Trace and Macro Elements Bioaccumulation in the Muscle and Liver Tissues of *Alburnus chalcoides* from the South Caspian Sea and Potential Human Health Risk Assessment

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Abstract: Danube bleak *Alburnus chalcoides* (Güldenstädt, 1772) is a commercially important fish species in the southern part of the Caspian Sea. However, little is known about the concentrations of trace element (TE) in its muscles. To address the issue, we caught 77 *A. chalcoides* specimens at three different fishing regions (including Astara, Anzali, Kiashahr) of the southern shoreline of the Caspian Sea from September 2017 through June 2018. An inductively coupled plasma optical emission spectrometry (ICP-OES) was used to measure element concentrations in fish muscle and liver tissues. In the case of liver, 27 elements were detected. So that, 9 elements including Ag, Ba, Be, Ce, Co, Sc, U, V and Y which were not detected in liver. In the case of muscle tissue, 22 elements were detected and 14 elements including Ag, Ba, Be, Bi, Cd, Ce, Co, La, Li, Sc, U, V, W and Y. The element values in the muscle of *A. chalcoides* were significantly lower (p<0.05) than the maximum permitted levels suggested by International standards.

Keywords: *A. chalcoides*, Caspian Sea, Trace Elements, Human Health

1. Introduction

Heavy metals are introduced to aquatic environments through natural and anthropological activities. These elements may sink toward the bottom sediment or accumulate directly in marine ecosystem. The accumulation process is affected by different environmental and biological factors such as salinity, temperature, season, size, sex and species. Heavy metals enter aquatics through skin, gill and food. The strict periodic surveillance of these elements is therefore advisable. Toxic metals pollution is less visible and direct than other types of marine pollution but its effects on marine ecosystems and humans are intense and very extensive [1]. Fish are notorious for their ability to concentrate heavy metals in their muscles and they also play important role in human nutrition. So, it is important to carefully screen the bioaccumulation and exposure levels of heavy metal to ensure that unnecessary elevated level of some toxic metals is not being transferred to human through fish consumption. The presence of metal in aquatic environment may lead to its accumulation in organisms through different mechanisms: via the direct uptake from water through gill or skin (bio-concentration), via the uptake of suspended particles (ingestion) and via the consumption of contaminated food (bio-magnification) [2]. More attention has been paid on developed and developing countries about anomalous distribution of metals in the water, sediments and fishes which are important in understanding the behavior of the metals and also the swapping between the sediments and
the water column [3].

The Caspian Sea is the largest land - enclosed drainage in the world, which supports substantial fisheries. However, it has been endangered by massive loads of contaminants. The various environmental pollutants produced by natural and anthropogenic sources are discharged from coastal catchment [4, 5]. The hazardous effects of heavy metals on human health are reviewed by international organizations such as the WHO. Bioaccumulation is defined as an increase in the concentration of pollutants by an increase in age, which sometimes is reflected in the size of the organisms [6]. Metal bioaccumulation is influenced by various environmental and biological factors particularly the feeding source [7, 8].

Danube bleak *Alburnus chalcoides* (Güldenstädt, 1772) belonging to Actinopterygii (ray-finned fishes), order Cypriniformes (Carps), family Cyprinidae (Minnows or carps), subfamily Alburninae is a Freshwater, brackish, pelagic and potamodromous fish (Ref. 51243) in temperate regions. (5°C - 20°C) (Ref. 12468). Its name originates from the city of Al Bura, where the fish was known (Ref. 45335). Its distribution: Europe and Asia: Caspian (mostly western to southern coast, rarely found in Ural and Volga). Populations from Aral Sea basin might belong to this species (Ref. 59043). It is reported from the Black Sea basin (Ref. 58342). Max length: 40.0 cm TL male/unsexed; (Ref. 5556); common length: 20.0 cm TL male/unsexed; (Ref. 1441); common length: 28 cm TL (female). It Inhabits lower reaches of rives, coastal lakes, estuaries, and brackish areas of sea and occur close to surface and tolerate salinities up to 14 ppt. Adults predominantly prey on planktonic crustaceans, terrestrial insects and small fish while larvae and young juveniles feed on zooplankton, algae and insect larvae. Spawn in small rivers or streams with heavy current on gravel bottom. There are numerous reports on heavy metal bioaccumulation in aquatic environments of Iran including Bibak et al. (2018) [9] who assessed element accumulation in sediments from the northern Persian Gulf; Eslami et al., (2011) [10] heavy metals in muscle and liver of *Perca fluviatilis* and *Tinca tinca* in Anzali Wetland; Heydari et al. (2011) [11] on *Acipenser stellatus*, Monsef et al., (2012a, 2012b) [12, 13] on *Rutilus frisii kutum*. There are also some reports on TE concentrations in *R. kutum* [12-15], but there is no report on TE contamination in Danube bleak, *A. chalcoides*. Therefore, the aim of this study was to determine the levels of some trace elements (TEs) in edible parts of *A. chalcoides* collected from the coast of the Caspian Sea and compare TE levels in fish caught from the different geographical localities as well as to define risk assessment of these elements for human health.

