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Trace and Macro Element Contaminations in Tissues of *Vimba persa* and *Alosa braschnikowi* From the South Caspian Sea and Potential Human Health Risk Assessment

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Abstract

Alosa braschnikowi and *Vimba persa* are commercially important fish species in the southern part of the Caspian Sea. However, little is known about the trace element (TE) concentrations in their muscles. To this end, a total of 82 pieces of *V. persa* and 74 *A. braschnikowi* were caught at three different fishing areas (Astara, Anzali, and Kiashahr) around the southwestern shorelines of the Caspian Sea from September 2017 through June 2018. An inductively coupled plasma optical emission spectrometry (ICP-OES) was used to measure the accumulation of 34 traces and macro elements in some tissues of the fish species. Then, comparisons of the element levels in these tissues were made for the three different fishing areas. Overall, in the case of *A. braschnikowi,* 30, 30, and 28 elements were detected in its skin, gonads, and kidneys, respectively, while in the case of *V. persa,* 27 and 28 elements were found in its muscle and liver, respectively. The majority of element levels measured in these tissues indicated no significant differences among the sampling areas ($P>0.05$) except for such elements as copper, lanthanum, and tungsten in skin, lanthanum, rubidium, and vanadium in gonads, as well as molybdenum and zinc in kidney ($P<0.05$). The capacity of observed elements put human health at risk were examined as well. The TE content in the tissues of *A. braschnikowi* and in the muscles of *V. persa* ($P<0.05$) differed significantly from the international standard levels ($P<0.05$). In this study, only lead indicated a target hazard quotient (THQ) index above 1. Therefore, high consumption of this fish (360 g per year) may be a matter of concern for the consumer. In the case of other evaluated elements, the THQ value was less than 1.

Keywords: Caspian Sea, Elements, ICP-OES, Fishing areas, Target hazard quotient

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1. Introduction

Heavy metals are considered as the important sources of hazardous pollutants in the aquatic ecosystems (1-5). These metals are usually discharged into the aquatic ecosystems and accumulate in water and sediments (6,7). A large quantity of metal ions is deposited in the sediment due to their adsorption, hydrolysis, and co-precipitation properties, while only a small amount of these elements can dissolve in water. The accumulated heavy metals in sediment can be chemically altered by organisms and converted into the organic complexes, some of which may put animal and human life at greater danger through the food chain (8-10). Industrial factories are constantly importing heavy metals from adjacent grounds into the coastal environments by river, inlets, and estuaries filled with run-off (10-14). The harmful effects of some metals are as follows:

Arsenic (As), similar to many elements, enters the environment through natural and anthropogenic sources (15). The World Health Organization (WHO), the Environmental Protection Agency (EPA), and several studies (16) have substantiated that inorganic As can increase the risk of lung, skin, bladder, liver, kidney, and prostate cancers in humans (17). Copper (Cu) is an essential element, high levels of which in the environment can cause accumulation in organisms (18-20) while lead (Pb) is one of the carcinogenic metals for humans. Children absorb Pb much more efficiently than adults do (4-5 times more), which affects their intelligence quotient (IQ) (17). Nickel (Ni) is not generally highly toxic, but high ingestion of it through contact can cause renal problems and skin allergies (21). Zinc (Zn) is also an essential micronutrient (22). The Caspian Sea is the largest continental water body in the world, and the Iranian

coast stretches for nearly 700 km from Azerbaijan in the West to Turkmenistan in the East (Fig. 1). Sea currents transport entrapped pollutants, which are discharged locally or in adjacent Azerbaijan or even Russia, along the Iranian coast (23). Furthermore, more than 10 million people reside in the coastal provinces of Iran that border the Caspian Sea and frequently consume the Caspian Sea sturgeon and other types of fish (23,24). Trace metals accumulated by fish via local aquatic food chains have the potential to enter the human food chain (25). Given the biological, conservational, and commercial importance of members of the biological community of the Caspian Sea, it is necessary to understand how an increase in the bioavailability of toxic contaminants such as trace metals might markedly affect the biota of the Caspian.

According to Borodin (1904), *Alosa braschnikowi* is one of the economic fish that inhabits in the temperate regions. Its maximum length is reported to be 500 cm (9). Its diet is typically made up of mollusks, small crustaceans, and insects. These species spawn more frequently in the spring and summer, while their spawning pattern was shown to be different in the subspecies. Despite the small size, *A. braschnikowi* has a high nutritional value and is even more advantageous than red meat in some cases. This fish plays an important role in the food chain and the health of the Caspian Sea ecosystem. Owing to the important role of this fish in the food chain, it is extremely important to study its benefits for human consumption.

Vimba bream, *Vimba persa* (Pallas, 1814), *Cypriniformes*, *Cyprinidae, Leuciscinae* is a type of freshwater, brackish, benthopelagic, and anadromous fish living in the subtropical areas with temperature range of 10-20 ˚C (64°N - 35°N, 12°E - 53°E) reaching to maximum total length (TL) of 50.0 cm. Its common length is 20.0 cm, maximum published weight is 1.4 kg, and maximum age is reported to be 15 years. It feeds mainly on small molluscs and insect larvae. Distribution of this fish ranges from Eurasia, involving Caspian, Black, Marmara, and Baltic Sea basins, which flows in riffles in shallow fast-flowing streams and rivers on gravels. A number of studies were conducted on heavy metal pollution in Iran. For example, Bibak et al (26) measured heavy metal pollutions in sediments of Persian Gulf, and Eslami et al (27) explored metals in tissues of *Perca fluviatilis* and *Tinca tinca* in Wetland of Anzali. In another study, Alipour et al (28) investigated *Rutilus rutilus caspicus* from Miankaleh International Wetland, the Caspian Sea. Likewise, Heydari et al (29) conducted a study on *Acipenser stellatus*, Monsefrad et al (30) on *Rutilus frisii kutum*, and Khanipour et al (11) focused on *Silurus glanis* from Anzali Wetland, the Southwest Caspian Sea. There are also some reports on TE concentrations in *R. kuum* (30-34); however, there seems lack of studies on metal pollution in *V. persa*. Accordingly, the purpose of the present study was to report the levels of some elements (trace and major) in different tissues of *V. persa* and *A. braschnikowi* sampled from the Caspian Sea.

