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Trace and major elements in muscle and liver tissues of *Alosa braschnikowy* from the South Caspian Sea and potential human health risk assessment

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Abstract

Even though the Caspian Sea is an important source of fish protein for the people around it and Caspian marine shad, *Alosa braschnikowi* (Borodin, 1904) constitutes a considerable part of fisheries catch, but few reports available about trace element bioaccumulation in fish tissues. Seventy eight individuals of *A. braschnikowy* were caught at three fisheries stations (Astara, Anzali and Kiashahr ports) around the southwestern coast of the Caspian Sea between September 2017 and January 2018. The trace and major elements accumulated in the fish tissues (liver and muscle) were measured using ICP-OES (Inductively coupled plasma optical emission spectrometry). Our results revealed that muscle in total accumulated 27 elements, while liver 33 elements highlighting an exhibiting role of liver in receiving and storing a greater amount of elements from environment. Comparisons of accumulated element levels in examined tissues were also conducted between fishing regions, and results showed no significant differences among the sampling regions. Knowing that exposure to some of the examined TEs can affect human health, we took into account their possible health risks. The level of all elements in the muscle of *A. braschnikowy* were significantly lower than the maximum concentrations permitted by WHO/FAO and FDA standards, suggesting that the muscle are safe for human consumption.

1. Introduction

Urbanization, industrial and agricultural development, has polluted the water environment [1]. An impairment of water quality by chemicals, heat, or bacteria and adversely affects water for domestic, agricultural, municipal, or industrial use [2]. Heavy metals are marked as the main elements posing a great threat to the aquatic environment [3]. They are released through various sources such as dyeing, metalworking, battery production, textiles, printing, horticulture, metallurgy. If the wastewater is not treated properly, heavy metal levels in the environment increase [4]. Those persistent inorganic compounds are capable to accumulate in organisms, where they can lead to the enzyme dysfunctions and formation of various diseases and toxicities [5,6].

Caspian Sea is the world's largest closed water body. It is a characterized by richness of various living aquatic organisms especially fishes and crustaceans. However, the lack of any natural linkage with other water bodies has made it very susceptible to external factors such as climatic conditions and human activities [7]. Since the Caspian Sea, more specifically the southern part, is one of the major resources of oil and gas stock, extraction and transportation of oil through the sea is considered as the main source of pollution in Caspian Sea coastal waters. The number of cities and industries which surrounded the Caspian Sea represents other sources of pollution. Pollution from these sources enter the sea either directly or through rivers [8]. All those considerably change natural habitats and deteriorated remarkably fish populations [9].

Caspian marine shad, *Alosa braschnikowi* [10] belonging to Actinopterygii (ray-finned fishes), Clupeiformes, Clupeidae, Alosinae, is a brackish; pelagic; oceanodromous fish inhabiting temperate regions reaching [11]. It occurs in brackish water and non-anadromous, but strongly migratory. Feeds on small clupeids, gobies, atherines, also crustaceans and occasionally insects and mollusks. Spawning patterns among the subspecies vary but occur mostly in spring and summer after an inshore migration and movement northward. Various spawning patterns are observed among the subspecies (mostly in spring and summer after an inshore migration and movement northward).

Fish are reported to be exposed to heavy metal accumulation in Iran including *Liza abu* and *Sphyraena Jello* [12]; *Perca fluviatilis* and *Tinca tinca* [13]; *Acipenser persicus* and *A. stellatus* [14]; *Mugil auratus* [15]; some other sturgeons such as *A. persicus* [16]; *Liza aurata* [17]; *Rutilus caspicus* [18]; *Liza saliens* [19]; *Huso huso* and *A. nudiventris* [20]; *Acipenser stellatus* [21]; and *Rutilus kutum* [22-23]. However there is no report on TE bioaccumulation in Caspian marine shad, *A. braschnikowi.*

Considering the importance of detecting TE in fish tissues due to the environmental and public health risks related to them, the aim of the present study was to determine the levels of some TE in edible parts of Caspian marine shad, *Alosa braschnikowi* collected from the coast of the Caspian Sea and compare their levels in fish caught from the different geographical localities as well as to define risk assessment of these elements for human health.

