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Research Article

Element Accumulations in Liver and Kidney Tissues of Some Bony Fish Species in the Southwest Caspian Sea

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Abstract

The Caspian Sea is the largest inland body of water in the world and so has both common characteristics of seas and lakes with over 153 fish species which inhabit the sea and its basin. However, little is known about the trace element (TE) contaminations (TECs) in its tissues. In the present study, 122 specimens of three fish species including *Rutilus caspius* (Roach, n=71), *Leuciscus aspius* (Asp, n=20), and *Tinca tinca* (Tench, n=31) were collected from three different fisheries regions (i.e. Astara, Anzali and Kiashahr) of the southern part of the Caspian Sea from September 2017 to June 2018. Inductively coupled plasma optical emission spectrometry (ICP-OES) was employed to measure TE levels in different fish tissues. An attempt was made to assess possible influences of habitat on element accumulation in the liver and kidney of three fish species in the southwest of the Caspian Sea basin. Some elements including Ca, K, Mg, P, S, Sc, and Sr showed different concentrations in the liver and kidney. Also their levels were significantly different between freshwater resident (Tench) and marine (Roach) species ($p < 0.05$). The differences among TECs in the liver and kidney of Roach, Asp and Tench were reduced to three components using principal component analysis (PCA). Results indicated that 83.60% of the total variability is related to TEs such as Cu, Fe, Sr, Ca, S, Na, Mg, K, and Al. The impact of habitat variability on the element accumulation was confirmed through linear chart obtained for liver and kidney (as body filtering organs) of Roach and Asp as marine residents as well as Tench as a freshwater resident. This could illustrate the borderline created by these habitats.

Keywords: *Rutilus caspius*, *Leuciscus aspius*, *Tinca tinca*, Trace elements, Caspian Sea

Introduction

The trace element (TE) pollution in water resources has long been found to be a serious environmental concern (Pagano et al., 2017; Capillo et al., 2018; Chorehi et al., 2013). Aquatic organisms can accumulate TEs in their bodies via respiration, adsorption and ingestion (Zhou et al., 2001; Boran et al., 2000). TE contamination is a serious problem in the coastal regions, due to waste disposal of discharges from agriculture, industries and some urban sources (Aliko et al., 2018; Burgos-Aceves et al., 2018). TE accumulation elevated in marine ecosystems as a direct result of anthropogenic activities (Seco-Gesto et al., 2007). TEs were categorized as potentially toxic (cadmium, arsenic, lead, mercury, nickel, etc.), probably essential (vanadium, cobalt) and essential (copper, selenium, iron, manganese, zinc) (Munoz-Olivas et al., 2001). Fish is considered as a suitable indicator for long term monitoring of TE contaminations in different water resources (Fazio et al., 2014; Sattari et al., 2019). Therefore, numerous studies have been conducted on TE accumulation in different fish species (Türkmen et al., 2007).

The Caspian Sea is the world's largest inland body of water and thus has characteristics common to both seas and lakes. It is bordered by Russia (Dagestan, Kalmykia, and Astrakhan oblasts), the Republic of Azerbaijan, Iran (Guilan, Mazandaran and Golestan provinces), Turkmenistan, and Kazakhstan (Vajargah et al., 2014; Sattari et al., 2019). Hence, not only it doesn’t contain fresh water, but also it is under intense pollutant threats from industrial and agricultural effluents as well as growing urbanization in the most riparian countries of the Caspian Sea (Karrari et al., 2012).

