Trophic transfer, bioaccumulation, and health risk assessment of heavy metals in Aras River: case study—Amphipoda–zander–human

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Abstract
Heavy metals (As, Pb, Cd, and Cu) were traced in a model of the aqueous food chain in the Aras River, located in northwest of Iran. The selected model included the zander (Sander lucioperca L.) and crustacean species known as amphipods (Gammarus sp.) which belong to the food chain of this ray-finned fish. A total of 172 samples (70 fish and 102 amphipods) were collected randomly and analyzed for heavy metals using atomic absorption spectrophotometry (AAS). The results showed that the accumulation of heavy metals in both taxa are in the order of As > Pb > Cd > Cu, and concentrations of heavy metals in fish muscle are higher than Gammarus sp. in all stations in different seasons. Specimens of station (1) displayed the highest heavy metal content due to local industrial activities. The recorded concentrations of As, Pb, and Cd exceed the permissible limits. There is a close correlation between the concentrations of heavy metals in the amphipods and zanders. Target hazard quotient (THQ), total target hazard quotient (TTHQ), and carcinogenic risk (CR) were calculated to assess risks to human health. The average of THQ for As (1.43) exceeded the international standards and presenting health risks to the consumers of this fish species. The TTHQ for heavy metals was estimated higher than 1. At all stations, the value of CR_{Cd} > 1 \times 10^{-3} indicating the degree of carcinogenicity of this metal in all parts of the Aras River. Therefore, according to our results, efficient control measures and regular biomonitoring should be established in this region.

Keywords Heavy metals · Food chain · Biomagnification · Aras River · Human health · THQ

Introduction
Heavy metals are frequently released into the freshwater ecosystems from natural sources such as volcanic eruptions, forest fires, and aerosol formation and artificial sources which include industrial activities, agricultural activities, petroleum refinery waste, and mining (Mohammed et al. 2011). Some metals such as chromium (Cr), nickel (Ni), copper (Cu), and iron (Fe) play a significant role in biological systems, while some heavy metals such as mercury, arsenic, lead, and cadmium are non-essential metals and extremely toxic (Ouyang et al. 2002; Hogan 2010). These metals exist at low concentrations in air, water, and soil, but human activities can increase their concentration in the ecosystems to harmful levels (Zhang et al. 2012). These metals bring in various long-term diseases for humans; for instance, excessive exposure to Cd may give rise to cancer, pulmonary diseases, and alveolitis, while high Zn content has critical effects on reproduction (Tchounwou et al. 2012).

According to previous studies, fish and aquatic invertebrates such as amphipods are suitable bioindicators for monitoring environmental contaminants such as heavy metals (Uysal et al. 2009; Kalyoncu et al. 2012). These organisms often are frequently exposed to metal contaminations and are valid indicative factors in freshwater ecosystems (Asuquo et al. 2004). Studies on bioaccumulation of pollutants in these organisms can be used to assess the trace metal pollution, biomagnification through food chains, and the...
quality of aquatic ecosystems (Kiffney and Clements 1994; Rashed 2001; Jelassi et al. 2015, Subotić et al. 2013a, b).

Dissolved metals in the environmental water are absorbed through fish gills, cuticle of amphipods, and other sensitive organs of aquatic organisms (Guerra-García et al. 2010). Heavy metals may enter the human body indirectly through the food chain (Malvandi and Alahabadi 2019). Zander feeding on amphipods can indirectly transfer heavy metals to human communities (Fig. 1).

Amphipoda is an order of crustaceans with 9000 described species found in marine, freshwater (such as Gammarus sp.), and terrestrial habitats. They are in the food chains of many freshwater organisms such as fish (Glazier et al. 2014). This order has a wide trophic range, feeding as herbivores, detritophages, and a variety of prey (Kunz et al. 2010). One of the predators of Gammarus in freshwater is zander. Zander is a species from the Percidae family frequently found in Aras River (Coad 2016). This fish is an important food resource for human consumers of the region (Nasehi et al. 2013).