2. Materials and methods

This study was conducted at five fisheries areas including Astara: 38° 42′ 25″ N, 48° 86′ 87″ E, Anzali: 37° 46′ 39″ N, 49° 47′ 99″ E, Kiashahr: 37° 42′ 20″ N, 49° 94′ 95″ E, Sari: 36° 78′ 39″ N, 53° 03′ 99″ E and Torkaman port 36° 89′ 28″ N, 54° 04′ 64″ E along the southern shoreline of the Caspian Sea (Figure 1). Samplings were carried out from September 2017 to June 2018 and a total of 74 specimens of *Alosa braschnikowi* were collected. The specimens were transported to the Fish Biology Laboratory, University of Guilan, Sowmeh Sara, Iran using a styrofoam cooler box at 4°C. Fish were washed using distilled water, dissected and pieces of muscle and liver tissues were placed in an oven at 80°C for 18 h to dry [24]. Age determination was carried out using fish scale during the process (Table 1). To extract elements 0.5 g of each tissue was digested in 10 ml 65% nitric acid using a microwave oven, passed through the Whatman filter paper No. 40 and diluted with distilled water to the required volume.
Inductively coupled plasma – optical emission spectrometry (ICP-OES) (Zarazma Co. Tehran, Iran) was used for the determination of element levels in the samples. Instrumental detection limits for trace

elements was 0.01 mg kg^{-1}, and for major elements (Al, Ca, Fe, K, Mg, Mn, Na and Si) was 0.1 mg kg^{-1}. The concentrations of TEs were expressed as the metal selectivity index (MSI) for each tissue.

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

A = absolute concentration of a metal in a tissue
T= Total concentration of all TEs in that tissue

