Research Paper

Evaluation of Trace Element Contaminations in the Skin Tissue of *Rutilus Kutum* (Kamensky, 1901) From the South of the Caspian Sea

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ABSTRACT

Background: The Caspian Sea is surrounded by Kazakhstan, Azerbaijan, Iran, Turkmenistan, and Russia. *Rutilus kutum* is a highly steamed fish species caught from the South Caspian Sea, but little data available on Trace Element (TE) concentrations in its skin.

Methods: We caught 51 R. kutum from three stations (i.e., Anzali, Astara, and Kiashahr) in the southern shoreline of the Caspian Sea from September 2017 to January 2018, and ICP-OES was used to assay TE concentrations in their skin.

Results: The Trace Element Concentrations (TECs) measured in the skin (except for potassium) did not display significant differences between the stations and only the concentration of potassium was higher in Kiashahr than in other areas.

Conclusion: TE levels in the *R. kutum* skin were lower than those in the previous reports from the Caspian Sea, which could not result in any risk to human health.

1. Introduction

The use of Trace Elements (TEs), primarily iron (Fe), zinc (Zn), copper (Cu), lead (Pb), nickel (Ni), and manganese (Mn), is increasing due to rapid industrialization, and the resulting contamination of the aquatic environment has become a global concern [1-3]. The accumulation of TEs is hazardous not only to fish growth and reproduction but also for humans. Due to increased industrial practice, heavy metals enter the water resources and are transferred to humans through the food chain. Minerals play important roles in osmoregulation, intermediary metabolism, and formation of the skeleton, healthy scales, teeth, and bones. Contamination with a wide range of pollutants and heavy metals, in particular, has become a serious problem threatening this important food source [4]. Heavy metals enter the living organisms through ingestion, breathing, and skin contact and are deposited in various organs, like

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muscles and bones resulting in intoxications by cumulative effect. Depending on environmental contamination, fish may contain small or large quantities of heavy metals. Accumulation depends on the species, fish age, sex, weight, and position in the food chain. A predator fish will be contaminated in a "pyramid". The Caspian Sea is a rich source of various aquatics, including crustaceans and fishes. However, it is very prone to climate changes and anthropogenic interventions [5]. The Caspian Sea provides almost the whole seafood of the population in the northern part of Iran [6-9].

Rutilus kutum (Kamensky, 1901) belonging to the family Cyprinidae is a highly steamed fish in the Caspian Sea [10]. The people around the South Caspian Sea consume its skin as well as flesh and gonads [11, 12]. The skin is a large and extensive organ that protects the body against environmental stressors and pathogens that can inhibit and interfere with the physiological functions of internal organs [13]. Although depending on the fish species, a very wide morphological and functional diversity has been declared regarding the details of the skin and its structural composition, its general structure is almost the same in different types of fish and consists of two main layers. The epidermis covers the outer surface of the fish bodies and the inner layer includes the dermis and hypodermis [14]. The skin of fish is the first line of body defense against environmental pollutants or pathogens. Mucus and scales of the fish are chemical and physical barriers to the skin [15]. However, pollutants can enter the body through the skin (i.e. heavy metals, pesticides, and pathogens). Evaluation of the skin of fish can provide better knowledge about fish health and pollutant effects than any single biochemical parameter. Histopathological changes or damages can be directly caused by environmental stressor parameters and their products (such as pathogens infections, and toxic compounds). Moreover, histopathological biomarkers can affect biotic factors, water quality, and fish health status. Finally, they can be reliable biomarkers for environmental stress [16]. However, few reports are available about TE concentrations in its skin tissue. In this study, TE concentrations in the skin of *R. kutum* from the southern shores of the Caspian Sea were addressed. The aim of the present study was to measure the concentrations of some TE in the skin of *R. kutum* collected from the southern shore of the Caspian Sea, compare their concentrations in different stations, and reveal the risk of these TEs for human health.