There are numerous reports regarding the heavy metal contamination in aquatic environments of Iran including: Pourang et al., (2005) on five sturgeon species in the Caspian Sea; Abtahi et al., (2005) on *Liza aurata* in the south Caspian Sea; Sadeghirad, (2007) on *Acipenser persicus* and

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Acipenser stellatus from the Caspian Sea; De Mora et al., (2004) on coastal sediments from the Caspian Sea; Amini Ranjbar and Sotudehnia, (2005) on *Mugil auratus* of the Caspian Sea; Askary Sary and Beheshti, (2012) on *Liza abu* from the Karoun and Karkheh rivers; Beheshti, (2011) on *Liza abu* collected from the Karkheh and Karoon rivers; Ebrahimzadeh et al., (2011) on *Liza saliens* collected from the Caspian Sea; Khanipour et al., (2018) on *Silurus glanis* collected from Anzali Wetland, the southwest Caspian Sea; and Alipour and Banagar, (2018) on fish obtained from Gorgan Bay, the southeast Caspian Sea. There are also some reports on TE accumulations in *R. kutum* (Shahryari et al., 2010; Eslami et al., 2014; Mirzajani et al., 2016; Sattari et al., 2019). Eslami et al., (2011) reported the existence of TEs in muscle and liver of *Perca fluviatilis* and *Tinca tinca* in Anzali Wetland; Bibak et al., (2018) worked on heavy metal levels in sediments of the northern part of the Persian Gulf. This study aimed to determine the levels of some target trace elements (TEs) in the livers and kidneys of three fish species which were collected from the geographically different coastal regions of the Caspian Sea.

Materials and Methods

This study was conducted in three fisheries regions including 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) along the south western coasts of the Caspian Sea. 122 specimens were collected from September, 2017 to January, 2018 from three different fish species including *Rutilus caspius* (Roach, n=71), *Leuciscus aspius* (Asp, n=20), and *Tinca tinca* (Tench, n=31) with gill net. The specimens were transported to the Fish Biology Laboratory, University of Guilan, Sowmeh Sara, Iran by a styrofoam cooler box at 4°C. Fish were washed using distilled water, dissected and pieces of muscle were dried in the oven (80°C for 18 h) (Vajargah et al., 2018b). Fish age was determined with scales during the process.

To extract TEs, 0.5 g of each tissue was digested in 10 ml of 65% nitric acid in a microwave oven. Then, specimens were passed through the Whatman filter paper No. 40 and were diluted in distilled water to the required volume. An inductively coupled plasma–optical emission spectrometry (ICP-OES) (Zarazma Co. Tehran, Iran) was employed to measure trace element levels in the specimens. Instrumental detection limits for trace and major (Al, Ca, Fe, K, Mg, Mn, Na and Si) element measurements were equal to 0.02 mgkg^{-1}, and 0.1 mg kg^{-1}, respectively.

Statistical analyses

After examining the normality of acquired data and homogeneity of variances in the fish tissues (liver and kidney) from different habitats, the variability of TE concentrations was investigated through one-way analysis of variances (ANOVA). For heterogeneous variables, the Kruskal-Wallis test was employed, otherwise, we used Man-Whitney U test (Zar, 1996).

Principle component analysis (PCA) was used to reduce the number of variables without losing much information (Quinn and Keouch, 2002). Eigenvalues against the number of principal components and also the values of cumulative variances were provided to define the important principle components and elements. Discriminant function analysis (DFA) was employed to calculate the exact place of each fish species which were related correctly. Ward's method, as a complementary method for DFA, was employed to construct cluster dendrogram using Euclidean distance (average linkage clustering). All statistical analyses were performed using SPSS version 16.0 (significance level α=0.05, SPSS Inc., Chicago, IL, USA).

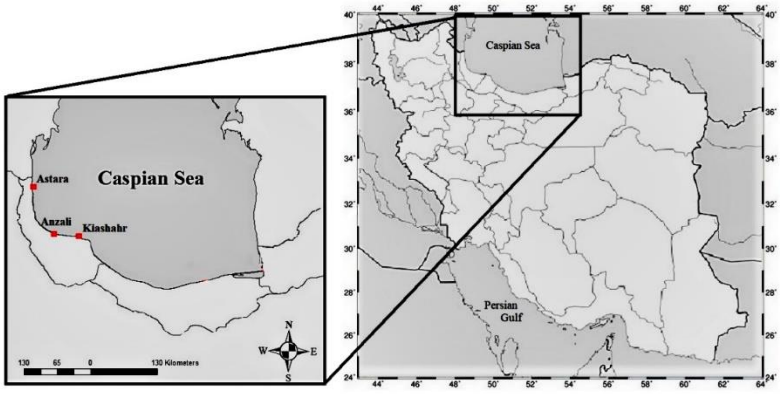

Figure 1. Map of the study area along the south western coasts of the Caspian Sea