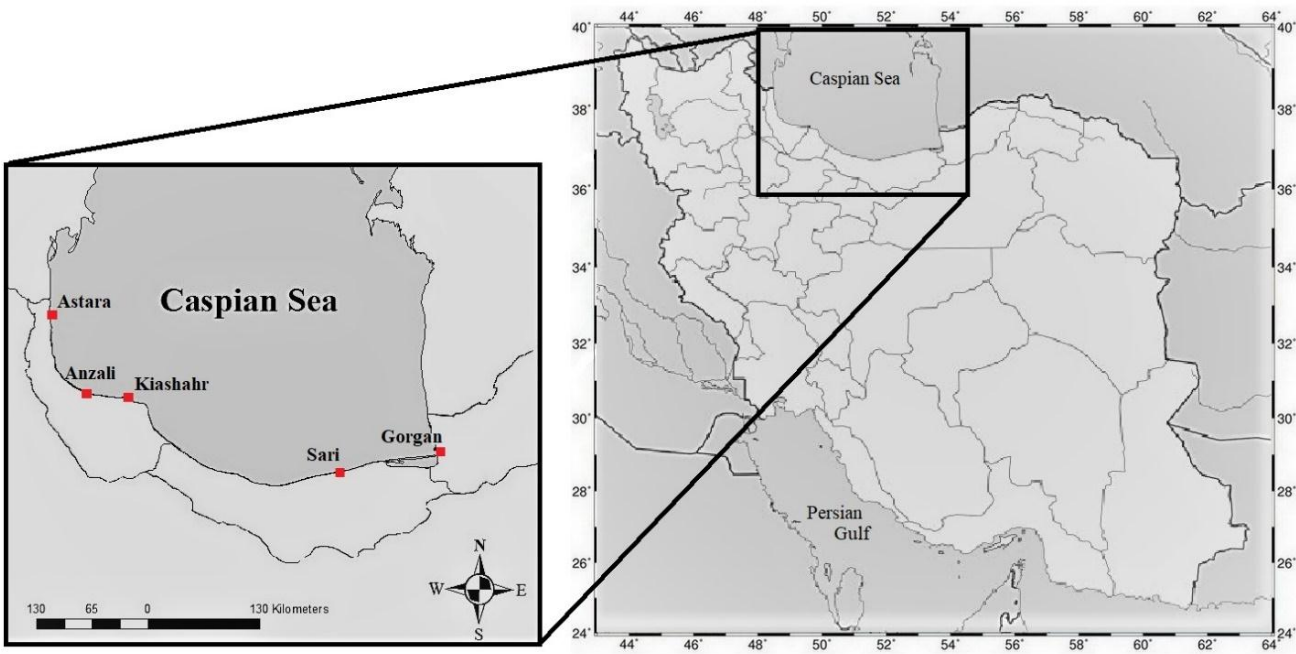

Fig. 1. Location of *Alosa braschnikowi* sampling areas in the southern coasts of the Caspian Sea.

Table 1: Morphometric characteristics of *Alosa braschnikowi* from the three study 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)
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

3. Statistical analysis

The trace and major elements (N = 36) were measured using ICP-OES, then examined statistically excluding Ag, Ba, Cd, Co and Mo which were not detected by the device (Tables 3 & 4). The normality of the data as well as homogeneity of variances was assessed using SPSS software. One-Way ANOVA was used when the fish age was considered as a covariate (ANCOVA) in order to determine the element possible variabilities. (Table 3). In the case of non-homogeneity of variances, we employed the Kruskal-Wallis test (Table 4, [25]).

The concentrations of Pb, Zn, Mn, Cu, Sn, Sb, Al, Cr, As, and Cd in the muscles were compared with maximum levels permitted by international standards using single student t-test [25]. All statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL). The significant level was considered as α = 0.05.

Table 2: Maximum permitted concentration in parts per million (ppm) recommended by Codex Alimentarius Commission (FAO & WHO, amended in 2018), US Food and Drug Administration (FDA, 2011).

Elements	Maximum permitted concentration in parts per million (ppm)
Lead	0.5
Cadmium	2
Arsenic	0.1
Chromium	1
Aluminum	100
Antimony	1
Tin	230
Copper	10
Manganese	0.5
Zinc	100
Selenium	1

4. Results and discussion

In the present study, a total of 74 *Alosa braschnikowi* specimens were dissected and their muscle and liver tissues were examined for 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), vanadium (V), tungsten (W), yttrium (Y) and zinc (Zn).

In the case of muscle tissue, 27 elements were detected. Nine elements (Ag, Ba, Be, Ce, La, U, V, W and Y) which were below limit of detection were excluded from statistically analyses. For liver tissue, 33 elements were detected. Elements that were not detected were Ag, U and Y (Tables 3-4). This observed discrepancy in number of accumulated elements between liver and muscle confirm inter-tissue differences and exhibiting the role of liver in receiving more elements from environment and preventing them to penetrate inside the other tissues. In Table 3, concentrations of accumulated elements in the muscle tissue from the three different regions (Astara, Anzali and Kiashahr) were presented. Comparisons between these regions showed no significant differences (ANOVA and Kruskal – Wallis tests, $p<0.05$). According to Table 4, the detected elements in the liver tissue, were measured in the three different regions including Astara, Anzali and Kiashahr but their concentrations in these regions were not significantly different (ANOVA and Kruskal – Wallis tests, $p<0.05$).

Table 3. Concentrations of trace elements in *Alosa braschnikowy* muscle at three fisheries regions of the South Caspian Sea. P is regarded as being significant (*) if <0.05