2. Materials and Methods

2.1. Sampling Location and Elements Measurement

Astara (38° 42′ 25″ N, 48° 86′ 87″), Anzali (37° 46′ 39″ N, 49° 47′ 99″), and Kiashahr (37° 42′ 20″ N, 49° 94′ 95″) were the regions from which samples were taken. Sampling lasted one year from September 2017 to June 2018. A total of 156 fish pieces were caught (82 pieces of *V. persa* and 74 *A. braschnikowi*) and were then transferred to the laboratory at cool box of 4°C. The tissues were dissected in the laboratory. Liver and muscle tissues were isolated

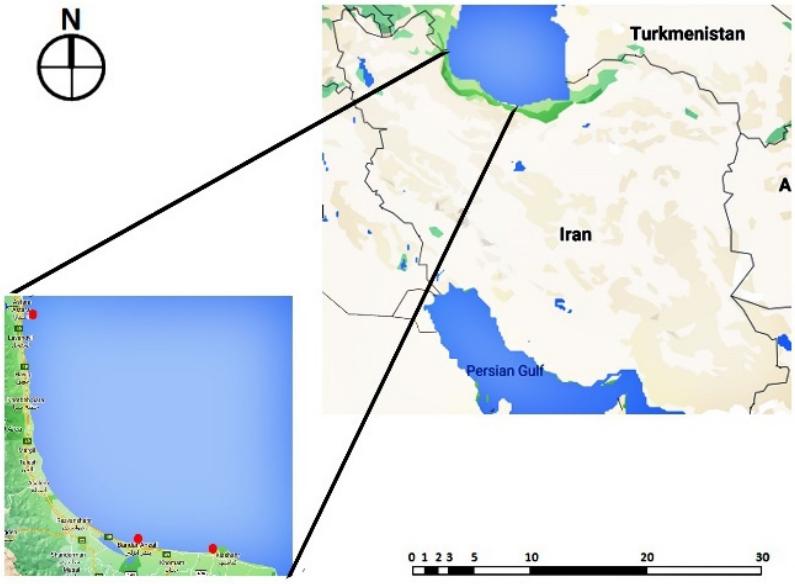

Fig. 1. Location of Fish Sampling Areas in the Southern Coasts of the Caspian Sea.

Table 1. Morphometric Characteristics of Fish Sampling From the Three Regions 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 Width (cm)
Alosa braschnikowi									
Average	252.72	30.23	27.19	25.74	6.68	1.94	1.28	6.36	2.96
SD	130.71	4.88	4.47	4.32	0.93	0.33	0.13	1.15	0.63
Max.	810	42	39	36.5	8.9	2.9	1.7	10.2	5
Min.	45	18	16	15	4.2	1.1	1	3.6	1.2
Vimba persa									
Average	65.61	18.04	16.21	14.90	3.55	1.01	0.93	3.99	1.91
SD	33.98	2.38	2.15	2.02	0.50	0.18	0.12	0.66	0.33
Max.	205	25	23	21.5	4.8	1.5	1.2	6.1	3
Min.	30	14	12.5	11.5	2.6	0.6	0.6	2.2	1.2

Note. SD: Standard deviation; Max: Maximum; Min: Minimum.

from *V. persa*, while askin, gonadal, and liver tissues were isolated from *A. braschnikowi* in the laboratory of Sowmehsara University, Guilan. Afterward, these tissues were dried in an oven (at 80°C for 18 hours). Then, samples were ground in a mortar. Subsequently, 1 g of powder was used for acid digestion, and nitric acid 65% was employed as a solvent for these tissues. The elements were assayed in Zarazma Company (Tehran, Iran) using an inductively coupled plasma-optical emission spectrometry (ICP-OES) device.

The metal selectivity index (MSI) was applied to explicit the concentration of elements in tissues as follows:

$$\mathrm{MSI} = \frac{a}{t} \times 100 \quad (1)$$

a = Absolute concentration of an element in a texture

t= Total absorption of all element in that texture

2.2. Statistical Analyses

The ICP-OES device was used to measure all elements in tissues (Tables 1 and 2). After examining the normality of the data and homogeneity of variances, analysis of covariance (ANCOVA) and Kruskal-Wallis tests were employed in this study. Finally, metal concentrations were compared with international standards (Tables 3 and 4) to determine the level of contamination and hazards of heavy metals in this study (36).

2.3. Target Hazard Quotient (THQ)

THQ was calculated via the following equation:

$$THQ = \frac{EF \times ED \times FIR \times C}{RFD \times W \times ATn} \times 10^{-3}$$

where EF: Frequency of exposure (In this study,360 days per year were considered)

ED: Duration of exposure (In this study, 70 years were considered for adults)

FIR: Rate of fish ingestion, (25 g day-1 for adults)

Table 2. Maximum Permitted Concentration in Parts Per Million (ppm) Recommended by Codex Alimentarius Commission (FAO & WHO, 2018) and US Food and Drug Administration (2011)

Elements	Maximum Permitted Concentration (ppm)
Pb	0.5
Cd	2
As	0.1
Cr	1
Al	100
Sb	1
Sn	230
Cu	10
Mn	0.5
Zn	100

Note. FAO: Food and Agriculture Organization; WHO: World Health Organization; Pb: Lead; Cd: Cadmium; As: Arsenic; Cr: Chromium; Al: Aluminium; Sb: Antimony; Sn: Tin; Cu: Copper; Mn: Manganese; Zn: Zinc.

C: Concentration of elements in the muscle of fish (mg kg-1)

RfD: Oral reference dose (mg kg-1 day 1)

W: Average body weight (kg, 70 kg for adults)

ATn: Mean exposure time for noncarcinogens (365 days year-1 ×ED).