2. Materials and Methods

We conducted this study at three localities: Kiashahr: 37° 42′ 20″ N, 49° 94′ 95″ E, Astara: 38° 42′ 25″ N, 48° 86′ 87″ E, and Anzali: 37° 46′ 39″ N, 49° 47′ 99″ E, around the west coasts of the Caspian Sea (Figure 1).

R. kutum (N=51) samples were collected from September 2017to January 2018. The samples were transported to the fish diseases laboratory, University of Guilan, Sowmehsara, Iran, using a Styrofoam box with ice at 4 °C [17]. We washed fish using distilled water [18]. After age determination (Table 1) and dissecting fish, skin samples were placed in an oven (80 °C for 18 h) to dry. In total, 0.5 g of skin was digested with nitric acid (10 mL, 65%) in an oven, followed by passing it through the Whatman paper (No. 40) and diluting it by distilled water to reach the exact volume. We employed an inductively coupled plasma-optical emission spectrometry (ICP-OES; Zarazma, Tehran, Iran) for measuring TE levels in the samples. Instrument detection limits for most metals were 0.02 mg/kg^{-1}, except for Al, Ca, K, Mg, Fe, Mn, Na, and Si (0.1 mg/kg^{-1}). The

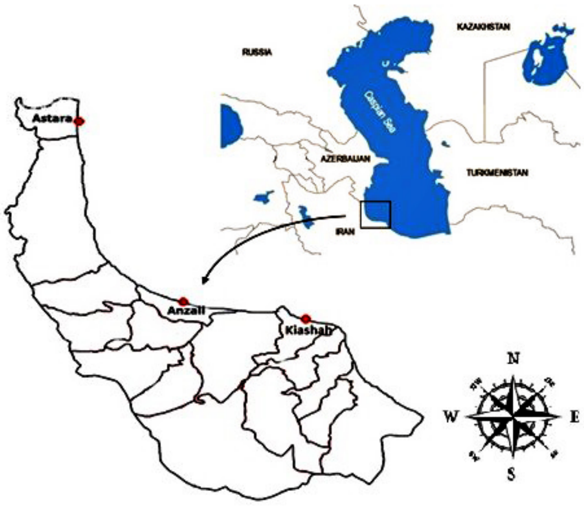

Figure 1. The locality of fish sampling stations in the South Caspian Sea

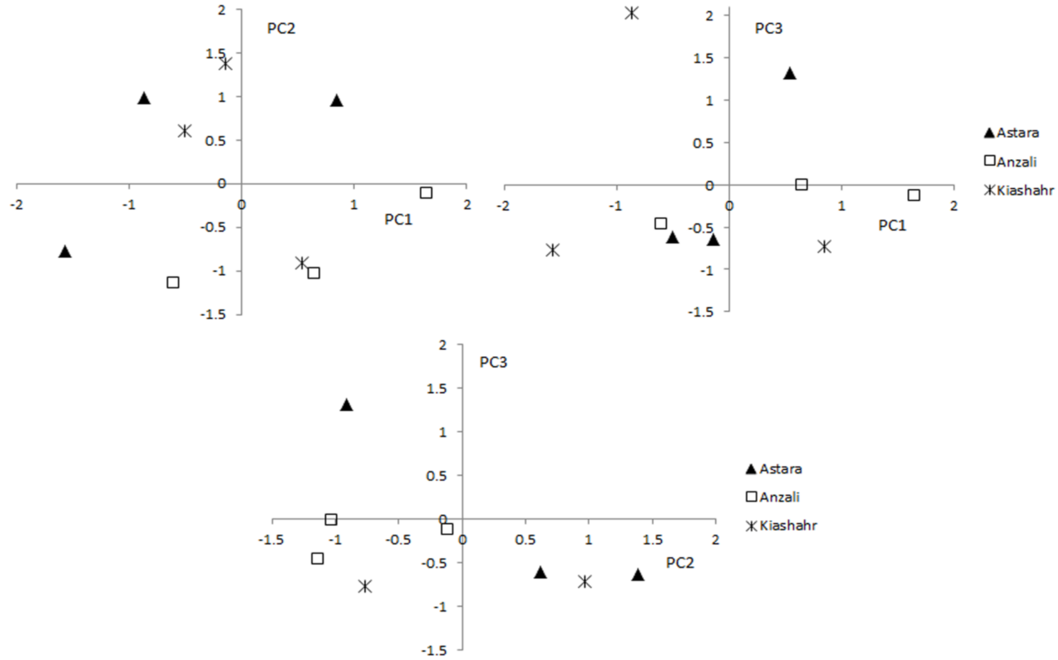

Figure 2. Principal component Analysis (PCA) of the concentrations of trace elements in the fish skin in different stations of the southern coasts of the Caspian Sea. The scatter plots show individual fish scores for PC1 vs. PC2 (A), PC1 vs. PC3 (B), and PC2 vs. PC3 (C), which explain the total variance of 67.03%.

reported element concentrations were compared with the Maximum allowed concentration as ppm (parts per million) presented by Codex Alimentarius Commission [17], US Food and Drug Administration (Table 2).