Elemental Variables (ppm)	Mean ± SE Range Anzali	 Astara	 Kiashahr	 Total	*P* value
Ag	BDL	BDL	BDL	BDL	-
Al	1.30±0.77 0.54-2.59	1.70±0.66 1.26-2.88	1.25±1.008 0.52-2.71	1.43 ±0.77 0.52-2.88	0.18**
As	0.06±0.01 0.04-0.08	0.06±0.02 0.03-0.09	0.06±0.03 0.03-0.11	0.06±0.02 0.03-0.11	0.96*
Ba	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	0.87**
Ba	BDL	BDL	BDL	BDL	-
Be	BDL	BDL	BDL	BDL	-
Ca	111.14±41.04 76.8-163.2	115.36±42.80 67.8-173.4	193.15±90.11 124.6-319.6	136.07±66.04 67.8-319.6	0.11*
Ce	BDL	BDL	BDL	BDL	-
Cd	0.01±0 0.01-0.01	BDL	BDL	0.01±0 0.01-0.01	0.14**
Co	0.01±0 0.01-0.01	BDL	BDL	0.01 ± 0 0.01-0.01	0.37**
Cr	0.06±0.03 0.04-0.11	0.06±0.03 0.04-0.13	0.03±0.01 0.01-0.04	0.05±0.03 0.01-0.13	0.13**
Cu	0.04±0.008 0.03-0.05	0.06±0.03 0.03-0.11	0.10±0.09 0.05-0.24	0.06±0.05 0.03-0.24	0.05**
Fe	1.37±0.35 1.03-1.87	1.84±1.42 1.12-4.4	1.71±1.16 0.93-3.44	1.63±1.01 0.93-4.4	0.93**
K	235.46±46.99 179-309.3	257.18±73.97 170-353	273.8±92.17 190-396	254.17±67.68 170-396	0.72*
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	0.64**
Mg	19.96±6.28 14-30.1	25.54±4.25 21.1-31.5	23.25±8.38 16.2-34.9	22.89±6.32 14-34.9	0.40*
Mn	0.06±0.01 0.05-0.08	0.08±0.02 0.06-0.11	0.05±0.03 0.03-0.11	0.06±0.02 0.03-0.11	0.99*
Mo	0.01±0 0.01-0.01	BDL	0.03±0.03 0.01-0.06	0.02±0.02 0.01-0.06	0.19**
Na	43.94±17.30 32.3-72.8	93.54±57.78 42-190.4	46.25±26.62 27.4-84.3	62.31±43.21 27.4-190.4	0.12*
Ni	0.01±0.01 0.01-0.03	0.01±0 0.01-0.01	0.01±0 0.01-0.01	0.01±0.007 0.01-0.03	0.84**
P	205.86±51.42 149-283.3	242.08±29.41 217.7-279	249.37±100.69 165.7-390	231.22±61.75 149-390	0.54*
Pb	0.03±0.005 0.03-0.04	0.02±0.005 0.02-0.03	0.05±0.01 0.05-0.07	0.03±0.01 0.02-0.07	0.15*
Rb	0.71±0.33 0.14-1.03	0.70±0.33 0.23-0.96	0.80±0.13 0.7-0.96	0.73±0.27 0.14-1.03	0.67**
S	130.4±39.57 101-200	132.1±23.76 102-152	348.45±371.67 120-898	193.30±207.13 101-898	0.23**
Sb	0.02±0.03 0.01-0.08	0.06±0.06 0.01-0.16	0.03±0.02 0.01-0.05	0.04±0.04 0.01-0.16	0.42**
Si	0.44±0.21 0.21-0.79	0.46±0.18 0.29-0.7	0.35±0.10 0.26-0.51	0.42±0.17 0.21-0.79	0.68*
Sn	0.05±0.04 0.02-0.12	0.08±0.01 0.07-0.09	0.08±0.04 0.05-0.13	0.07±0.03 0.02-0.13	0.76*
Sr	0.16±0.18 0.06-0.49	0.25±0.15 0.13-0.49	0.26±0.20 0.06-0.47	0.22±0.17 0.06-0.49	0.29**
Th	0.07±0.03 0.04-0.12	0.05±0.01 0.04-0.06	0.06±0.01 0.04-0.07	0.06±0.02 0.04-012	0.53*
Ti	0.04±0.02 0.02-0.07	0.02±0.005 0.02-0.03	0.02±0.01 0.01-0.03	0.03±0.01 0.01-0.07	0.42**
U	BDL	BDL	BDL	BDL	-
V	BDL	BDL	BDL	BDL	-
W	BDL	BDL	BDL	BDL	-