Accumulation of heavy metals in zander and Amphipoda is heavily studied (Ahsanullah and Williams 1991; Alundağ and Yiğit 2005; Yıldırım et al. 2008; Fiałkowski et al. 2009; Mazej et al. 2010; Güll et al. 2011; Özpıralak et al. 2012; Subotić et al. 2013a, b; Noël et al. 2013; Strode and Balode 2013; Bach et al. 2014; Alipour and Banagar, 2018; Bessa et al. 2017; Khemis et al. 2017; Jelassi et al. 2019; Kontchou et al. 2020); however, only a limited studies are done in the Aras River (Nasehi et al. 2013; Farsani et al. 2019). Based on the mentioned literature, the accumulation of heavy metals in tissues of different organisms depends on the species, feeding behavior, ecological and physiological conditions, age, sexual characteristics, and migration.

This study aimed to (1) assess the concentration of heavy metals (As, Pb, Cd, Cu) in the edible tissues of zander and its prey (Gammarus), (2) compare seasonal changes of concentrations of these elements, (3) assess cancer and non-cancer risks to humans associated with consumption of zander, and (4) introduce an index group to assess these harmful metals.

Methods and materials

Study area and sampling sites

The study was carried out along Aras River (Fig. 2). Aras River with a length of 1072 km originates from Turkey and stretches for 460 km at the common borderline of Iran, Armenia, and Azerbaijan. This river drains into the Caspian Sea and has one of the most important aquatic
ecosystems in the world with a complex ecological diversity and natural resources (Çelekli et al. 2020). Also, this river is considered as an important drinking water resource for several countries in the region including Iran, Armenia, Azerbaijan, and Turkey. Therefore, it is necessary to investigate the bioaccumulation of heavy metals in the tissue of its inhabitants.

**Sampling**

Based on the habitat, human activities, and topographic characteristics, Aras River was separated into four sections. The field sampling was conducted within the period from October 2017 to November 2020. Totally 172 samples (70 adult fish and 102 Amphipoda) were collected from 21 sites (Table 1). The study and sampling area are shown in Figure 2. The sampling sites were chosen randomly. Samples of fish were collected from the local fishermen retrieving their fishing gear and were washed several times with distilled water. Amphipoda samples were obtained at the same sampling locations and collected by hand and mesh.

**Heavy metal analyses**

After collection, the specimens were put in plastic bags containing powdered ice, transferred to the laboratory, and kept in a freezer at – 20 °C until they were tested. In the laboratory, their total weights were determined using a digital scale with an accuracy of 0.001 g. Extracted muscle (1 ± 0.001 g) and cuticle tissue samples (1 ± 0.001 gr) were stored at – 20 °C. The dissected tissues were separately placed in Petri dishes and put in an oven at 110 °C to dry for 24 h. Dried tissues were grounded in a China mortar and pestle, and 1 g of each one was poured in a 100-ml Erlenmeyer flask, and then 10 ml of 60% nitric acid was added, and the flask was kept for 2 h at room temperature for preliminary digestion. Samples were then put on

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**Table 1** Number of specimens of *Gammarus* sp. and zander per station in different seasons

| Stations | Gammarus sp. | Zander |
|----------|--------------|--------|
|          | Spring | Summer | Autumn | Winter | Spring | Summer | Autumn | Winter |
| St1      | 7      | 7       | 6      | 6      | 5      | 5      | 4      | 4      |
| St2      | 6      | 6       | 6      | 6      | 4      | 4      | 5      | 5      |
| St3      | 6      | 7       | 7      | 6      | 4      | 5      | 4      | 4      |
| St4      | 7      | 6       | 6      | 7      | 5      | 4      | 4      | 4      |
a hot plate at 140 °C for 5 h for the complete digestion of the samples. Then, they were passed through a 42-micron paper filter and water was added to adjust the volume at 25 ml (Mehmood et al. 2020). We included 10 blanks in digestion and analysis procedures as controls. Results of element levels are expressed as μg/g of dry weight.

**Chemical analysis**

Concentrations of the heavy metals in the prepared samples were determined using an atomic absorption spectrometry (Model: Aurora, Trace A11200). Limits of detection for the metals were as follows: As and Pb 0.05 μg/L, Cd 0.01 μg/L, and Cu 0.1 μg/L. Determined element concentration (μg/g of dry weight) in fish muscle tissue in this study was compared to the maximum allowable concentration (MAC) in fish meat used for human diets published by various organizations and other global studies (Table 5).