3. Results and Discussion

3.1. Sampling and Studied Elements

In this study, fish specimens were dissected and their muscle and liver (for *V. persa*) as well as gonad, skin, and kidney (for *A. braschnikowi*) were examined for 34 elements including silver (Ag), aluminium (Al), As, barium (Ba), beryllium (Be), bismuth (Bi), calcium (Ca), cadmium (Cd), caesium (Ce), cobalt (Co), chromium (Cr), and Cu. The other elements were iron (Fe), potassium (K), lanthanum (La), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), Ni, phosphorus (P), Pb, rubidium (Rb), sulphur (S), antimony (Sb), scandium (Sc), silicon (Si), tin (Sn),

Table 3. Concentration of Trace Elements in *V. persa* Muscle at Three Fisheries Regions Around the South Caspian Sea

Elemental Variables (ppm)	Mean ± SE Range				*P* value
	Anzali	Astara	Kiashahr	Total	
Ag	BDL	BDL	BDL	BDL	-
Al	2.27±0.77 0.96-2.96	1.56±0.64 0.65-2.45	2.02	1.87±0.72 0.65-2.96	0.26*
As	0.09±0.03 0.05-0.14	0.06±0.02 0.04-0.1	BDL	0.07±0.03 0.04-0.14	0.69*
Ba	BDL	BDL	BDL	BDL	-
Be	BDL	BDL	BDL	BDL	-
Bi	BDL	BDL	BDL	BDL	-
Ca	309±179.59 89.8-522.5	250.01±223.56 26.9-573.8	196.3	268.56±192.50 26.9-573.8	0.83*
Cd	0.08±0 0.08-0.08	0.01±0 0.01-0.01	BDL	0.04±0.04 0.01-0.08	0.85**
Ce	BDL	BDL	BDL	BDL	-
Co	BDL	0.02±0.01 0.01-0.04	BDL	0.02±0.01 0.01-0.04	0.21**
Cr	0.07±0.01 0.05-0.09	0.07±0.01 0.06-0.11	0.07	0.07±0.01 0.05-0.11	0.97*
Cu	0.08±0.02 0.05-0.11	0.07±0.03 0.03-0.13	BDL	0.07±0.03 0.03-0.13	0.26*
Fe	1.40±0.50 0.92-2.11	1.47±0.31 1.11-1.99	0.97	1.40±0.39 0.92-2.11	0.52*
K	262.8±73.91 167.4-345.1	226±67.12 137.9-350	197.8	237.98±67.44 137.9-350	0.57*
La	BDL	BDL	BDL	BDL	-
Li	0.01±0 0.01-0.01	0.01±0 0.01-0.01	BDL	0.01±0 0.01-0.01	0.81**
Mg	27.76±9.29 14.3-35.6	33.17±9.61 21.7-45.7	22.8	30.29±9.33 14.3-45.7	0.47*
Mn	0.08±0.07 0.03-0.13	0.12±0.02 0.08-0.14	BDL	0.10±0.04 0.03-0.14	0.25**
Mo	BDL	0.02±0.02 0.01-0.04	BDL	0.02±0.02 0.01-0.04	0.39**
Na	57.06±19.42 35-77	74.15±26.35 41.2-124.1	54.0	66.03±23.60 35-124.1	0.44*
Ni	BDL	0.03±0.03 0.01-0.07	BDL	0.03±0.03 0.01-0.07	0.22**
P	326.62±130.28 164.1-464.3	380±108.74 241.7-542.6	250.9	349.53±114.63 164.1-542.6	0.52*
Pb	0.04±0 0.04-0.04	0.02±0.01 0.01-0.03	BDL	0.02±0.01 0.01-0.04	0.70**
Rb	0.30±0.21 0.09-0.52	0.66±0.14 0.52-0.8	BDL	0.48±0.25 0.09-0.8	0.67**
S	194.26±68.43 106-261.3	211.97±68.11 134.1-297.4	164.9	201.53±63.86 107-297.4	0.78*
Sb	0.06±0.02 0.02-0.09	0.05±0.04 0.01-0.11	0.09	0.05±0.03 0.01-0.11	0.64*
Si	0.33±0.08 0.22-0.45	0.28±0.21 0.05-0.69	0.26	0.30±0.16 0.05-0.69	0.86*
Sn	0.07±0 0.07-0.07	0.05±0.01 0.05-0.07	BDL	0.06±0.01 0.05-0.07	0.66**
Sr	2.68±1.92 0.05-4.9	2.31±2.02 0.34-5.41	1.73	2.41±1.83 0.05-5.41	0.89*
Th	0.04±0 0.04-0.04	0.03±0.02 0.01-0.05	BDL	0.03±0.01 0.01-0.05	0.63**
Ti	0.02±0 0.02-0.02	0.02±0.02 0.01-0.05	BDL	0.02-0.01 0.01-0.05	0.58**
V	BDL	BDL	BDL	BDL	-

Table 3. Continued.

Elemental Variables (ppm)	Mean ± SE Range				*P* value
	Anzali	Astara	Kiashahr	Total	
W	BDL	0.01±0 0.01-0.01	BDL	0.01±0 0.01-0.01	0.65**
Zn	0.97±0.39 0.47-1.39	1.04±0.26 0.63-1.41	0.69	0.99±0.31 0.47-1.41	0.59*

Note. ANOVA: Analysis of variance; SE: Standard error; *P* is regarded as being significant if<0.05. *Tested by one-way ANOVA; **Tested by Kruskal-Wallis. BDL: Below detectable level; Ag: Silver; Al: Aluminium; As: Arsenic; Ba: Barium; Be: Beryllium; Bi: Bismuth; Ca: Calcium; Cd: Cadmium; Ce: Caesium; Co: Cobalt; Cr: Chromium; Cu: Copper; Fe: Iron; K: Potassium; La: Lanthanum; Li: Lithium; Mg: Magnesium; Mn: Manganese; Mo: Molybdenum; Na: Sodium; Ni: Nickel; P: Phosphorus; Pb: Lead; Rb: Rubidium; S: Sulphur; Sb: Antimony; Si: Silicon; Sn: Tin; Sr: Strontium; Th: Thorium; Ti: Titanium; V: Vanadium; W: Tungsten; Y: Yttrium; Zn: Zinc.

strontium (Sr), thorium (Th), titanium (Ti), vanadium (V), tungsten (W), yttrium (Y), and Zn.