Y	BDL	BDL	BDL	BDL	-
Zn	0.53±0.19 0.33-0.83	0.94±0.82 0.54-2.42	0.80±0.85 0.04-2.02	0.75±0.64 0.04-2.42	0.39**

*. Tested by ANOVA **. Tested by Kruskal–Wallis, BDL: below detectable level

Even though there were no significant differences in accumulated elements in liver and muscle between examined regions correspondence analysis revealed that Kiashahr separate from Astara and Anzali according to first dimension (Fig. 2). Second dimension distinguished liver and muscle tissue only exception was for muscle tissue of individuals from Astara (Fig. 1).

Table 4. Concentration of trace elements in fish liver at three fisheries regions of the South Caspian Sea. P is regarded as being significant (*) if <0.05

Elemental Variables (ppm)	Mean ± SE Range				*P* value
	Anzali	Astara	Kiashahr	Total	
Ag	BDL	BDL	BDL	BDL	-
Al	1.81±0.57 0.97-2.3	1.86±1.03 0.29-3.09	1.72±0.86 0.87-2.87	1.80 ±0.76 0.29-3.09	0.96*
As	0.07±0.02 0.05-0.1	0.05±0.02 0.03-0.08	0.07±0.01 0.06-0.09	0.06±0.02 0.03-0.1	0.14*
Ba	0.01±0 0.01-0.01	0.01±0.005 0.01-0.02	0.01±0 0.01-0.01	0.01±0.003 0.01-0.02	0.96**
Ca	76.36±33.50 29.5-115	73.08±27.03 31.1-99.3	131.17±53.42 63.5-190.7	89.88±43.44 29.5-190.7	0.73*
Cd	0.01±0 0.01-0.01	1.01±0 0.01-0.01	0.01±0 0.01-0.01	0.01 ± 0.0 0.01-0.01	0.90**
Co	BDL	1.01±0 0.01-0.01	0.01±0 0.01-0.01	0.01 ± 0 0.01-0.01	0.17**
Cr	0.05±0.01 0.04-0.08	0.06±0.02 0.03-0.09	0.05±0.02 0.04-0.09	0.05±0.02 0.03-0.09	0.54**
Cu	0.11±0.01 0.09-0.15	0.09±0.02 0.07-0.14	0.15±0.05 0.1-0.22	0.12±0.03 0.07-0.22	0.63*
Fe	4.44±1.50 3.04-7.08	6.87±3.76 0.94-10.6	6.04±1.09 4.94-7.54	5.68±2.51 0.94-10.6	0.28*
K	128.58±21.48 107.2-156	132.6±44.68 95-188.2	160.97±38.21 106-187	138.56±35.31 95 -188.2	0.48**
La	BDL	0.1±0 0.1-0.1	BDL	0.1±0 0.1-0.1	0.36**
Li	0.01±0 0.01-0.01	0.01±0.007 0.01-0.02	BDL	0.01±0.005 0.01-0.02	0.37**
Mg	14.76±4.08 10.8-20.4	10.27±9.68 1.43-24.6	15.39±4.31 9.96-20.5	13.43±6.49 1.43-24.6	0.43*
Mn	0.08±0.02 0.06-0.11	0.03±0.005 0.03-0.04	0.08±0.02 0.06-0.11	0.06±0.02 0.03-0.11	0.05*
Mo	0.01±0 0.01-0.01	0.01±0 0.01-0.01	0.01±0 0.01-0.01	0.01±1.83e-18 0.01-0.01	0.90**
Na	66.15±14.97 50.2-89.8	61.1±19.81 44.4-83.8	71.65±15.56 49.6-83.7	65.93±16.18 44.4-89.8	0.65*
Ni	0.01±0 0.01-0.01	0.01±0 0.01-0.01	0.01±0 0.01-0.02	0.01±0 0.01-0.01	0.65**
P	159.81±42.43 118-239.6	138.64±55.19 91-198.7	189.75±46.16 130-241	160.74±48.84 91-241	0.31*
Pb	0.07±0.01 0.06-0.09	0.07±0.01 0.06-0.09	0.06±0.005 0.06-0.07	0.07±0.01 0.06-0.09	0.98**
Rb	0.61±0.41 0.05-1.12	0.64±0.42 0.09-1.06	0.91±0.10 0.79-0.98	0.68±0.37 0.05-1.12	0.89**
S	129.9±26.63 105-171.7	135.36±35.08 95-180.1	326±376.87 116-890	184.01±197.23 95-890	0.48**
Sb	0.02±0.01 0.01-0.05	0.05±0.04 0.01-0.1	0.03±0.03 0.01-0.07	0.03±0.03 0.01-0.1	0.20**
Si	0.39±0.13 0.19-0.53	0.36±0.18 0.18-0.61	0.42±0.14 0.26-0.61	0.38±0.14 0.18-0.61	0.85*
Sn	0.05±0.02 0.04-0.09	0.05±0.01 0.04-0.07	0.09±0.03 0.06-0.13	0.06±0.02 0.04-0.13	0.40*