**Quality control and quality assurance**

All labware was washed with 10% HNO3, and only ultrapure water was used for sample digestion and preparation. All samples were analyzed in duplicate to improve the accuracy of the results. The standard deviation was less than 9% in batch treatments. Results are expressed as mean ± standard deviation. Sample spiking and recovery rates for As, Pb, Cd, and Cu, were 92.5, 95.4, 97.2, and 93.5%, respectively. Quality of analyses was controlled using the certified reference material (DOLT-4-certified) fish muscle, supplied by the National Research Council of Canada (NRC).

**Statistical analyses**

Statistical analyses were performed using SPSS (Ver. 22). The mean and standard error of heavy metal concentrations were calculated. One-way analysis of variance (ANOVA) was performed to compare As, Pb, Cd, and Cu concentrations among stations by season and organism. T-test was used to compare the concentrations of heavy metals between Gammarus sp. body and fish muscle tissue in different stations by season. Mann-Whitney U test was used for comparison of heavy metal total concentrations between Gammarus sp. body and fish muscle tissue in different stations by season. Mann-Whitney U test was used for comparison of heavy metal total concentrations between Gammarus sp. body and fish muscle tissue among seasons; and then, a pairwise comparison was performed using the Mann-Whitney U test. The Pearson correlation test was used to evaluate the correlation between the concentration of heavy metals in the body of Gammarus sp. and fish muscle. For all tests, a two-tailed P value of < 0.05 was considered significant.

**Risk assessment analyses**

**The target hazard quotients (THQ)**

Target hazard quotients (THQ) is an index for assessing the potential non-carcinogenic effects of food materials such as fish species exposed to heavy metal contaminations. If the value of this index is less than 1, it means that the consumed fish have fewer undesirable effects on human, whereas values higher than 1 indicate that eating the fish will have very harmful effects on human. It is calculated following the USEPA (2012) equations:

\[
\text{THQ} = \frac{EF \times ED \times FIR \times C \times \frac{Bw \times TA}{RfD}}{10^{-3}}
\]

where \(EF\) is exposure frequency (365 days/year), \(ED\) exposure duration (70 years); \(FIR\) fish ingestion rate (36 g/person/day; FAO 2005); \(C\) metal concentration (μg/g); \(RfD\) reference oral dose (Table 1; mg/kg bw day-1; USEPA 2012); \(Bw\) average body weight for adult consumer (67 kg); and \(TA\) mean exposure time for non-carcinogens (EF × ED).

**Total target hazard quotient (TTHQ)**

Since the pollution may have been caused by two or more heavy metals, the total THQ index (TTHQ) is used to evaluate the effects of these metals. It was calculated as the sum of the hazard quotients (Chien et al. 2002):

\[
\text{TTHQ} = \text{THQ}_{(As)} + \text{THQ}_{(Pb)} + \text{THQ}_{(Cd)} + \text{THQ}_{(Cu)}
\]

**Carcinogenic risk (CR)**

CR is the cancer risk index for an individual who has used all his/her life the class of food materials that have been exposed to carcinogens (USEPA 2010). Among the studied metals, cadmium, lead, and arsenic are classified as carcinogens (Kortei et al. 2020). This index is calculated using the following formula:

\[
\text{CR} = \frac{EF \times ED \times FIR \times C \times CSF}{Bw \times TA} \times 10^{-3}
\]

According to the New York State Department of Health [NYSDOH (New York State Department of Health) 2007], the CR categories are described as, if CR ≤ 1x10^-6 = Low; 10^-4 to 10^-3 = moderate; 10^-3 to 10^-1 = high; ≥10^-1 = very high. Here, CSF is cancer slope factors (mg/kg day) (Table 2).

Since only zander is directly in the food chain of the people in the region, THQ, TTHQ, and CR were calculated only for this fish species.
The concentrations of heavy metals in the studied specimens

Table 3 presents the concentrations of the studied heavy metals in zander muscles and in the body of the amphipod specimens.

**Arsenic**

The lowest concentration of arsenic (0.58 μg/g of dry weight) in zander was recorded at station 1 in summer and autumn; and the highest concentration (1.25 μg/g of dry weight) was found at station 1 in summer. The values in *Gammarus* sp. specimens (0.40–0.96 μg/g of dry weight) were similar to those in zander (Table 3). In addition, the average concentration (0.838 μg/g of dry weight) in zander muscles were larger compared to those in studies conducted in other regions such as the Danube River in Serbia (0.66 μg/g of dry weight) and the Seine, Escaut, and Rhône Rivers (0.119 μg/g of dry weight) in France (Table 5).