3.2. Tissue Element Analyses in Vimba persa

Table 4 illustrates that the element levels in the fish gonads were not significantly different between the sampling areas. Table 5 also depicts the extent of MSI in all the sampling areas. According to this table, the rate of this index in Astara and Anzali was almost the same while different from Kiashahr. The amount of MSI for each

Table 4. The Trace Element Levels in the V. *persa* Liver at Three Fishing Regions Around the South Caspian Sea

Elemental Variables (ppm)	Mean ± SE Range				*P* Value
	Anzali	Astara	Kiashahr	Total	
Ag	BDL	BDL	BDL	BDL	-
Al	1.60±0.64 0.91-2.35	2.21±1.51 0.38-4.68	1.76	1.94±1.17 0.38-4.68	0.70*
As	0.04±0.01 0.03-0.05	0.03±0.01 0.02-0.06	BDL	0.03±0.01 0.02-0.06	0.61**
Ba	BDL	0.02±0 0.02-0.02	BDL	0.02±0 0.02-0.02	0.39**
Be	BDL	BDL	BDL	BDL	-
Bi	BDL	BDL	BDL	BDL	-
Ca	35.18±23.16 13.9-76.3	62.7±39.01 15.8-125.8	22.1	50.14±34.9 13.9-125.8	0.34**
Cd	0.01±0 0.01-0.01	0.01±0.005 0.01-0.02	-	0.01±0.005 0.01-0.02	0.53**
Ce	BDL	BDL	BDL	BDL	-
Co	0.01±0.01 0.01-0.01	0.01±0.01 0.01-0.01	BDL	0.01±0 0.01-0.01	0.81**
Cr	0.35±0.58 0.05-1.4	0.09±0.04 0.04-0.17	0.09	0.19±0.36 0.04-1.4	0.93**
Cu	0.25±0.12 0.12-0.45	0.48±0.36 0.2-1.17	0.47	0.39±0.29 0.12-1.17	0.30**
Fe	11.88±8.31 5.35-24.1	12.37±7.47 5.92-27.84	23.22	13.01±7.77 5.35-27.84	0.42*
K	138.9±40.80 95.9-204.2	136.22±53.84 91.6-247	187.4	141.19±46.89 91.6-247	0.62*
La	BDL	BDL	BDL	BDL	-
Li	BDL	0.03±0.01 0.02-0.04	BDL	0.03±0.01 0.02-0.04	0.39**
Mg	14.26±6.64 7.1-23.4	25.11±12.28 13.5-48.6	19.6	20.51±10.90 7.1-48.6	0.25*
Mn	0.12±0.02 0.11-0.14	0.09±0.06 0.05-0.19	0.11	0.10±0.05 0.05-0.19	0.61**
Mo	0.01±0 0.01-0.01	0.01±0.005 0.01-0.02	BDL	0.01±0.005 0.01-0.02	0.53**
Na	59.92±37.48 24.1-122.6	78.45±44.11 47.4-174.9	81.5	71.56±39.16 24.1-176.9	0.30**

Table 4. Continued.

Elemental Variables (ppm)	Mean ± SE Range				*P* Value
	Anzali	Astara	Kiashahr	Total	
Ni	0.08±0.07 0.02-0.17	0.02±0.02 0.01-0.06	BDL	0.05±0.06 0.01-0.17	0.40**
P	169.94±60.17 85.9-224.1	195.24±124.21 100-469.7	274.1	191.57±98.45 85.9-469.7	0.66*
Pb	0.05±0 0.05-0.05	0.09±0.04 0.06-0.15	BDL	0.08±0.04 0.05-0.15	0.45**
Rb	0.45±0.26 0.27-0.64	0.56±0.46 0.18-1.44	BDL	0.53±0.40 0.18-1.44	0.35*
S	123.02±57.88 57.4-209.7	158.18±74.24 80.7-313	159.6	144.76±65.75 57.4-313	0.67*
Sb	0.06±0.01 0.03-0.07	0.05±0.03 0.01-0.09	0.08	0.05±0.02 0.01-0.09	0.45**
Si	0.44±0.25 0.27-0.88	0.35±0.14 0.18-0.55	0.38	0.39±0.18 0.18-0.88	0.77*
Sn	0.06±0 0.06-0.06	0.06±0.02 0.04-0.09	BDL	0.06±0.02 0.04-0.09	0.58**
Sr	0.18±0.13 0.06-0.42	0.38±0.19 0.19-0.78	0.15	0.28±0.19 0.06-0.78	0.15*
Th	0.03±0 0.03-0.03	0.06±0.02 0.05-0.08	BDL	0.05±0.02 0.03-0.08	0.45**
Ti	0.06±0 0.06-0.06	0.05±0.03 0.02-0.08	BDL	0.05-0.02 0.02-0.08	0.63**
V	BDL	BDL	BDL	BDL	-
W	0.01±0 0.01-0.01	BDL	BDL	BDL	0.44**
Zn	1.38±0.36 1.03-1.85	1.67±1.24 0.9-4.35	1.17	1.56±0.91 0.9-4.35	0.64**

Note. SE: Standard error; ANOVA: Analysis of variance; *Tested by one-way ANOVA; **Tested by Kruskal-Wallis.
BDL: Below detectable level; Ag: Silver; Al: Aluminium; As: Arsenic; Ba: Barium; Be: Beryllium; Bi: Bismuth; Ca: Calcium; Cd: Cadmium; Ce: Caesium; Co: Cobalt; Cr: Chromium; Cu: Copper; Fe: Iron; K: Potassium; La: Lanthanum; Li: Lithium; Mg: Magnesium; Mn: Manganese; Mo: Molybdenum; Na: Sodium; Ni: Nickel; P: Phosphorus; Pb: Lead; Rb: Rubidium; S: Sulphur; Sb: Antimony; Si: Silicon; Sn: Tin; Sr: Strontium; Th: Thorium; Ti: Titanium; V: Vanadium; W: Tungsten; Zn: Zinc.

Table 5. Metal Selectivity Index in the Muscle of *V. persa* at Three Fisheries Regions of the South Caspian Sea

Elements	Astara	Anzali	Kiashahr
Al	2.12	3.07	3.62
As	0.09	0.13	0.09
Ca	338.32	417.02	351.88
Cr	0.10	0.10	0.13
Cu	0.09	0.11	0.00
Fe	2.00	1.89	1.74
K	305.82	354.67	354.57
Mg	44.89	37.46	40.87
Mn	0.16	0.11	0.00
Na	100.35	77.01	96.80
P	514.22	440.80	449.75
S	286.84	262.17	295.59
Sb	0.07	0.08	0.16
Si	0.38	0.45	0.47
Sr	3.13	3.63	3.10
Zn	1.42	1.31	1.24

Note. Ag: Silver; As: Arsenic; Ca: Calcium; Cr: Chromium; Cu: Copper; Fe: Iron; K: Potassium; Mg: Magnesium; Mn: Manganese; Na: Sodium; P: Phosphorus; S: Sulphur; Sb: Antimony; Si: Silicon; Sr: Strontium; Zn: Zinc.

element can be observed in Table 5.