Sr	0.09±0.06 0.03-0.2	0.11±0.09 0.06-0.29	0.11±0.05 0.06-0.19	0.10±0.07 0.03-0.29	0.46**
Th	0.06±0.02 0.05-0.09	0.06±0.01 0.05-0.07	0.07±0.05 0.03-0.13	0.06±0.02 0.03-0.13	0.84*
Ti	0.03±0.01 0.02-0.05	0.02±0.01 0.01-0.04	0.04±0.02 0.02-0.06	0.03±0.01 0.01-0.06	0.62*
U	BDL	BDL	BDL	BDL	-
V	BDL	1.01±0 0.01-0.01	BDL	0.1±0 0.1-0.1	0.36**
W	0.01±0 0.01-0.01	BDL	0.01±0 0.01-0.01	0.1±0 0.1-0.1	0.19**
Y	BDL	BDL	BDL	BDL	-
Zn	1.28±0.34 0.89-1.9	1.08±0.37 0.72-1.68	1.51±0.52 1.06-2.25	1.27±0.41 0.72-2.25	0.31*

*. Tested by ANOVA, **. Tested by Kruskal–Wallis , BDL: below detectable level

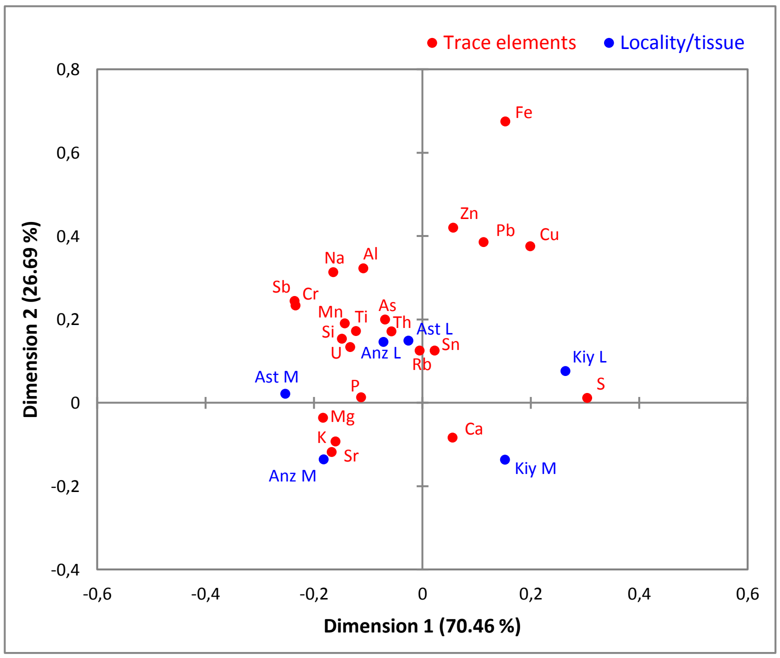

Fig 2. Correspondence analysis of trace elements in muscle (M) and liver (L) of examined individuals from three regions (Kiashahr- Kiy, Astara- Ast and Anzali- Anz).