**Cadmium**

The lowest amount of this metal in zander (0.36 μg/g of dry weight) was observed at station 4 in summer and the highest value (1.1 μg/g of dry weight) was found at station 2 in summer (Table 3). The corresponding values for *Gammarus* sp. specimens were recorded at station 4 in summer (0.22 μg/g of dry weight) and at station 2 in summer and autumn (0.84 μg/g of dry weight) in France (Table 5).

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### Table 2

Cancer slope factors and reference oral doses for some heavy metals. The acceptable range of the risk limit is $10^{-6}$ to $10^{-4}$

| Heavy metal | Reference doses | Oral slope factors | References |
|-------------|-----------------|--------------------|------------|
| Arsenic     | $3 \times 10^{-4}$ | 1.5                | USEPA 2012; Kortei et al. 2020 |
| Lead        | $4 \times 10^{-3}$ | $8.5 \times 10^{-3}$ |           |
| Cadmium     | $1 \times 10^{-3}$ | 6.3                | USEPA 2012; IRIS 2019b; Kortei et al. 2020 |
| Copper      | $4 \times 10^{-2}$ |                    |            |

### Table 3

Comparison of As, Pb, Cd and Cu concentration (μg/g of dry weight) among stations by season and organism. Boldface numbers indicate statistically significant differences