Several information is available on elements in aquatic environments of the Caspian Sea; however, there is a few information about elements in *V. persa*. As such, this study is the first report about its contamination in Iran. It was found that the average concentrations of As, Ca, Cd, Co, K, Mg, Mo, P, S, and Sr in muscle tissue were higher than in liver, while Al, Cr, Cu, Fe, Li, Na, Ni, Pb, Rb, Si, Th, Ti, and Zn in liver were shown to be higher than in muscle even though the differences between these tissues were not significant ($P>0.05$). The presence of higher levels of Cd, Pb, Cu, and Zn in liver compared with muscle may be due to some physiological functions, which form complexes in liver such as metallothionein, a protein formed in liver with higher affinity to bind with some elements such as Zn and Cu. The element levels in the liver, as an important organ in the element detoxification in the body, were measured as well. Various studies have shown that the liver stores these elements at high levels (37).

3.3. Tissue Element Analyses in *Alosa braschnikowi*

The level of 30 elements in the skin were examined, and the related data are provided in Tables 6-8. Some element levels including Ag, Ba, Be, Ce, and Y in the skin were less

than detectable. It was also true for the fish gonads. In kidney, in addition to the aforementioned elements in the skin and liver, La and V were also lower than the detection limit. Only 28 elements were used in the statistical analyses of kidney. The levels of W, Cu, and L in the skin were significantly different in all three sampling areas ($P<0.05$) while no significant difference was reported for the other elements ($P>0.05$).

Table 6. The Trace Element Levels in the *Alosa braschnikowi* Skin at Three Fishing Regions Around the South Caspian Sea

Elemental Variables (ppm)	Mean ± SE Range				*P* Value
	Anzali	Astara	Kiashahr	Total	
Al	3.66±1.68 1.95-5.9	2.74±0.70 2.25-3.24	1.63±0.86 0.79-2.52	2.78±1.48 0.79-5.9	0.21*
As	0.07±0.02 0.04-0.1	0.05±0.007 0.05-0.06	0.08±0.02 0.07-0.11	0.07±0.02 0.04-0.11	0.42*
Ba	0.01±0.005 0.01-0.02	0.01±0.007 0.01-0.02	0.01±0 0.01-0.01	0.01±0.004 0.01-0.02	0.26**
Ca	124.35±74.07 33.6-214.5	97.35±10.25 90.1-104.6	168.66±40.18 125-204.1	133.12±57.50 33.6-214.5	0.41*
Cd	0.01±0 0.01-0.01	0.01±0 0.01-0.01	BDL	0.01±0 0.01-0.01	0.31**
Co	0.01±0.007 0.01-0.02	0.01±0.007 0.01-0.02	0.01±0 0.01-0.01	0.01±0.005 0.01-0.02	0.51*
Cr	0.05±0.005 0.05-0.06	0.04±0.007 0.04-0.05	0.05±0.01 0.04-0.07	0.05±0.009 0.04-0.07	0.48*
Cu	0.07±0.04 0.04-0.14	0.04±0 0.04-0.04	0.03±0 0.03-0.03	0.05±0.03 0.03-0.14	0.03**
Fe	5.57±1.43 3.6-6.89	1.93±0.55 1.54-2.32	5.64±1.92 4.28-7.84	4.78±2.08 1.54-7.84	0.06*
K	82.85±26.19 45.4-106	91.15±2.05 89.7-92.6	122.66±2.08 121-125	97.96±24.77 45.4-125	0.07*
La	BDL	0.01±0 0.01-0.01	BDL	0.01±0 0.01-0.01	0.01**
Li	0.02±0 0.02-0.02	0.02±0.02 0.01-0.04	BDL	0.02±0.01 0.01-0.04	0.09**
Mg	13.16±3.73 7.94-16.8	22.1±12.86 13-31.2	10.44±1.37 8.92-11.6	14.24±6.91 7.94-31.2	0.24**
Mn	0.15±0.04 0.09-0.2	0.08±0.06 0.04-0.13	0.09±0.03 0.06-0.13	0.12±0.05 0.04-0.2	0.18*
Mo	0.01±0 0.01-0.01	0.01±0 0.01-0.01	0.01±0 0.01-0.01	0.01±0 0.01-0.01	0.51**
Na	29.8±11.91 14.4-42.2	30.6±9.61 23.8-37.4	26.63±4.67 21.4-30.4	28.92±8.56 14.4-42.2	0.88*
Ni	0.01±0.005 0.01-0.02	0.02±0.02 0.01-0.04	0.01±0 0.01-0.01	0.01±0.01 0.01-0.04	0.11*
P	108.77±61.05 55.4-190.1	151.65±92.84 86-217.3	115.4±23.56 94.8-141.1	120.51±54.18 55.4-217.3	0.70*
Pb	0.04±0.02 0.01-0.08	0.04±0.02 0.03-0.06	0.06±0.02 0.05-0.09	0.05±0.02 0.01-0.09	0.57*
Rb	1.06±0.57 0.74-1.92	0.68±0.17 0.56-0.81	0.73±0.18 0.53-0.88	0.87±0.41 0.53-1.92	0.88**
S	329.75±406.94 113-940	115.5±4.94 112-119	398±491.95 107-966	304.88±367.60 107-966	0.44**
Sb	0.03±0.01 0.01-0.04	0.03±0.02 0.01-0.05	0.01±0.005 0.01-0.02	0.02±0.01 0.01-0.05	0.38*
Si	0.32±0.22 0.12-0.54	0.18±0.01 0.17-0.19	0.38±0.04 0.36-0.44	0.31±0.16 0.12-0.54	0.42*
Sn	0.07±0.04 0.02-0.11	0.13±0.07 0.08-0.18	0.08±0.01 0.08-0.1	0.09±0.04 0.02-0.18	0.34*
Sr	0.14±0.04 0.09-0.2	0.12±0.05 0.08-0.16	0.10±0.02 0.09-0.13	0.12±0.04 0.08-0.2	0.50*

Table 6. Continued.