MSI in fish muscle from Astara and Anzali showed almost similar values, while values for MSI displayed different values in Kiashahr (Table 5). Al, As, Cr, Ni and Zn in Kiashahr exhibited lower values, while S, Cu and Pb were higher in this region in comparison with Astara and Anzali. The MSI values of other elements showed similar values in all the three regions.

Numerous reports have been published on heavy metal pollution in aquatic environments of Iran, but there is no report on elements contamination in *A. braschnikowy* from the South Caspian Sea. This is the first report about its contamination in Iran. Few elements have been measured in these reports (including Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb, and Zn) mostly using atomic absorption spectrophotometry.

The maximum average concentrations of Cr, Mo, Sr were assayed in muscle tissue while Cu and Fe were found in liver. In both tissues, the highest level was related to Fe which was 8.15 mg/kg in muscle and 28.4 mg/kg in liver, while Ba, Ni and Cd had low levels.

In the present study, the Cd, Pb, Cu and Zn levels in muscle tissue were lower than in the liver which may be due to some physiological functions (forming complexes) [26]. One of these complexes is called metalothionein, a protein which forms in liver has affinity to bind with some elements such as Zn and

Cu. Liver is a main organ in detoxification of toxins as well as accumulation of elements and their transfer into kidney to excrete from urine. The liver is also organ with higher metabolic functions, highly vascularized and very important in exhibit pathologic effects of pollutants in fish. The results of different studies illustrated the liver affinity for accumulation of elements in higher levels [26]. Jaric et al. [27] in a study on *Acipenser ruthenus* from Danube River reported that Al, Ba, Sr concentrations in fish muscle were higher than in liver tissue, while As, Cd, Cu, Fe, Mo, Ni, Zn and Li in liver had higher levels than in muscle which different from our results. Visnjic-Jeftic et al. (2010) [28] studied the bioaccumulation of heavy metals and microelements in *Alosa immaculate* tissues from Danube River and reported that there were significant differences between fish tissues from the point view of element bioaccumulations. So that Cd, Cu, Zn and Fe exhibited higher concentrations in liver, while in muscle had higher level in muscle. Hence only Cu and Fe had similar accumulation with the results of our study. In the present study, lower concentration of Zn in liver tissue may be in association with increased level of 17 β - estradiol in female fish. The increased secretion of this hormone in reproduction period has been reported to result in decreased level of Zn in liver [29] followed by its elevated amount in circulation and gonads. However, its level was reduced in the liver tissue of both male and female which may be due to the formation of its complex with methalothionin.

Yilmaz et al. [26] studied the accumulation of some elements including Cu, Fe, Mn, Zn, Cr and Al in liver, gill, intestine, skin and muscle of *Tinca tinca* from a lake (Siddikli Kucukbogaz) in Turkey and reported that the highest levels of Cu and Fe were found in liver which is in line with the results of the present study.

Arsenic (AS): Lethal oral consumptions of As to humans are lower than animals. So that, the oral arsenic LD_{50} is 15-112 mg/kg [30].The arsenic levels in fish species of Iran were reported in literature (Table 6). The As mean level (±SD) in the present study was 0.06 ± 0.02 mg kg^{-1} d.w. in both muscle and liver tissues of *A. braschnikowi* which was lower than international standards [31].