| Heavy metal | Stations   | Gammarus sp. | Zander |
|-------------|------------|---------------|--------|
|             | Spring Mean ± SE | Summer Mean ± SE | Autumn Mean ± SE | Winter Mean ± SE | Spring Mean ± SE | Summer Mean ± SE | Autumn Mean ± SE | Winter Mean ± SE |
| Pb          |            |               |        |                |            |               |        |                |
| St1         | 0.51 ± 0.02 | 0.51 ± 0.03   | 0.56 ± 0.02 | 0.49 ± 0.02   | 0.51 ± 0.02 | 0.72 ± 0.05   | 0.84 ± 0.04   | 0.70 ± 0.03   |
| St2         | 0.51 ± 0.14 | 0.45 ± 0.12   | 0.49 ± 0.12 | 0.50 ± 0.08   | 0.51 ± 0.14 | 0.63 ± 0.14   | 0.75 ± 0.16   | 0.77 ± 0.09   |
| St3         | 0.62 ± 0.98 | 0.66 ± 0.09   | 0.61 ± 0.10 | 0.63 ± 0.13   | 0.62 ± 0.10 | 0.93 ± 0.08   | 0.81 ± 0.07   | 0.89 ± 0.12   |
| St4         | 0.40 ± 0.03 | 0.46 ± 0.04   | 0.50 ± 0.06 | 0.62 ± 0.07   | 0.50 ± 0.07 | 0.65 ± 0.04   | 0.75 ± 0.07   | 0.84 ± 0.06   |
| F (p)       | 1.01 (0.421) | 1.53 (0.257) | 0.49 (0.695) | 0.80 (0.518) | 0.37 (0.776) | 2.83 (0.895) | 0.20 (0.895) | 1.03 (0.412) |
| Cd          |            |               |        |                |            |               |        |                |
| St1         | 0.59 ± 0.03 | 0.44 ± 0.04   | 0.37 ± 0.03 | 0.35 ± 0.03   | 0.83 ± 0.05 | 0.77 ± 0.04   | 0.64 ± 0.09   | 0.50 ± 0.03   |
| St2         | 0.79 ± 0.02 | 0.84 ± 0.03   | 0.84 ± 0.01 | 0.83 ± 0.01   | 0.93 ± 0.05 | 1.10 ± 0.07   | 1.03 ± 0.06   | 1.02 ± 0.03   |
| St3         | 0.43 ± 0.04 | 0.27 ± 0.02   | 0.49 ± 0.04 | 0.50 ± 0.05   | 0.73 ± 0.05 | 0.42 ± 0.03   | 0.66 ± 0.07   | 0.68 ± 0.05   |
| St4         | 0.42 ± 0.08 | 0.22 ± 0.01   | 0.29 ± 0.04 | 0.37 ± 0.04   | 0.69 ± 0.10 | 0.36 ± 0.03   | 0.44 ± 0.06   | 0.44 ± 0.03   |
| F (p)       | 12.17 (0.001) | 111.05 (0.0001) | 48.41 (0.0001) | 38.50 (0.0001) | 2.68 (0.094) | 53.91 (0.0001) | 11.95 (0.0001) | 55.84 (0.0001) |
| Cu          |            |               |        |                |            |               |        |                |
| St1         | 0.32 ± 0.03 | 0.37 ± 0.06   | 0.34 ± 0.03 | 0.44 ± 0.05   | 0.48 ± 0.04 | 0.61 ± 0.04   | 0.54 ± 0.03   | 0.69 ± 0.05   |
| St2         | 0.51 ± 0.15 | 0.54 ± 0.15   | 0.52 ± 0.17 | 0.63 ± 0.10   | 0.71 ± 0.17 | 0.75 ± 0.14   | 0.69 ± 0.15   | 0.88 ± 0.05   |
| St3         | 0.62 ± 0.09 | 0.67 ± 0.11   | 0.55 ± 0.08 | 0.61 ± 0.10   | 0.87 ± 0.12 | 0.85 ± 0.07   | 0.67 ± 0.07   | 0.78 ± 0.15   |
| St4         | 0.35 ± 0.05 | 0.43 ± 0.03   | 0.37 ± 0.04 | 0.35 ± 0.02   | 0.55 ± 0.04 | 0.64 ± 0.04   | 0.54 ± 0.05   | 0.54 ± 0.01   |
| F (p)       | 2.46 (0.113) | 1.74 (0.212) | 1.14 (0.371) | 3.10 (0.067) | 2.68 (0.094) | 1.67 (0.226) | 0.93 (0.454) | 2.92 (0.077) |
| As          |            |               |        |                |            |               |        |                |
| St1         | 0.49 ± 0.03 | 0.44 ± 0.04   | 0.40 ± 0.03 | 0.47 ± 0.02   | 0.79 ± 0.06 | 0.64 ± 0.05   | 0.58 ± 0.05   | 0.73 ± 0.06   |
| St2         | 0.71 ± 0.13 | 0.96 ± 0.06   | 0.84 ± 0.10 | 0.74 ± 0.11   | 0.97 ± 0.11 | 1.25 ± 0.23   | 1.10 ± 0.09   | 0.97 ± 0.05   |
| St3         | 0.75 ± 0.11 | 0.54 ± 0.09   | 0.58 ± 0.08 | 0.58 ± 0.05   | 1.16 ± 0.23 | 0.81 ± 0.07   | 0.84 ± 0.09   | 0.78 ± 0.04   |
| St4         | 0.48 ± 0.05 | 0.48 ± 0.04   | 0.47 ± 0.04 | 0.45 ± 0.05   | 0.71 ± 0.06 | 0.69 ± 0.06   | 0.78 ± 0.08   | 0.66 ± 0.05   |
| F (p)       | 2.55 (0.105) | 16.25 (0.0001) | 7.46 (0.004) | 3.94 (0.036) | 2.25 (0.135) | 4.72 (0.021) | 7.04 (0.006) | 7.40 (0.005) |
µg/g of dry weight). The average value for this metal (0.7 µg/g of dry weight) in zander at the four studied stations was smaller than other studies including those in Kayseri and Lake Beyşehir in Turkey (2.52 µg/g of dry weight; Yıldırım et al. 2008 and 2.17 µg/g of dry weight; Özparlak et al. 2012, respectively) and larger than those in studies conducted in other regions such as the Danube River in Serbia (0.005 µg/g of dry weight; Subotić et al. 2013a, b), Seine, Escaut, and Rhône Rivers in France (0.001 µg/g of dry weight; Noël et al. 2013); Sidi Salem Reservoir in Tunisia (0.171 µg/g of dry weight; Khemis et al. 2017); Hirfanlı Dam in Turkey (0.25 µg/g of dry weight; Gül et al. 2011); Caspian Sea (0.09 µg/g of dry weight; Alipour and Banagar. 2018); and the Velenjsko jezero in Slovenia (˂ 0.01 µg/g of dry weight; Mazej et al. 2010).