Results

In the present study, several specimens from three different fish species were dissected and their kidney and liver tissues were examined for the presence of 36 elements including: Silver (Ag), Aluminum (Al), Arsenic (As), Barium (Ba) Beryllium (Be), Bismuth (Bi), Calcium (Ca), Cadmium (Cd), Cesium (Ce), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Potassium (K), Lanthanum (La), Lithium (Li), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Sodium (Na), Nickel (Ni), Phosphorus (P), lead (Pb), Rubidium (Rb), Sulfur (S), Antimony (Sb), Scandium (Sc), Silicon (Si), Tin (Sn), Strontium (Sr), Thorium (Th), Titanium (Ti), Uranium (U), Vanadium (V), Tungsten (W), Yttrium (Y) and Zinc (Zn).

Some elements such as Ca, K, Mg, P, S, Sc and Sr exhibited different concentrations between liver and kidney (*$p<0.05$, Table 1). Their levels were also displayed significant differences between Tench, as a freshwater resident, and Roach, as a marine species (Table 2). The variability of TECs in different edible tissues of Roach, Asp and Tench was reduced to three components using PCA (PC1= 46.32%, PC2 =22.48% and PC3=14.79%) (Figures 2-3). It was found that 83.60% of total variation is related to TEs such as Cu, Fe, Sr, Ca, S, Na, Mg, K and Al (Table 3). The three-dimensional diagram illustrated the weight of each component in PCA is shown in Figure 3 (Figure 3). The first component was mainly influenced by Na, Mg, K and S (Table 3). Ca and Sr had special contributions in PC2, while the highest values in PC3 were obtained for Cu, Fe and Al (Table 3).

Table 1. Mean of concentration±SD for different elements in the liver and kidney of the sea water fish and lagoon fish of the Iranian Caspian Sea (*$p<0.05$).

Elements	Elemental concentration (ppm; Median ± SD)			*p* value
	T. tinca	***R. caspius***	***L. aspius***	
Al	019 ± 0.20	0.22 ± 0.11	0.05 ± 0.05	0.46
Ca	9.06 ± 0.38	25.84 ± 9.73	97.30 ± 73.30	0.02
Cr	0.00 ± 0.00	0.02 ± 0.01	0.09 ± 0.01	0.12
Cu	0.07 ± 0.05	0.17 ± 0.12	0.00 ± 0.00	0.36
Fe	1.59 ± 0.59	4.82 ± 1.92	0.63 ± 0.25	0.17
K	38.33 ± 12.97	114.62 ± 8.01	115.30 ± 67.60	0.03
Mg	4.03 ± 0.81	15.06 ± 2.33	17.05 ± 5.25	0.02
Na	20.57 ± 5.78	56.16 ± 6.25	48.50 ± 19.70	0.58
P	36.27 ± 11.60	117.00 ± 13.94	220.60 ± 119.9	0.02
S	29.73 ± 10.18	99.18 ± 8.56	92.70 ± 52.00	0.03
Sb	0.08 ± 0.08	0.04 ± 0.02	0.09 ± 0.09	0.59
Si	0.00 ± 0.00	0.30 ± 0.06	0.26 ± 0.06	0.02
Sr	0.05 ± 0.02	0.26 ± 0.12	0.72 ± 0.63	0.02
Zn	0.70 ± 0.12	2.08 ± 0.28	1.20 ± 0.30	0.08

Table 2. Pair-wise comparisons (Mann–Whitney U-test) of the significant elements among the fish belongs to sea water and lagoon of the Iranian Caspian Sea.