Elemental Variables (ppm)	Mean ± SE Range				*P* Value
	Anzali	Astara	Kiashahr	Total	
Th	0.08±0.05 0.04-0.16	0.06±0.007 0.06-0.07	0.11±0.01 0.09-0.12	0.08±0.03 0.04-0.16	0.46*
Ti	0.24±0.09 0.11-0.33	0.08±0.02 0.07-0.1	0.18±0.12 0.06-0.31	0.18-0.10 0.06-0.33	0.25*
V	0.01±0 0.01-0.01	BDL	BDL	0.01±0 0.01-0.01	0.45**
W	0.02±0.01 0.01-0.03	BDL	0.03±0.005 0.03-0.04	0.02±0.01 0.01-0.04	0.01*
Zn	2.17±0.96 1.22-3.29	0.56±0.09 0.5-0.63	3.57±0.19 3.34-3.69	2.28±1.31 0.5-3.69	0.09*

Note. SE: Standard error; ANOVA: Analysis of variance; P is regarded as being significant (*) if <0.05. *Tested by one-way ANOVA; **Tested by Kruskal–Wallis; BDL: Below detectable level.
Ag: Silver; Al: Aluminium; As: Arsenic; Ba: Barium; Be: Beryllium; Bi: Bismuth; Ca: Calcium; Cd: Cadmium; Ce: Caesium; Co: Cobalt; Cr: Chromium; Cu: Copper; Fe: Iron; K: Potassium; La: Lanthanum; Li: Lithium; Mg: Magnesium; Mn: Manganese; Mo: Molybdenum; Na: Sodium; Ni: Nickel; P: Phosphorus; Pb: Lead; Rb: Rubidium; S: Sulphur; Sb: Antimony; Si: Silicon; Sn: Tin; Sr: Strontium; Th: Thorium; Ti: Titanium; V: Vanadium; W: Tungsten; Zn: Zinc.

Table 7. The Trace Element Levels in the *Alosa braschnikowi* Gonads at Three Fishing Regions Around the South Caspian Sea

Elemental Variables (ppm)	Mean ± SE Range				*P* Value
	Anzali	Astara	Kiashahr	Total	
Al	1.01±0.55 0.62-1.83	3.03	0.69±0.25 0.54-0.98	1.14±0.86 0.54-3.03	0.20**
As	0.03±0.01 0.02-0.05	0.02	0.05±0.01 0.04-0.06	0.04±0.01 0.02-0.06	0.17*
Ba	0.01±0.005 0.01-0.02	0.01	0.01±0.005 0.01-0.02	0.01±0.004 0.01-0.02	0.78**
Ca	125.3±3.61 120.8-129.6	97.5	88.63±63.16 42.9-160.7	108.07±38.63 42.9-160.7	0.51*
Cd	0.01±0.007 0.01-0.02	BDL	0.01±0 0.01-0.01	0.01±0.004 0.01-0.02	0.54*
Co	0.01±0 0.01-0.01	BDL	0.01±0 0.01-0.01	0.01±0 0.01-0.01	0.55**
Cr	0.05±0.008 0.04-0.06	0.04	0.05±0.01 0.04-0.07	0.05±0.01 0.04-0.07	0.63*
Cu	0.06±0.005 0.06-0.07	0.04	0.05±0.01 0.04-0.06	0.05±0.01 0.04-0.07	0.13**
Fe	1.87±0.07 1.79-1.95	1.47	3±1.43 2.14-4.66	2.24±1.0001 1.47-4.66	0.05**
K	288.25±44.02 239-335	156	271.66±54.37 211-316	265.25±61.34 154-335	0.13*
La	BDL	0.1	BDL	0.1±0 0.1-0.1	0.03**
Li	0.02±0 0.02-0.02	0.02	BDL	0.02±0 0.02-0.02	0.17**
Mg	15.77±3.20 12.3-19.9	18.7	16.66±4.40 11.7-20.1	16.47±3.30 11.7-20.1	0.78*
Mn	0.06±0.01 0.05-0.08	0.02	0.04±0.03 0.02-0.08	0.05±0.02 0.02-0.08	0.22*
Mo	0.01±0 0.01-0.01	0.01	0.01±0 0.01-0.01	0.01±0 0.01-0.01	0.82**
Na	39.27±2.98 36.8-43.4	27.8	50.63±13.78 37.8-65.2	42.1±11.09 27.8-65.2	0.15*
Ni	0.02±0.01 0.01-0.03	BDL	0.01±0.005 0.01-0.02	0.01±0.008 0.01-0.03	0.63*
P	232.75±36.83 190-279	137	227.66±47.60 173-260	218.87±48.26 137-279	0.20*
Pb	0.05±0.03 0.01-0.09	0.08	0.07±0.03 0.05-0.11	0.06±0.03 0.01-0.11	0.57*
Rb	0.92±0.02 0.89-0.95	0.9	0.73±0.07 0.66-0.8	0.85±0.10 0.66-0.95	0.00*

Table 7. Continued.