**Lead**

The lowest amount of this metal in zander (0.5 µg/g of dry weight) was measured at station 4 in spring and the highest (0.93 µg/g of dry weight) at station 3 in summer. The corresponding values for *Gammarus* sp. specimens were (0.40 µg/g of dry weight) at station 4 in spring and (0.66 µg/g of dry weight) at station 3 in summer (Table 3). The average value of concentration (0.71 µg/g of dry weight) in zander was smaller than those reported from Beyşehir Lake in Turkey (0.162 µg/g of dry weight; Özparlak et al. 2012) and larger than those recorded from Kayseri Lake in Turkey (˂ 0.03; Yıldırım et al. 2008), Seine, Escaut, and Rhône Rivers in France (0.008 µg/g of dry weight; Noël et al. 2013), Sidi Salem Reservoir in Tunisia (0.046 µg/g of dry weight; Khamis et al. 2017), Hirfanlı Dam in Turkey (0.65 µg/g of dry weight; Gül et al. 2011), Caspian Sea (0.53 µg/g of dry weight; Alipour and Banagar. 2018), and Velenjsko jezero in Slovenia (˂ 0.03 µg/g of dry weight; Mazej et al. 2010).

**Copper**

The smallest amount of this metal (0.48 µg/g of dry weight) was recorded at station 1 in spring and the largest (0.88 µg/g of dry weight) at station 2 in winter. The corresponding values for *Gammarus* sp. specimens were (0.34 µg/g of dry weight) at station 1 in autumn and (0.67 µg/g of dry weight) at station 3 in spring, respectively (Table 3).

**Seasonal variations in heavy metal concentrations**

Statistically significant differences were not found among *Gammarus* sp. specimens and zander muscle tissues of various stations in each season for Pb and Cu concentrations. However, significant differences were found among *Gammarus* sp. specimens of various stations in each season for concentrations of Cd and As (except in spring, for the concentrations of Cd); also, the concentrations of Cd and As were different among stations for zander samples in each season, except spring (Table 4). A pairwise comparison showed that Cd concentration of *Gammarus* sp. specimens from stations 1 and 2 are statistically different from each other and from stations 3 and 4 in the spring and summer; also, stations 2 and 3 are different from each other and from the other two stations in the autumn and winter. Station 1 was the only site where the measured concentrations in zander significantly differed from all other stations in every season.

Concentrations of the measured heavy metals in fish muscle were consistently higher than its amphipod prey at each station in different seasons (Table 4). No pattern was found to the significance of these differences. However, it was observed that at station 1 for all heavy metals (except Pb in spring), the concentrations in the muscle of fish significantly differed from the concentrations in *Gammarus* sp. body. Also, the concentrations of Cd in the muscle of zander were significantly higher than *Gammarus* sp. body in most stations by season (Table 4). The differences in concentrations of heavy metals among seasons in *Gammarus* sp. body are not statistically significant; with the exception of lead (Kruskal–Wallis χ² = 18.65, p < 0.0005), this pattern was also observed for other heavy metals in fish muscle. A pairwise comparison showed that the amount of Pb in the fish muscle in spring is significantly lower than other seasons.

The total concentration of each heavy metal in the muscle of fish was significantly higher than *Gammarus* sp. body

| Table 4 | Comparison of Pb, Cd, Cu, and Zn concentrations between fish muscle and *Gammarus* sp. body by season and station. S: significant; NS: not significant |
|---------|---------------------------------------------------------------|
|          | Heavy metal | Spring | Summer | Autumn | Winter |
|          |             | St1    | St2    | St3    | St4    | St1 | St2 | St3 | St4 | St1 | St2 | St3 | St4 |
| Pb       |             | NS     | NS     | NS     | NS     | S   | S   | S   | S   | S   | NS  | NS  | NS  | NS  |
| Cd       |             | S      | S      | S      | NS     | S   | S   | S   | S   | S   | NS  | NS  | S   | S   |
| Cu       |             | S      | NS     | NS     | S      | S   | S   | S   | NS  | NS  | NS  | S   | NS  | NS  | S   |
| As       |             | S      | NS     | NS     | S      | S   | N   | S   | S   | S   | S   | S   | S   | S   | S   |