Statistical analysis

The TEs were examined statistically except for Li, which was not detected by ICP-ES. After examining the normality of the data and homogeneity of variances, one-way ANOVA was employed to assess differences in TE concentrations, followed by Tukey's post-hoc test to reveal the sources of differences. In cases where normality of variances was not met, the Kruskal-Wallis test was performed. We used Principal Component Analysis (PCA) for reducing the number of variables, by preserving the data.

We provided Eigenvalues against the principle component numbers as well as the cumulative variance values to determine the important principle components

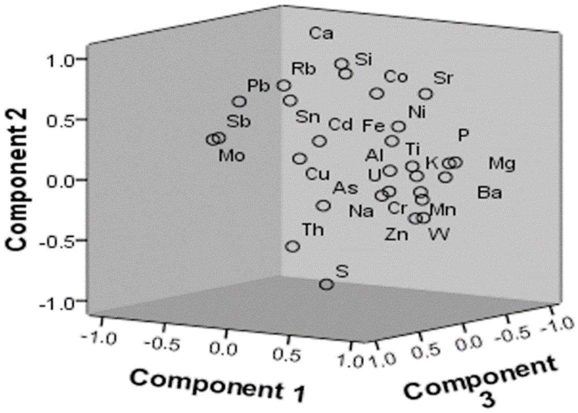

Figure 3. Loaded characteristics for PC1, PC2, and PC3 resulting from principal component analysis (PCA) of multi-elemental analyses of the fish skin in different stations of the southern coasts of the Caspian Sea

Table 1. Morphometric characteristics of *Rutilus kutum* from the three studied areas of the South Caspian Sea

Values	Total Weight (g)	Total Length (cm)	Fork Length (cm)	Standard Length (cm)	Head Length (cm)	Snout Length (cm)	Eye Diameter (cm)	Body Height (cm)	Body Thickness (cm)
Mean	1531.30	82.41	75.74	41.79	14.40	4.47	2.27	15.61	7.96
Max	35220.00	1895.50	59.00	52.10	18.10	10.28	5.10	19.10	9.00
Min	435.00	37.50	33.50	31.50	5.70	1.50	0.90	6.50	4.00
SD	195.68	3.28	2.98	2.82	0.63	0.32	0.13	0.96	0.54

and elements. Within-group linkage was employed for drawing cluster dendrogram using Euclidian distance (according to mean linkage procedure). It was also used as a complementary method to carry out the diagnostic analysis. All statistical analyses were done by SPSS. The significant level was considered as α=0.05.