Elemental Variables (ppm)	Mean ± SE Range				*P* Value
	Anzali	Astara	Kiashahr	Total	
S	125.75±19.06 103-142	134	160.66±43.50 111-192	139.87±31.63 103-192	0.40*
Sb	0.02±0.005 0.02-0.03	0.01	0.01±0 0.01-0.01	0.01±0.007 0.01-0.03	0.41**
Si	0.25±0.03 0.22-0.3	0.11	0.18±0.13 0.08-0.33	0.20±0.08 0.08-0.33	0.36*
Sn	0.08±0.04 0.02-0.12	0.06	0.05±0.03 0.02-0.09	0.06±0.03 0.02-0.12	0.63*
Sr	0.07±0.03 0.05-0.11	0.05	0.07±0.01 0.06-0.08	0.07±0.02 0.05-0.11	0.67*
Th	0.05±0.01 0.03-0.06	0.02	0.05±0.02 0.03-0.07	0.04±0.01 0.02-0.07	0.30*
Ti	0.02±0.01 0.02-0.04	0.03	0.02±0.01 0.01-0.04	0.02±0.01 0.01-0.04	0.93*
U	BDL	BDL	BDL	BDL	-
V	BDL	0.1	BDL	0.1±0 0.1-0.1	0.03**
W	0.05±0.02 0.03-0.09	0.03	0.04±0.01 0.3-0.05	0.04±0.02 0.03-0.09	0.41*
Zn	5.01±1.64 3.39-7.24	2.66	3.81±0.71 3-4.37	4.27±1.44 2.66-7.24	0.31*

Note. SE: Standard error; ANOVA: Analysis of variance; P is regarded as being significant (*) if <0.05. *Tested by ANOVA; **Tested by Kruskal-Wallis.
BDL: Below detectable level. Ag: Silver; Al: Aluminium; As: Arsenic; Ba: Barium; Be: Beryllium; Bi: Bismuth; Ca: Calcium; Cd: Cadmium; Ce: Caesium; Co: Cobalt; Cr: Chromium; Cu: Copper; Fe: Iron; K: Potassium; La: Lanthanum; Li: Lithium; Mg: Magnesium; Mn: Manganese; Mo: Molybdenum; Na: Sodium; Ni: Nickel; P: Phosphorus; Pb: Lead; Rb: Rubidium; S: Sulphur; Sb: Antimony; Si: Silicon; Sn: Tin; Sr: Strontium; Th: Thorium; Ti: Titanium; V: Vanadium; W: Tungsten; Zn: Zinc.

Table 8. Concentrations of Trace Elements in *A. braschnikowi* Kidney at Three Fishing Regions of the South Caspian Sea

Elemental Variables (ppm)	Mean ± SE Range				*P* Value
	Anzali	Astara	Kiashahr	Total	
Al	1.35±0.78 0.61-2.12	0.9	1.81±1.39 0.92-3.42	1.46±0.96 0.61-3.42	0.74*
As	0.03±0.01 0.02-0.05	0.04	0.03±0.01 0.02-0.04	0.03±0.01 0.02-0.05	0.52**
Ba	0.01±0.005 0.01-0.02	0.02	0.01±0 0.01-0.01	0.01±0.004 0.01-0.02	0.17**
Ca	81.6±21.11 56.4-105.3	63.4	85.53±25.37 62.6-112.8	80.8±20.69 56.4-112.8	0.71*
Cd	0.06±0.06 0.01-0.13	0.02	0.01±0 0.01-0.01	0.04±0.04 0.01-0.13	0.15**
Co	0.02±0.008 0.01-0.03	0.02	0.01±0 0.01-0.01	0.01±0.007 0.01-0.03	0.14**
Cr	0.05±0 0.05-0.05	0.04	0.04±0.01 0.03-0.05	0.04±0.007 0.03-0.05	0.13**
Cu	0.10±0.02 0.07-0.12	0.12	0.07±0.04 0.04-0.12	0.09±0.03 0.04-0.12	0.37**
Fe	4.91±0.75 4.32-6	5.6	4.7±3.60 2.54-8.86	4.92±2.009 2.54-8.86	0.94*
K	136.25±28.07 100-166	146	123.96±85.56 67.4-222.4	132.86±49.94 67.4-222.4	0.93*
Li	BDL	BDL	0.03±0 0.03-0.03	0.03±0 0.03-0.03	0.43**
Mg	7.01±1.33 5.25-8.45	9.18	14.05±9.78 4.55-24.1	9.92±6.35 4.55-24.1	0.49**
Mn	0.04±0.008 0.03-0.05	0.02	0.03±0.01 0.02-0.05	0.03±0.01 0.02-0.05	0.37*

Table 8. Continued.

Elemental Variables (ppm)	Mean ± SE Range				*P* Value
	Anzali	Astara	Kiashahr	Total	
Mo	0.01±0 0.01-0.01	0.02	0.01±0 0.01-0.01	0.01±0.003 0.01-0.02	0.03**
Na	60.8±18.64 48.3-88.5	56.4	41.93±31.70 20.1-78.3	53.17±22.91 20.1-88.5	0.62*
Ni	0.01±0.005 0.01-0.02	BDL	0.01±0 0.01-0.01	0.01±0.005 0.01-0.02	0.06*
P	117.5±20.04 89-132	116	102.66±74.96 54-189	111.75±42.83 54-189	0.92*
Pb	0.07±0.01 0.06-0.1	0.08	0.07±0.02 0.05-0.09	0.07±0.01 0.05-0.1	0.93*
Rb	0.86±0.07 0.78-0.97	1.96	0.89±0.25 0.61-1.09	1.01±0.40 0.61-1.96	0.25**
S	146.75±11.44 131-157	153	137.66±47.92 110-193	144.12±27.29 110-193	0.89*
Sb	0.02±0.02 0.01-0.06	0.02	0.02±0.02 0.01-0.05	0.02±0.01 0.01-0.06	0.94**
Si	1.45±2.53 0.11-5.26	0.14	0.33±0.32 0.09-0.7	0.87±1.78 0.09-5.26	0.95**
Sn	0.04±0.01 0.03-0.05	0.04	0.04±0.02 0.03-0.07	0.04±0.01 0.03-0.07	0.93*
Sr	0.03±0.005 0.03-0.04	0.02	0.04±0.02 0.02-0.07	0.03±0.01 0.02-0.07	0.48*
Th	0.05±0.03 0.01-0.1	0.1	0.04±0.01 0.04-0.06	0.05±0.03 0.01-0.1	0.36*
Ti	0.02±0.008 0.01-0.03	BDL	0.04±0.03 0.01-0.08	0.02±0.02 0.01-0.08	0.24**
W	0.01±0 0.01-0.01	BDL	0.01±0 0.01-0.01	0.01±0 0.01-0.01	0.82**
Zn	0.98±0.17 0.74-1.12	0.92	0.32±0.16 0.13-0.43	0.72±0.36 0.13-1.12	0.00*

Note. SE: Standard error; P is considered significant (*) if <0.05. *Tested by ANOVA; **Tested by Kruskal-Wallis.
BDL: Below detectable level. Ag: Silver; Al: Aluminium; As: Arsenic; Ba: Barium; Be: Beryllium; Bi: Bismuth; Ca: Calcium; Cd: Cadmium; Ce: Caesium; Co: Cobalt; Cr: Chromium; Cu: Copper; Fe: Iron; K: Potassium; La: Lanthanum; Li: Lithium; Mg: Magnesium; Mn: Manganese; Mo: Molybdenum; Na: Sodium; Ni: Nickel; P: Phosphorus; Pb: Lead; Rb: Rubidium; S: Sulphur; Sb: Antimony; Si: Silicon; Sn: Tin; Sr: Strontium; Th: Thorium; Ti: Titanium; V: Vanadium; W: Tungsten; Zn: Zinc.