Elements	Tench-roach			Tench-asp			Roach-asp		
	M-W	Z	*p*	M-W	Z	*P*	M-W	Z	*p*
Ca	0.00	-2.24	<0.05	0.00	-1.73	>0.05	1.00	-1.55	>0.05
k	0.00	-2.24	<0.05	1.00	-1.16	>0.05	5.00	0.00	>0.05
Mg	3.00	-2.24	<0.05	1.00	-1.73	>0.05	0.00	-0.78	>0.05
P	0.00	-2.24	<0.05	0.00	-1.73	>0.05	3.00	-0.78	>0.05
S	0.00	-2.24	<0.05	0.00	1.00	>0.05	1.00	5.00	>0.05
Sr	0.00	-2.24	<0.05	0.00	-1.73	>0.05	4.50	-0.20	>0.05
Si	0.00	-2.29	<0.05	0.00	-1.94	>0.05	4.50	-0.20	>0.05

M-W and Z are grouping variable scores for Mann–Whitney U and Kruskal–Wallis tests (*$p<0.05$).

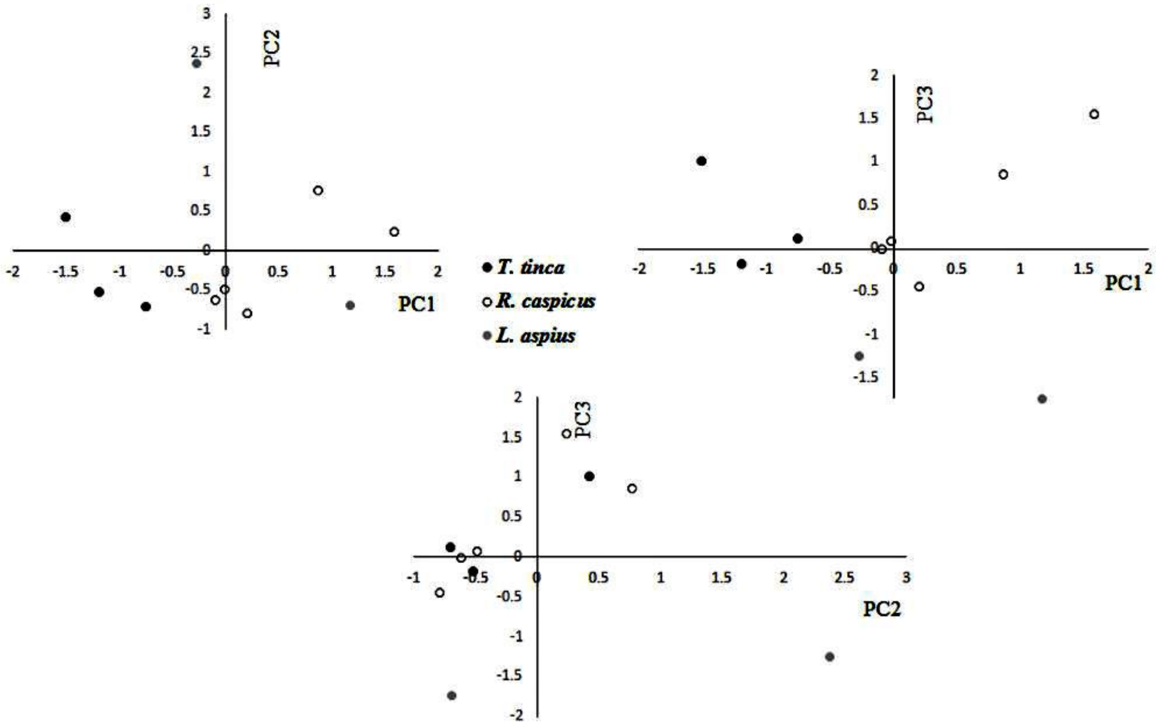

Figure 2. Principal component analysis (PCA) of elemental concentrations of the fish liver and kidney between habitats in the coastal water of the Iranian Caspian Sea. Scatter plots demonstrate individual fish scores for PC1 vs. PC2, PC1 vs. PC3, and PC2 vs. PC3 which together explain 83.60% of the total variance, this graph obtained by SPSS version 16.0.