2. Material and Methods

This study was conducted at five fisheries areas including Astara: 38° 42ʹ 25ʺ N, 48° 86ʹ 87ʺ E, Anzali: 37° 46ʹ 39ʺ N, 49° 47ʹ 99ʺ E, Kiashahr: 37° 42ʹ 20ʺ N, 49° 94ʹ 95ʺ E, Sari: 36° 78ʹ 39ʺ N, 54° 04ʹ 64ʺ E and Torkaman port 36° 89ʹ 28ʺ N, 54° 04ʹ 64ʺ E along the southern shoreline of the Caspian Sea.

Samplings were carried out from September 2017 to June 2018 and a total of 77 specimens of *A. chalcoides* 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. 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.
An inductively coupled plasma – optical emission spectrometry (ICP-OES) (Zarazma Co. Tehran, Iran) was used for the determination of TE concentrations in the samples. Instrumental detection limits for trace elements was 0.02 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.

\[
\text{MSI} = \frac{A}{T} \times 100
\]

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

Statistical analysis

3. Results and Discussion

In the present study, a total of 77 \textit{A. chalcoides} 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), thoriurium (Th), titanium (Ti), uranium (U), vanadium (V), tungsten (W), yttrium (Y) and zinc (Zn).

In the case of liver, 27 elements were detected. So that, 9 elements including Ag, Ba, Be, Ce, Co, Sc, U, V and Y which were not detected in liver, were excluded from statistically analyses. In the case of muscle tissue, 22 elements were detected and 14 elements including Ag, Ba, Be, Bi, Cd, Ce, Co, La, Li, Sc, U, V, W and Y which were not detected in muscle, excluded from analyses. (Tables 3-4).

According to Table 3, in total, 28 elements were detected from the \textit{A. chalcoides} liver. The detected elements in the liver were measured in the three different regions including Astara, Anzali and Kiashahr but their accumulation (except Cu, La and W) in these regions were not significantly different (ANOVA and Kruskal – Wallis tests, p<0.05).

In the case of muscle, 27 elements were detected in Astara, while 25 elements were found in Anzali and 20 elements in Kiashahr, indicating the higher amounts of pollution in Astara than in Anzali and kiashahr respectively. Noteworthy, Astara is closed to some adjacent littoral states of the Caspian Sea which may play an important role in contaminating the sea by exploring and/or exploiting petroleum, reflecting in detection of elements in aquatic organism’s body.

| Table 1. Morphometric characteristics of Alburnus chalcoides from the study regions of the South Caspian Sea. |
|--------------------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                                                   | Total weight     | Total length     | Fork length      | Standard length  | Head Length      | Snout length     | Eye diameter     | Body height       | Body width       |
| Average                                          | 76.78            | 19.38            | 4.14             | 3.38             | 7.84             | 2.24             | 1.93             | 8.75             | 4.56             |
| Max                                              | 395              | 34               | 2.35             | 2                | 3.80             | 1.00             | 1.00             | 4.90             | 2.10             |
| Min                                              | 15               | 13               | 1.65             | 1.65             | 3.30             | 0.9              | 0.80             | 3.50             | 1.90             |
| SD                                               | 83.92            | 5.17             | 4                | 3.80             | 0.89             | 0.31             | 0.13             | 0.88             | 0.47             |