Table 7 depicts that Rb, La, and V levels were significantly different in the sampling areas ($P<0.05$) while other elements did not exhibit significant differences in these areas ($P>0.05$).

Table 8 illustrates that the Mo and Zn levels were significantly different in the sampling areas ($P<0.05$), while other elements did not show significant differences in the sampling areas ($P>0.05$).

Visnjic et al studied elements in the *Alosa* tissues, reporting that the element levels in its liver were significantly higher than in its other tissues. In their study, Zn, Cu, Ca, and iron were reported to be at higher levels of accumulation in the fish liver (38), similar to the results obtained for the Cu and Fe in the present study. The results of the present study are also consistent with the results of a study conducted by Yilmaz et al (39) who investigated the element accumulations in the tissue of *Tinca tinca.* They found that the Cu and Fe levels in the fish liver were higher than in other tissues. In addition, they reported that Zn, Cu, Ca, and Fe had a higher level of accumulation in the fish liver, similar to the results obtained regarding the Cu and Fe in the present study. Other studies can be seen in Table 9.

The obtained results from THQ are presented in Table 10.

In the case of THQ higher than 1, the exposure is potentially cause for concern. Albeit, it should not be considered as a direct risk estimate (Table 10).

In this study, only Pb exhibited a THQ index above 1. Therefore, high consumption of this fish (360 days annually) may cause concern for the consumer. In the case of other assayed elements, the THQ value was less than 1 (Table 10). Alipour et al studied the level of THQ in *Sander lucioperca*, *Liza auratus*, *Alosa caspia*, *Cyprinus carpio,* and *Liza saliens* in the Gorgan bay, reporting that the THQ level was less than 1 (THQ<1). Storelli (45) measured the Cd, Hg, and Pb levels in fish from the Adriatic Sea, reporting that, except for Pb, THQ of these elements was below 1 (THQ<1), similar to the results obtained in the present study. Likewise, Majlesi et al (46) examined Pb levels in *Esox Lucius*, *Oncorhynchus mykiss,*

Table 9. The Comparison Between the Results of the Present Study and Results in the Literature

Element Fish Species	As (Mean ± SD)	Cu (Mean ± SD)	Pb (Mean ± SD)	Mn (Mean ± SD)	Ni (Mean ± SD)	Fe (Mean ± SD)	Zn (Mean ± SD)	Reference
Rutilus caspius	0.25±0.08	0.69±0.40				5.38±2.29	7.15±1.5	Alipour et al (40)
Neogobius gorlap	0.24±0.09	2.37±1.01				7.88±2.64	10.21±2.25	Alipour et al (40)
Esox lucius		0.21±0.02	0.004 ±0.0001				2.55±0.18	Imanpour Namin et al (41)
Alosa caspia			0.34±0.09			1118±128.9		Alipour and Banagar (42)
Clupeonella engraulformis			0.015±0.007					Taghvai Jelodar et al (16)
Alburnus chalcoides		1.46±1.97		3.2±4.1	0.42	80.9±66.5	38.5±30.4	Mirzajani et al (43)
Vimba persa								Present study
Permitted by FAO/WHO	0.1	10	0.5	0.5			100	FAO/WHO 2018 (44)

Note. SD: Standard deviation; As: Arsenic; Cu: Copper; Pb: Lead; Mn: Manganese; Ni: Nickel; Fe: Iron; Zn: Zinc; FAO: Food and Agriculture Organization; WHO: World health Organization.

Table 10. Estimated THQ of Some Elements due to the Consumption of Fish Species

		Vimba persa		
		Astara	Anzali	Kiyashahr
	RFD		THQ	
Cd	0.001	1.20	1.02	-
Cr	1.5	0.006	0.006	0.006
Cu	40	0.002	0.002	-
Pb	0.002	1.20	1.54	-
Fe	0.7	0.027	0.025	0.017
Zn	300	0.004	0.004	0.002

Note. THQ: Target hazard quotient; Cd: Cadmium; Cr: Chromium; Cu: Copper; Pb: Lead; Fe: Iron; Zn: Zinc; RFD: Oral reference dose (mg/kg-day) .

and *Cyprinus carpio*, reporting that THQ level of Pb was lower than 1 (THQ < 1), which was not in line with the results obtained in the present study.

The study of element levels in the marine environments is extremely important, especially in fish as an important human food item. Biomonitoring should be done annually to monitor the entry of these metals into the environment. In this study, two commercially-important fish species in the Caspian Sea were examined in terms of the element accumulations.

4. Conclusion

In the north of Iran, skin and gonads of some fish species are consumed by people. It is of great importance study the amount of heavy metals in these tissues as well as in liver and muscle. In the present study, the mean element levels in fish muscles were lower than the provisional tolerable daily/weekly/monthly intake of the TEs, as permitted by the United States Environmental Protection Agency (USEPA, 2006) and the FAO and WHO (2018). The element levels in the *V. persa* muscles were lower than the levels reported in the previous studies from the Caspian Sea. Further, in this study, element levels in the gonad in the skin and kidney tissues of *A. braschnikowi* were lower than those permitted by Codex Alimentarius Commission. These levels could not pose any threats to human health. As such, it is recommended that TEs monitoring in aquatic organisms and the Caspian environment should be regularly conducted to find out the TE trends over time.

Authors' Contributions

MS: Supervision, writing, reviewing, and editing; MB: Methodology, sample analysis, sampling, sample perpetration, investigation, and writing; MFV: Methodology, sample analysis, sampling, sample perpetration, and investigation.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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