3. Results and Discussion

In the present study, a total of 51 skin specimens of *R. kutum* were examined and 26 out of 36 TEs were detected, including arsenic (As), calcium (Ca), cadmium (Cd), aluminum (Al), chromium (Cr), Cu, Fe, magnesium (Mg), manganese, potassium (K), sodium (Na), phosphorus (P), Ni, rubidium (Rb), sulfur (S), cobalt (Co), antimony (Sb), Pb, silicon (Si), strontium (Sr), thorium (Th), titanium (Ti), tin (Sn), thallium (Tl), zinc (Zn), and tungsten (W) (Tables 3 and 4).

Based on Tables 3 and 4, the TE concentrations assayed in the skin (except for K) did not display significant differences among the three studied areas. Only the concentration of K was higher in the Kiashahr station than in Astara and Anzali.

Table 2. Maximum allowed concentration as ppm (parts per million)

Elements	Maximum Permitted Concentration in Parts per Million (ppm)
Lead	0.5
Cadmium	2
Mercury	0.5
Arsenic	0.1
Chromium	1
Aluminum	100
Tin	230
Antimony	1
Copper	10
Manganese	0.5
Zinc	100
Selenium	1

Table 3. Elemental concentrations of the fish skin in three different stations in the Caspian Sea, Iran

Elemental Variables (ppm)	Mean±SD			Z	P
	Astara	Anzali	Kiashahr		
Cr	0.04±0.01	0.05±0.001	0.04±0.02	1.54	0.46
Mo	0.01±0.01	0.001±0.001	0.001±0.07	2.13	0.35
Ni	0.01±0.001	0.01±0.001	0.01±0.02	0.00	1.00
Rb	0.74±0.16	0.53±0.09	0.87±0.02	5.65	0.06
S	78.25±33.28	112.76±15.82	79.46±4.91	2.49	0.29
Sb	0.043±0.01	0.02±0.02	0.001±0.08	0.70	0.70
Sn	0.05±0.06	0.03±0.01	0.03±0.03	2.95	0.23

A P<0.05 is regarded as significant (Kruskal-Wallis test).

The variability of TE concentrations in different areas by PCA was related to four components (PC1=32.74%, PC2=20.20%, PC3=14.09%, and PC4=10.96%) (Figures 2 and 3) and it was found that 77.99% of the total variability was related to TEs, such as Mg, Mn, Ti, W, Zn, Ca, Mo, and Cd (Table 5).

The three-dimensional diagrams displayed the weight of all components in PCA. The first component was mainly influenced by Mg, Mn, Ti, W, and Zn (Table 5). Ca and Mo had special values in PC2 and PC3, respectively, while the highest values in PC4 belonged to Al (Table 5).

Drawing dendrogram (Figure 4) grouped the Astara, Kiashahr, and Anzali stations into two main subgroups. Although there were no differences between Astara and Kiashahr stations, Anzali was different from these two stations and considered in a distinct group (Figure 4).

Among the studied elements, the important elements mentioned in other studies, including Al, As, Cu, Cr, Mn, Mg, Pb, Ni, Sn, and Zn were compared. As shown in Figure 5, the highest levels of As, Al, Cr, Mg, Mn, and Zn were observed in Anzali. The highest amount of Cu, Ni, and Sn was reported in Astara, while the high-