| 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                |

| Table 3. Concentrations of trace elements in Alburnus chalcoides liver at three fisheries regions of the South Caspian Sea. P is regarded as being significant if <0.05. |
|--------------------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Elemental variables (ppm)                        | Anzali Mean±SE Range | Astara Mean±SE Range | Kishahr Mean±SE Range | Total Mean±SE Range | P value |
| Ag                                               | BDL              | BDL              | BDL              | BDL              | -                |
| Al                                               | 1.73±1.15        | 3.85±3.61        | 2.96±1.15        | 3.25±2.75        | 0.64*            |
| As                                               | 0.91-2.55        | 1.48-11.66       | 1.79-4.11        | 0.91-11.66       | 0.23**           |
| Mn                                               | 0.04±0.02        | 0.04±0.007       | 0.06±0.02        | 0.05±0.01        |                   |
| Cr                                               | 0.03-0.06        | 0.03-0.05        | 0.05-0.09        | 0.03-0.09        |                   |
According to Table 4, in total, 24 elements were detected in *A. chalcoides* muscle exhibiting fewer number of elements in the fish muscle than in its liver. The detected elements in the muscle were measured in the three different regions.
including Astara, Anzali and Kiashahr but their concentrations in these regions were not significantly different (One – Way ANOVA and Kruskal – Wallis tests, p<0.05). Among the regions, 24 elements were detected in Astara, while 20 elements were found in Anzali and 14 elements in Kiashahr, indicating the lower amounts of pollution in kiashahr than in Anzali and Astara respectively.

Noteworthy, Sefidrud River which provides the main source of tap (plumbing) water in this area, and consequently nearly free from industrial and urban effluents, enters the Caspian Sea adjacent to the kiashahr which may be a reason for lower number of detected elements in the fish caught from this region.

Table 4. Concentrations of trace elements in Alburnus chalcoides muscle at three fisheries regions of the South Caspian Sea. P is regarded as being significant if <0.05.

| Elemental variables (ppm) | Anzali | Astara | Kiashahr | Total | P value |
|--------------------------|--------|--------|----------|-------|---------|
| Ag                       | BDL    | BDL    | BDL      | BDL   | -       |
| Al                       | 1.44±1.44 | 1.82±1.40 | 3.12     | 1.88±1.31 | 0.63*   |
| As                       | BDL    | 0.05±0.13 | BDL      | 0.06±0.02 | 0.56*   |
| Ba                       | BDL    | BDL    | BDL      | BDL   | -       |
| Be                       | BDL    | BDL    | BDL      | BDL   | -       |
| Bi                       | BDL    | 160.45±95.24 | 158.08±104.4 | 86.7 | 0.07±0.05 | 0.97*   |
| Ca                       | 93.1-227.8 | 158.08±104.4 | 72.5-347 | 86.7 | 0.05-0.2 | 0.56*   |
| Cd                       | BDL    | BDL    | BDL      | BDL   | -       |
| Co                       | BDL    | BDL    | BDL      | BDL   | -       |
| Cr                       | 0.06±0.02 | 0.09±0.05 | 0.14     | 0.09±0.04 | 0.44*   |
| Cu                       | 0.06±0.03 | 0.05±0.01 | 0.06     | 0.05±0.01 | 0.80*   |
| Fe                       | 1.32±1.04 | 1.31±1.04 | 1.45     | 0.58±2.1 | 0.97*   |
| K                        | 200.3±110.5 | 277.3±92.67 | 277.3±92.67 | 181.6 | 249.6±93.06 | 0.50*   |
| La                       | 122.1-278.5 | 180.3-450.6 | 180.3-450.6 | 122.1-450.6 | -       |
| Li                       | BDL    | BDL    | BDL      | BDL   | -       |
| Mg                       | 23.25±12.65 | 26.65±9.88 | 17.2     | 24.84±9.56 | 0.69*   |
| Mn                       | 0.03±0.02 | 0.04±0.008 | BDL      | 0.04±0.01 | 0.15**  |
| Mo                       | 0.02±0.05 | 0.03±0.05 | 0.05±0.01 | 0.03±0.02 | 0.71**  |
| Na                       | 56.05±37.26 | 58.83±17.99 | 58.83±17.99 | 55.25±21.26 | 0.57*   |
| Ni                       | 29.7-82.4 | 37.9-85.3 | 37.9-85.3 | 29.7-85.3 | -       |
| P                        | 236.75±97.93 | 248.9±89.3 | 248.9±89.3 | 234.3±88.1 | 0.57*   |
| Pb                       | 167.5-306 | 187.7-426.2 | 187.7-426.2 | 141.5-426.2 | 0.61**  |
| Rb                       | 0.03±0.03 | 0.04±0.04 | 0.03±0.05 | 0.03±0.05 | 0.36**  |
| Sr                       | 154.3±61.23 | 163.8±73.89 | 163.8±73.89 | 116.8 | 156.2±64.1 | 0.92**  |
| Th                       | 111-197.6 | 105.5-308.8 | 105.5-308.8 | 116.8 | 105.5-308.8 | -       |
| Ti                       | 0.03±0.02 | 0.03±0.02 | 0.03±0.02 | 0.03±0.02 | 0.38*   |
The amount order of elements in the fish muscle in the present study was Zn>Cu>Pb>Cd which is different from those reported by Khanipour et al. (2018) [23] in catfish, (Zn>Cu>Cd>Pb) from Anzali Wetland, Southwest Caspian Sea.