Lead (Pb): A provisional tolerable weekly intake of Pb as 0.025 mg/kg body weight was recommended bt the Joint FAO/WHO Food Standards Program Codex Committee (2011), while the European Community suggested maximum Pb levels suggested as 0.2 mg kg^{-1} f.w. in seafood. Some authors have documented Pb levels in the fish muscle (Table 6). The Pb levels (± SD) in the present study were 0.03 ± 0.01 and 0.07 ± 0.01 mg kg^{-1} in the muscle and liver of *A. braschnikowi* respectively, lower than those permitted by FAO/WHO standards [31].

Copper (Cu): Copper as 3.5 mg/kg body weight weekly has been recommended by the FAO/ WHO Expert Committee for Food Additives [32]. Some authors have reported the mean concentrations of Cu in fish species from Iran (Table 6). The mean levels (± SD) of Cu in the present study were 0.06 ± 0.05 and 0.12 ± 0.03 mg kg^{-1} in the muscle and liver of *A. braschnikowi* respectively, lower than those permitted by FAO/ WHO standards (amended in 2018) [33].

Chromium (Cr): Cr is proposed as 50-200 µg for daily consumption [32]. The dietary Cr level is important for insulin function and lipids metabolism [4]. Some documents were reported about the mean Cr levels (Table 6). The mean Cr levels (± SD) in the present study were 0.05 ± 0.03 and 0.05 ± 0.02

mg kg^{-1} in muscle and liver tissues of *A. braschnikowi* respectively, lower than those recommended by FAO/WHO standards [33,34].

Cobalt (Co): There are some reports in literature about the mean Co levels in different fish species (Table 6), but in the present study, the mean level (± SD) of cobalt was 0.01 ± 0.0 mg kg^{-1} in both muscle and liver tissues of *A. braschnikowi*.

Iron (Fe): The dietary allowance for iron was suggested to be 10 mg/day for elderly peoples by US National Academy of Science (RDA 1989) [31] the mean Fe levels in muscles of different species were documented (Table 6). The mean levels (± SD) of Fe in the present study were 1.63 ± 1.01 and 5.68 ± 2.51 mg kg^{-1} in the muscle and liver tissues of *A. braschnikowi* respectively.

Manganese (Mn): It pointed out that Mn deficiency is critical and causes some clinical signs such as severe birth defects, convulsions, asthma etc. [36]. There are some reports about the Mn levels (Table 6). The mean level (± SD) of Mn in the present study was 0.06 ± 0.02 mg kg^{-1} in both muscle and liver of *A. braschnikowi* respectively, lower than those suggested by FAO/ WHO standards [30-34].

Nickle (Ni): 100-300 µg Ni for daily intake was recommended by the World Health Organization [33]. Bulgarian Food Codex suggested the maximum Ni level for fish as 0.5 mg/kg. Some reports documented Ni levels in muscle of some fish species (Table 6). In the present study, the mean Ni levels (± SD) were assayed as 0.01 ± 0.007 and 0.01 ± 0.0 mg kg^{-1} in muscle and liver of *A. braschnikowi* respectively which were lower than those permitted by Codex Alimentarius Commission [33].

Vanadium (V). Janadele & Kardani, (2016) [37] only reported vanadium levels (± SD) in the fish muscle in Iran. However, in the present study, vanadium was not detected *in A. braschnikowi* muscles, but just found in the fish liver and its mean level (± SD) was 0.1 ± 0.0.

Zinc (Zn): The PTWI for Zn was documented to be 7 mg kg^{-1} body weight/week by FAO/WHO (2004). The mean Zn levels were documented in the fish muscles from Iran (Table 6). In the present study, the mean zinc levels (± SD) were 0.75 ± 0.64 and 1.27 ± 0.41 mg kg^{-1} in muscle and liver of *A. braschnikowi* respectively, lower than those suggested FAO/WHO standards [33].

Conclusion

Based on presented results we can conclude that the mean levels of accumulated element 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) and the FAO/WHO. The element levels in the *A. braschnikowi* muscles were also lower than the values reported in the previous studies from the Caspian Sea. These levels could not result in any threat to human health. We recommend that TEs monitoring in aquatic organisms and the Caspian environment should be conducted to find out the TE trends over the time.

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