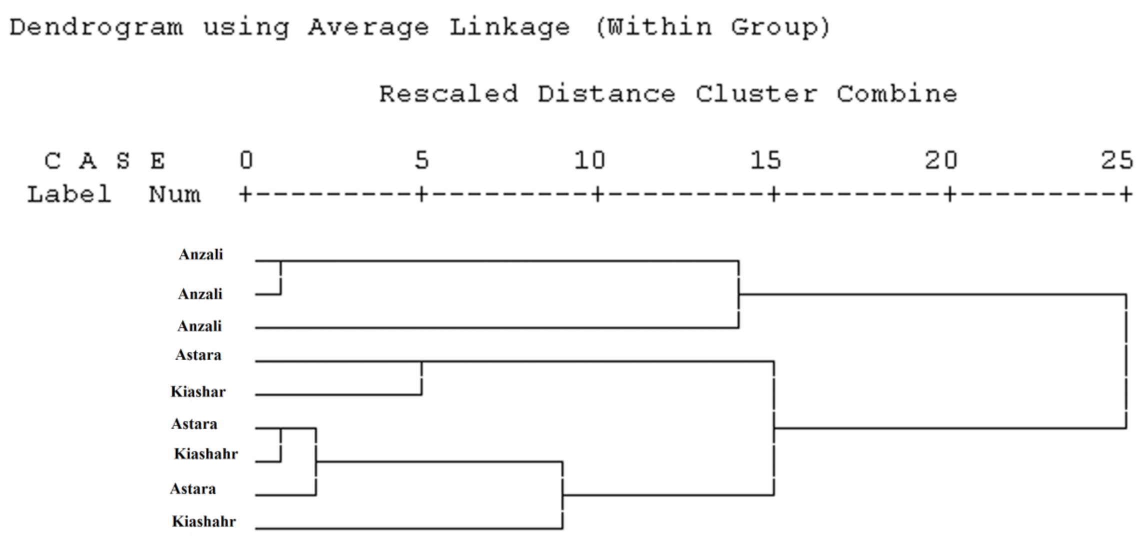

Figure 4. Dendrogram according to cluster analysis of elemental concentrations in the fish skin in different stations of the southern coasts of the Caspian Sea.

Table 4. Elemental concentrations of the skin in three different stations in the Caspian Sea, Iran

Elements (ppm)	Mean±SD			P
	Astara	Anzali	Kiashahr	
Al	11.97±1.50	12.07±3.09	8.80±2.47	0.59
As	0.05±0.001	0.07±0.01	0.06±0.01	0.10
Ca	181.83±35.54	94.97±13.45	148.23±16.62	0.11
Cd	0.01±0.001	0.001±0.001	0.001±0.001	1.00
Co	0.01±0.001	0.01±0.001	0.01±0.001	1.00
Cu	0.05±0.01	0.04±0.01	0.04±0.01	0.84
Fe	1.38±0.27	1.22±0.32	1.19±0.37	0.91
K	108.43±11.25a	104.87±3.02a	68.47±6.72b	0.02*
Mg	11.80±0.97	16.33±4.33	9.68±3.17	0.37
Mn	0.90±0.40	1.20±0.61	0.60±0.36	0.68
Na	28.70±4.09	30.10±2.78	25.00±3.30	0.59
P	126.23±21.86	247.73±84.44	135.57±63.69	0.37
Pb	0.05±0.01	0.03±0.01	0.07±0.02	0.39
Si	0.39±0.09	0.17±0.01	0.35±0.09	0.18
Sr	0.42±0.13	0.41±0.09	0.33±0.13	0.85
Th	0.08±0.01	0.08±0.01	0.07±0.01	0.77
Ti	0.02±0.01	0.01±0.001	0.03±0.01	0.82
W	0.001±0.001	0.01±0.001	0.001±0.001	0.73
Zn	0.87±0.32	1.24±0.36	0.72±0.28	0.54

*Significant (ANOVA).

est amount of Pb was determined in Kiashahr. Based on these results, Anzali was the most polluted area in terms of observed elements. Anzali port is in a better commercial and fishing position than the other two areas. The presence of shipping and fishing industries in Anzali has increased pollution in this area.

In northern Iran, fish skin is also consumed by humans, therefore, it is very important to investigate its contamination with heavy metals. Especially, due to the industrialization of the port and the existence of shipping and factories in these areas, and on the other hand, due to the high number of tourists visiting these areas, the existence of pollution has become inevitable.