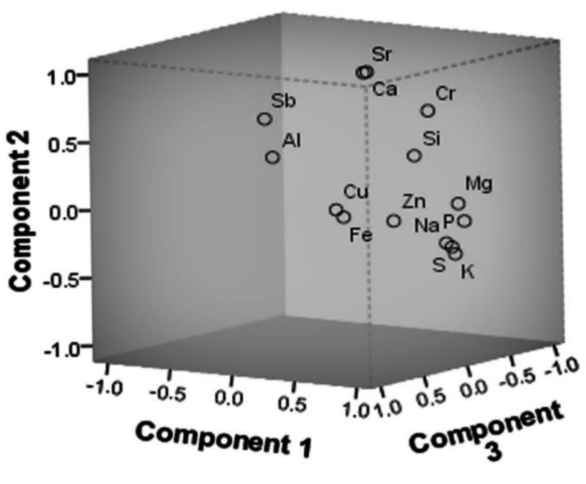

Figure 3. Characteristic load for PC1, PC2 and PC3 obtained by multi-elemental principal components analysis (PCA) for the elemental concentrations of the fish liver and kidney between habitats in the south Caspian Sea water, this graph obtained by SPSS version 16.0.

Table 3. Characteristic load for PC1, PC2 and PC3 obtained by principal component (PCA) analysis for elemental concentrations of the fish liver and kidney between habitats (sea water and lagoon) in the water of Iranian Caspian Sea

Elemental variables	*PC1	PC2	PC3
Al	.012	.418	.629
Ca	.087	.903	-.361
Cr	.576	.661	-.360
Cu	.544	.084	.666
Fe	.615	.039	.683

K	.906	-.337	-.179
Mg	.965	.044	-.138
Na	.933	-.233	-.041
P	.777	-.152	-.473
S	.952	-.267	-.078
Sb	-.287	.622	.286
Si	.844	.437	.188
Sr	.174	.931	-.199
Zn	.718	-.046	.256

***** Principle Components

The matrix composed of element concentrations in liver and kidney tissues of Roach, Asp, and Tench was described with two discriminant components. These experiments successfully discriminate the two investigated habitats (Wilk's Lambda=0.001, X^2=27.63, df=14 and p<0.05, Figure 4). Cluster analysis, as a complementary method, divided the fish into two sub-groups. No variation was found for element concentrations in Roach and Asp, while Tench was placed in a distinct subgroup (Figure 5).

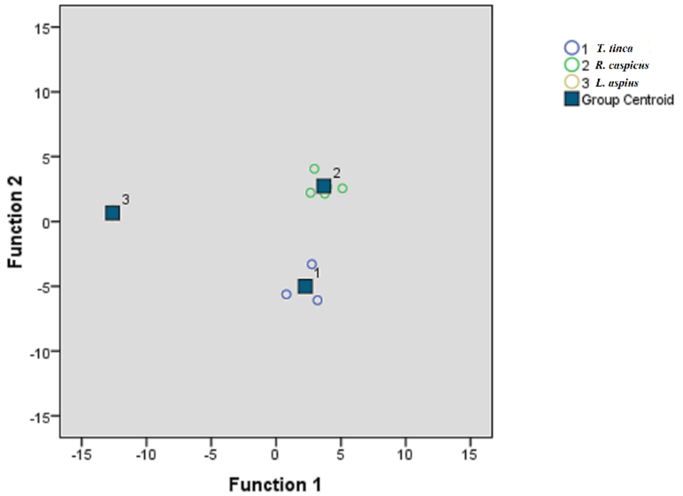

Figure 4. Plot of discriminant functions 1 and 2 for the elemental concentrations of the fish liver and kidney between habitats in the coastal water of the Iranian Caspian Sea, this graph obtained by SPSS version 16.0.

