹ FW.

The Pb concentrations in the fish muscles have been reported by many researchers (30,37,38) (Table 7). The Pb concentrations in the present study was 0.07 ± 0.02 mg

kg⁻¹ in the Caspian kutum muscles, which are lower than the FAO/WHO permitted levels (amended in 2018) (41). Cu is essential for human health. However, very high intake of this element can lead to adverse health problems including liver and kidney damages (28). The FAO and WHO Expert Committee on Food Additives have recommended the PTWI of 3.5 mg/kg body weight for Cu. The mean concentrations of Cu were reported by few researchers (26,29,31) (Table 7). The concentrations of Cu in the present study ranged from 1.00 to 2.72 mg kg⁻¹ in the *R. kutum* muscles, which are lower than the FAO/WHO permitted levels (amended in 2018).

Cr is a necessary TE for metabolism. The proposed daily intake for Cr is 50-200 µg (47). The dietary level of Cr is very essential for insulin function and lipid metabolism (43).

There are some reports on the mean Cr levels (25,29,34) (Table 7). The concentrations of Cr in the present study were 0.04-0.17 mg kg⁻¹ in the fish muscles, which are lower than the FAO/WHO permitted levels (amended in 2018). Cobalt (Co): Some studies investigated the mean Co levels in different fish species (29,32). In the present study, cobalt was not found in the *R. kutum* muscles.

Iron (Fe): The US National Academy of Science (NAS) suggests the permitted daily intake of 10 mg/day for Fe in the elderly (43).

The mean concentrations of Fe in muscles of different species were reported (24,29,30) (Table 7). The concentration of Fe in the present study ranged from 7.85 to 24.72 mg kg⁻¹ in *R. kutum* muscles.

Mn is an important TE for organisms. Mn deficiency is very critical and causes severe birth defects, convulsions, asthma and so on (44). Some studies have reported the Mn levels (29,32,34) (Table 7). The Mn levels in the present study ranged from 0.18 to 1.27 mg kg⁻¹ in *R. kutum* muscles, which are lower than the FAO/WHO permitted levels (amended in 2018).

Nickle (Ni): The World Health Organization (WHO) (45)

Table 7. Comparison of the TEs concentrations in muscles of *R. kutum* studied in this study with those in other fish species investigated in other studies

Fish Species	As	Cu	Pb	Mn	Ni	Fe	Zn	Reference
<i>Alosa pontica</i>	0.38	-	0.34	-	-	-	-	24
<i>Rutilus caspicus</i>	0.25	0.69	-	-	-	5.38	7.15	21
<i>Neogobius gorlap</i>	-	4.48	0.43	-	-	501.7	22.58	(25-27)
<i>Cyprinus carpio</i>	-	-	1.7	-	-	380.7	125.6	(27,28)
<i>Liza saliens</i>	0.07	1.3	8.6	-	-	914.6	-	(27,29,30)
<i>L. abu</i>	-	3.22	1.40	-	-	-	12.75	(26)
<i>Barbus grypus</i>	-	4.48	-	-	-	-	15.32	(26)
<i>Esox lucius</i>	-	0.21	0.004	-	-	-	2.55	(31)
<i>Sander lucioperca</i>	-	9.3	0.53	0.77	1.16	455.6	23.3	(27,29)
<i>Silurus glanis</i>	-	-	0.49	-	04	7.12	22.35	(32)
<i>Alosa caspia</i>	-	-	0.34	-	-	1118	-	(27)
<i>Clupeonella engrauliformis</i>	-	-	0.015	-	-	-	-	(33)
<i>C. grimmi</i>	-	-	0.014	-	-	-	-	(33)
<i>C. cultriventris</i>	-	-	0.016	-	-	-	-	(26)
<i>Alburnus chalcoides</i>	-	1.46	-	3.2	0.42	80.9	38.5	(29)
<i>R. kutum</i>	-	1.01	-	0.45	-	-	17.2	(34)
<i>R. kutum</i>	-	2.7	-	-	-	-	22.3	(15)
<i>R. kutum</i>	-	-	-	-	-	-	15.4	(35)
<i>R. kutum</i>	-	-	-	-	0.52	-	-	(36)
<i>R. kutum</i>	-	1.68	-	0.78	2.65	5.6	0.63	(37)
<i>R. kutum</i>	-	0.013	-	-	-	0.08	0.4	(30)
<i>R. kutum</i>	-	-	0.22	2.6	0.81	140.1	26.8	(29)
<i>R. kutum</i>	0.04	1.0	0.0	018	0.02	7.85	1.74	Present study
Permitted by the FAO/WHO	0.1	10	0.5	0.5	-	-	100	(38)

recommended permitted daily intake of 100-300 µg for Ni. Bulgarian Food Codex suggested the maximum permitted level of 0.5 mg/kg for Ni in fish (46-4). Some reports have been published on the Ni levels in muscles of some fish species (15,29,31,34) (Table 7). In the present study, the Ni levels in the Caspian kutum muscles were 0.02-0.04 mg kg⁻¹, which are lower than the FAO/WHO permitted levels (amended in 2018).

Vanadium (V): There is only one report on the vanadium levels in the muscles of fish species in Iran (26) (Table 7). In the present study, vanadium was not found in the fish muscles.

Zinc (Zn): The Expert Committee on Food Additives (FAO/WHO, 2004) recommended the PTWI of 7 mg/kg body weight for Zn. Some studies have reported the mean levels of Zn in muscles of some fish species (26,27,29,31,37) (Table 7). The Zn concentrations in the present study were 1.74-3.86 mg kg⁻¹ in the fish muscles, which are lower than the FAO/WHO permitted levels (amended in 2018).

Conclusion

In this study, the mean concentrations of TEs in the fish muscles were lower than the TEs tolerable daily/weekly/

monthly intake, allowed by the US Environmental Protection Agency (USEPA) and the FAO/WHO. The TEs concentrations in *R. kutum* muscles were less than those reported by the previous studies. According to the results, these concentrations cannot pose a threat to human health. So, it is recommended to monitor TEs concentrations in aquatics and in the Caspian Sea to find out the TEs trends over time.

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Ethical issues

The authors hereby certify that all data collected during the research are as expressed in the manuscript, and no data from the study has been or will be published elsewhere separately.

Competing interests

The authors declare that they have no conflict of interests.

Authors' contributions

All authors contributed in data collection, analysis, and interpretation. All authors reviewed, refined, and approved the manuscript.

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