Few studies have been done on TE levels in *R. kutum* caught from the South Caspian Sea [19-23]. Few elements have been assayed in these published data (such as Cd, Co, Fe, Pb, Cu, Cr, Mn, Ni, and Zn) mostly using other instruments, such as atomic absorption spectrophotometry. Thus, in the present study, we examined skin samples of 51 *R. kutum* specimens for 36 TEs by ICP-OES. We compared TE concentrations in different fishing stations, including Anzali, Astara, and Kiashahr located in the southwestern part of the Caspian Sea. The

Table 5. Loaded characteristics for PC1, PC2, PC3, and PC4 resulting from PCA for elemental concentrations of the fish skin in three different stations of the Caspian Sea, Iran

Elements	PC1	PC2	PC3	PC4
Al	0.832	0.174	0.433	0.218
As	0.078	-0.236	0.119	-0.428
Ba	0.715	-0.007	-0.370	-0.225
Ca	-0.029	0.874	-0.242	0.387
Cd	-0.026	0.272	0.016	0.899
Co	0.409	0.705	-0.021	-0.152
Cr	0.779	-0.071	0.005	-0.275
Cu	0.360	0.278	0.794	0.232
Fe	0.790	0.403	0.349	0.135
K	0.525	-0.009	-0.314	0.623
Mg	0.877	0.149	-0.252	-0.108
Mn	0.894	-0.100	0.151	0.003
Mo	-0.330	0.367	0.802	-0.294
Na	0.115	-0.239	-0.500	0.431
Ni	0.522	0.430	-0.109	-0.363
P	0.816	0.131	-0.270	-0.325
Pb	-0.323	0.640	0.511	-0.050
Rb	-0.433	0.666	-0.156	-0.357
S	0.198	-0.856	0.256	0.167
Sb	-0.356	0.364	0.700	-0.195
Si	-0.049	0.780	-0.315	0.060
Sn	-0.145	0.620	0.182	0.508
Sr	0.551	0.664	-0.378	-0.031
Th	0.172	-0.494	0.607	0.245
Ti	0.845	0.177	0.194	-0.297
W	0.874	-0.258	0.108	-0.079
Zn	0.854	-0.256	0.171	0.056

PCA: Principal Component Analysis.

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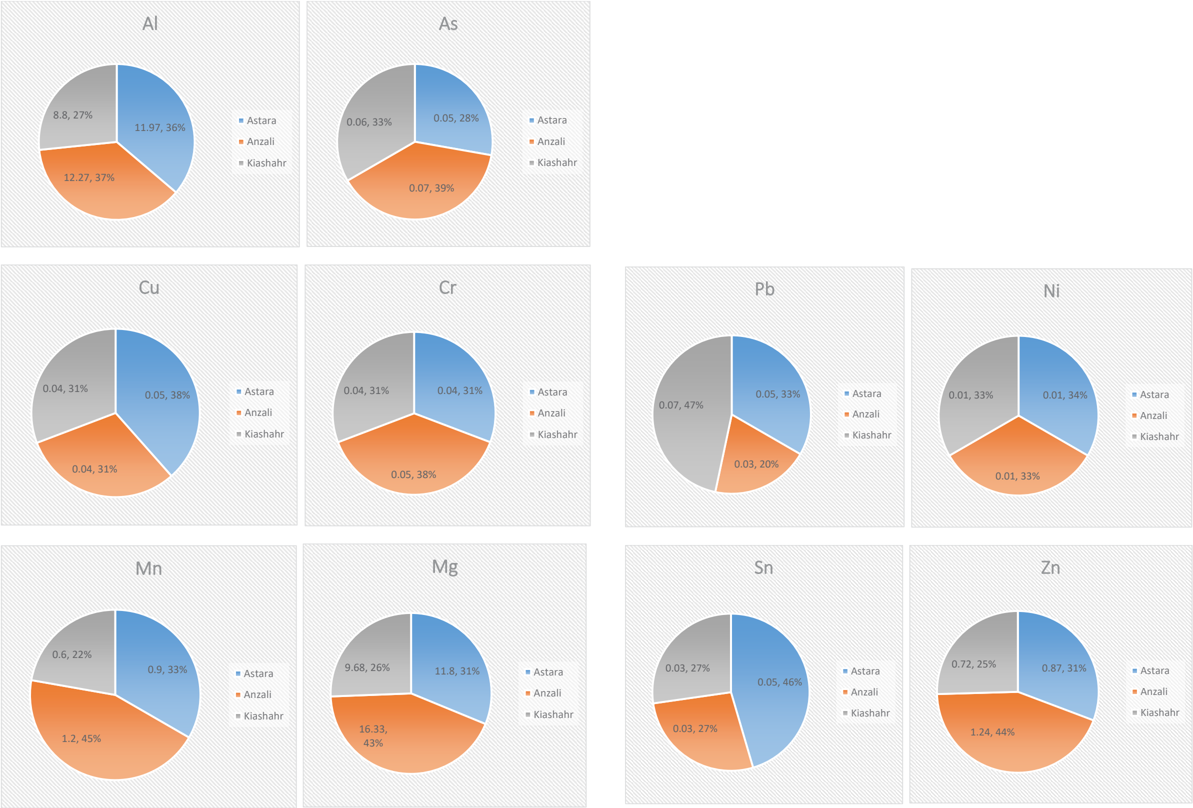