Ali et al. (2011) [24] studied five elements including Cr, Cd, Pb, Zn, Cu in the muscle tissues of A. chalcoides (0.52, 0.1, 1.28, 11.43 and 3.38 mg/kg respectively) which were higher than those reported in the present study (0.47, 0.25, 0.23, 6.1 and 0.255 mg/kg respectively).

In the present study, Ca and Mg were higher in Astara than in the other regions, while Cr, Zn, and Cu had higher levels in kiashahr and S, Pb and K displayed higher concentrations in Anzali. Meanwhile, some elements including Ag, Be, Bi, Ce, La, Sc, U, V and Y were below detectable levels in all the other regions, while Cr, Zn, and Cu had higher levels in kiashahr.

Canli and Atli (2003) [25] reported that in some fish species including P. reticulata Pb in muscle tissue was higher than Cd, while in the present study Cd was higher than Pb which may be due to different pollution sources.

Arsenic (AS): According to ATSDR (1998a) [26] and EPA (2010) [27] lethal oral doses of As in animals are higher than those in humans. The oral LD₅₀ values for arsenic ranged 15 - 112 mg kg⁻¹. The arsenic levels in different fish species were reported by some authors (Table 5). 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. chalcoides which was lower than those permitted by Codex Alimentarius Commission (Table 2) [22].

Lead (Pb): Recommended a provisional tolerable weekly intake of Pb is 0.025 mg/kg body weight [28], while the maximum Pb levels in seafood recommended by the European Community is 0.2 mg kg⁻¹ f.w. in fish. Pb levels in the fish tissues have been documented by some authors (Table 5). 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. chalcoides respectively which was lower than those permitted by international standards (Table 2) [22].

Copper (Cu): Recommended PTWI for copper is 3.5 mg/kg body weight per week [28]. The mean levels of Cu in some fish tissues were reported by some authors (Table 5). The mean levels 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. chalcoides respectively which were lower than those permitted by international standards (Table 2) [22].

Chromium (Cr): The proposed daily feeding of Cr is 50-200 µg [29]. The level of Cr in diet is important in insulin function and also in metabolism of lipids [30]. There are some reports about the mean Cr levels in different fish species (Table 5). 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. chalcoides respectively which were lower than those permitted by international standards including Codex Alimentarius Commission [22].

Cobalt (Co): There are some reports about the mean Co accumulation in different fish species (Table 5), 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. chalcoides

Iron (Fe): The US National Academy of Science [29]. Proposed the allowance for iron in diet to be 10 mg/day for elderly persons. The mean concentrations of Fe in the different species tissues were reported by some authors (Table 5). The mean levels 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. chalcoides respectively.