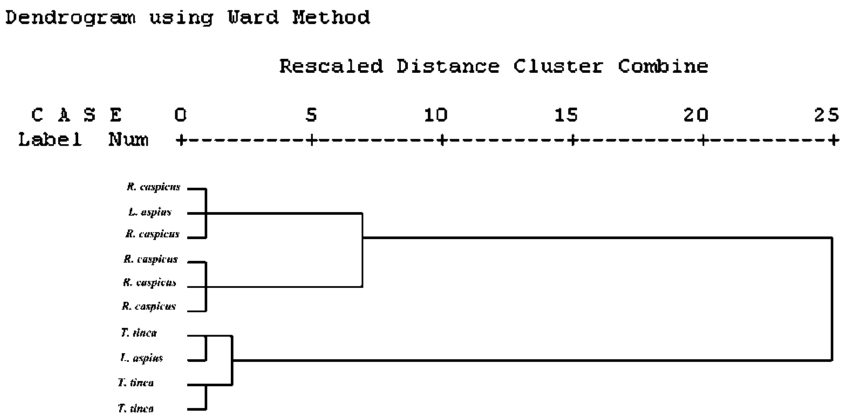

Figure 5. Dendrogram derived from cluster analysis of the elemental concentrations of the fish liver and kidney between habitats in the coastal water of the Iranian Caspian Sea, Cluster analysis, as a complementary method, divided the fish into two sub-groups. No variation was found for element concentrations in Roach and Asp, while Tench was placed in a distinct subgroup, this graph obtained by SPSS version 16.0.

Discussion

In the present study, an attempt was made to assess possible influences of habitat on the elements accumulation in the liver and kidney tissues of some fish species in the southwest of the Caspian Sea basin. Linear charts of element accumulations in the body filtering organs of Roach, Asp, and Tench exhibited their enough variability based on the habitats and also illustrated the borderline created by these habitats. The consequences of different elements bioaccumulation on fish tissues depend on sex, maturation stage, size, tissue type, habitat and fish diet (Azevedo et al., 2009). Previous studies revealed that element bioaccumulation in various fish tissues take place at different levels; but, in short time periods, filtering organs such as liver exhibit higher levels of these elements (Afonso et al., 2017, Alamdar et al., 2017; Salgado-Ramírez et al., 2017). This is while, gills and gut are the first organs receiving these elements (Tiphaine et al., 2018). Therefore, liver could be considered as the main organ for element aggregation monitoring studies (Salgado-Ramírez et al., 2017)

Since metal elements find their way to the aquatic environment and deposit in sediments, the demersal and benthivorous fish species are more susceptible to element bioaccumulation than planktivorous fish (Trevizani et al., 2019).

Little is known about Cu accumulation in fish species. However, it has been found in the higher than normal levels in the food chain which is distinguishable from low-level elements. It seems that raising in Metallothionein levels could be considered as an exact indicator of Cu existence in the ambient environment (Marijić and Raspor, 2007). This is reduced upon the migration of the fish from saltwater to freshwater (Ohji et al., 2007). It is also true for Sr which is found in higher amounts in saltwater in comparison to freshwater. So that, Strontium levels are higher in fish tissues with long residence times in sea water, regardless of the fish diet.

Meanwhile, there is a positive correlation between Sr and Ca. So that, its concentration is raised by low temperature and high salinity (Walther and Thorrold, 2006), instead of the fish diet in Asp tissues. Overall, the present study provides some basic information about elements bioaccumulation in the fish filtering tissues from different ecosystems with various salinity levels. These data could be applicable in determining the focal points of contaminations.

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