Figure 5. Comparison of the elements in sampling areas

potentials of the observed ECs to pose human health risks were also examined. The TE measured in the skin (except for K) did not show significant differences between the three studied areas; however, the concentration of K was significantly higher in Kiashahr than in Astara and Anzali sampling stations. The statistical analysis of the data revealed that the differences in the skin chemistry make it possible to separate fish species collected from the three studied sites.

Some authors have suggested that fish species may differ based on TE levels in their tissues probably due to their metabolism, activities, discrete growth rates, diet, feeding behaviors, and habitats [24-26]. The species with higher exposure to TEs in sediments and also their interactions with benthic invertebrates lead to bioaccumulation of higher TE levels in their tissues [27]. The highest levels of metals (Cu, Zn, Cd, Pb, and Ni) were observed in benthic feeders than in pelagic carnivores and planktivores [28]. Various environmental and biological factors, seasonal changes, and food availability affect TE bioaccumulation [23]. Anan et al. [19] suggested that the metabolic rate and dilution of TEs during growth may be responsible for this relationship. Thus, smaller fishes exhibit higher metabolic rates and as a result, accumulation of TEs by consuming food and water faster than larger fishes. The Cd, Cu, and Zn levels in muscles of common carp (C. carpio) and also goldfish (Carassius auratus,) as benthic fishes were reported to be higher than pelagic ones, such as *Hypophthalmichthys nobilis* and *H. molitrix* [29]. Roméo et al. [30] suggested that Cd, Cu, Mg, and Zn concentrations in the edible parts of pelagic fish species are lower than those of benthic species. Because skin, muscle, and gonad are consumed by people around the southern coasts of the Caspian Sea; thus, these edible parts for humans should be treated with utmost concern.

4. Conclusion

The mean TE levels in gonads in the present study were lower than those suggested by the United States Environmental Protection Agency (USEPA) and also by the World Health Organization (WHO)/ Food and Agriculture Organization (FAO). TE levels in the *R. kutum* skin were lower than those in the previous reports from the Caspian Sea, which could not result in any risk to human health. Thus, we suggest that the TEs should be monitored in other aquatics in the Caspian Sea to determine the TE level trends in the future.

Ethical Considerations

Compliance with ethical guidelines

The study protocol was approved by the Ethical Committee of the University of Guilan.

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Authors' contributions

Investigation, validation, and methodology: Mohammad Forouhar Vajargah; Supervision and conceptualization: Masoud Sattari; Visualization and data analysis: Javid Imanpour Namin; Writing and data analyses: Mehdi Bibak.

Conflict of interest

The authors declare that they have no conflict of interest.

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