Manganese (Mn) deficiency leads to severe birth defects, convulsions and asthma [31]. There are some reports about the Mn levels in different fish tissues (Table 5). The mean accumulation (± SD) of Mn in the present study was 0.06 ± **

| Element variable (ppm) | Anzali Mean ± SD | Astara Mean ± SD | Kiahshahr Mean ± SD | Total Mean ± SD | P value |
|------------------------|----------------|----------------|----------------|----------------|--------|
| U                      | BDL            | 0.05±0.008     | BDL            | 0.05±0.007     | 0.78** |
| V                      | BDL            | 0.05±0.07      | BDL            | 0.05±0.07      |        |
| W                      | BDL            | BDL            | BDL            | BDL            | -      |
| Y                      | BDL            | BDL            | BDL            | BDL            | -      |
| Zn                     | 1.21±1.12      | 1.11±0.33      | 1.89           | 1.22±0.54      | 0.47*  |

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

Table 5. Comparison of trace element concentrations in the muscle of Alburnus chalcoides in this study with those of different fish species from the literatures.

| 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 |
|---------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------|
|         | R. kutum     | 0.04±0.20    | 1.0±2.72     | 0.0-0.02     | 3.2±4.1      | 0.42         | 80.9±66.5    | 38.5±30.4    | [20]      |
|         | A. braschnikowy | 0.06±0.02   | 0.01±0.0    | 0.03±0.01   | 0.06±0.02    | 0.01±0.007  | 1.63±1.01   | 0.75±0.64    | [21]      |
|         | A. chalcoides | 0.00±0.0    | 0.10±0.5    | 0.05±0.0    | 1.63±1.01    | 0.75±0.64    | 100          |              | [22]      |

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| Element | Fish species | As Mean ± SE | Cu Mean ± SD | Pb Mean ± SD | Mn Mean ± SD | Ni Mean ± SD | Fe Mean ± SD | Zn Mean ± SD | Reference |
|---------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------|
|         | R. kutum     | 0.04±0.020   | 1.0±2.72     | 0.0-0.02     | 3.2±4.1      | 0.42         | 80.9±66.5    | 38.5±30.4    | [20]      |
|         | A. braschnikowy | 0.06±0.02   | 0.01±0.0    | 0.03±0.01   | 0.06±0.02    | 0.01±0.007  | 1.63±1.01   | 0.75±0.64    | [21]      |
|         | A. chalcoides | 0.00±0.0    | 0.10±0.5    | 0.05±0.0    | 1.63±1.01    | 0.75±0.64    | 100          |              | [22]      |

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0.02 mg kg\(^{-1}\) in both muscle and liver of \(A.\) chalcoides respectively which was lower than those permitted by international standards including Codex Alimentarius Commission [22].

Nickle (Ni): The World Health Organization [32] proposed 100-300 μg Ni for daily dietary consumption. The maximum Ni accumulation permitted for fish is 0.5 mg kg\(^{-1}\) based on Bulgarian Food Codex [32]. There are some reports about the tissue Ni levels in some fish species (Table 5). The mean Ni levels (± SD) in the present study were measured as 0.01 ± 0.007 and 0.01 ± 0.0 mg kg\(^{-1}\) in muscle and liver of \(A.\) chalcoides respectively which were lower than those permitted by international standards such as Codex Alimentarius Commission [22].

Vanadium (V). There is only one report concerning to the vanadium levels (± SD) in the fish tissues in Iran [33]. However, in the present study, vanadium was not detected in \(A.\) chalcoides muscles, but just found in the fish liver and its mean level (± SD) was 0.1 ± 0.0.

Zinc (Zn) [29] proposed the PTWI for Zn to be 7 mg kg\(^{-1}\) body weight/week. The mean concentrations of Zn in some fish species were reported by some authors from Iran (Table 5). The mean zinc levels (± SD) in the present study were 0.75 ± 0.64 and 1.27 ± 0.41 mg kg\(^{-1}\) in muscle and liver of \(A.\) chalcoides respectively which were lower than those recommended by Codex Alimentarius Commission (Table 2) [22].

4. Conclusion

In the present study, Fe exhibited the highest amount in kidney of \(A.\) chalcoides than the other elements, while Sr was higher in fish muscle than in liver. It was also found that pb, Cr and Cu was lower than levels permitted by international standards [22]. Pb in fish caught from Kiashahr was lower than the other regions, while Fe in this region was higher than in the other regions.

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