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Table 5 Comparison of Pb, Cd, Cu, and As concentration (μg/g of dry weight) between Gammarus sp. body and zander muscle

|       | Cd Gammarus sp. | Zander | Pb Gammarus sp. | Zander | Cu Gammarus sp. | Zander | As Gammarus sp. | Zander |
|-------|----------------|--------|----------------|--------|----------------|--------|----------------|--------|
| Mean ± SE | 0.50 ± 0.03 | 0.70 ± 0.03 | 0.53 ± 0.02 | 0.71 ± 0.03 | 0.48 ± 0.02 | 0.67 ± 0.03 | 0.59 ± 0.03 | 0.84 ± 0.03 |
| Z     | -4.395        | -5.237 | -5.497         | -6.040 |                |        |                |        |
| p     | < 0.0005      | < 0.0005 | < 0.0005      | < 0.0005 |                |        |                |        |

Table 6 Comparison of heavy metals concentration (μg/g of dry weight) in muscle of zander in this study with different standards and other studies worldwide

| Standards                     | Fish species | Cd | As | Pb | Cu | References               |
|-------------------------------|--------------|----|----|----|----|--------------------------|
| FAO/WHO limits                | S. lucioperca| 2.52 | 0.03 |     |    | FAO/WHO 2003             |
| European commission (EC)      | S. lucioperca| 0.005 | 0.66 | 1.3 |    | EC 2006                  |
| CODEX                         | S. lucioperca| 2.17 | 1.62 | 3.38 |    | FAO/WHO Codex Alimentarius 2009 |
| Kayseri, Turkey               | S. lucioperca| 0.643 | 0.678 |    |    | ALTUNDAĞ AND YİĞİT 2005  |
| Danube River, Serbia          | S. lucioperca| 0.09 | 0.53 |    |    | ÖZPARLAK ET AL. 2012     |
| Beyşehir Lake, Turkey         | S. lucioperca| 0.25 | 0.4  | 0.65 |    | ALTUNDAĞ AND YİĞİT 2005  |
| Caspian Sea, Iran             | S. lucioperca| 0.171 | 0.046 |    |    | ALTUNDAĞ AND YİĞİT 2005  |
| Velenjsko jezero, Slovenia    | S. lucioperca| 0.01 | 0.03 |    |    | MAZEJ ET AL. 2010        |
| Hirfanlı dam, Turkey          | S. lucioperca| 0.001 | 0.119 |    |    | NOËL ET AL. 2013         |
| Sidi Salem, Tunisia           | S. lucioperca| 0.003–0.01 | 0.007–0.42 | 0.23–3.3 |    | POPOV ET AL. 2012       |
| Aras river, Iran              | S. lucioperca| 0.7 | 0.838 | 0.71 | 0.67 | PERESN STUDY             |

(Table 5). The values obtained for concentrations of As, Pb, and Cd in zander muscle tissues were higher than the permissible limits in the standards set by various organizations (Table 6).

Health risk assessment

The THQs of the heavy metals in all stations were: As > Cd > Pb > Cu.

Values of target hazard quotient (THQ) and total target hazard quotient (TTHQ) were calculated for zander. The values of THQ for arsenic were higher than 1 at all stations among the four studied heavy metals. The values of TTHQ were higher than 1 at all stations, but were the highest (> 2) at station 2. The values of CR_{Cd} at all stations were more than $10^{-3}$, indicating the high level of human carcinogenicity for this metal in Aras River. CR_{As} with a value more than $10^{-4}$ ranked second in this regard indicating an intermediate level of human carcinogenicity (Table 7).

Our data showed positive relationships between heavy metal concentrations in the prey body (amphipod) and predator muscle (zander) (Table 8); the values of the studied

Table 7 Values of target hazard quotient (THQ), total target hazard quotient (TTHQ), and lifetime cancer risk (CR) calculated for zander. Blue highlighted values exceed recommendations

| Station | THQ_{As} | TR_{As} | THQ_{Pb} | TR_{Pb} | THQ_{Cd} | TR_{Cd} | THQ_{Cu} | TTHQ |
|---------|----------|---------|----------|---------|----------|---------|----------|------|
| ST1     | 1.17     | 5.2×10^{-4} | 0.08    | 3.15×10^{-6} | 0.35    | 2.3×10^{-3} | 0.00 | 1.6 |
| ST2     | 1.83     | 8.2×10^{-4} | 0.08    | 3.01×10^{-6} | 0.52    | 3.4×10^{-3} | 0.00 | 2.43 |
| ST3     | 1.52     | 6.8×10^{-4} | 0.1     | 3.54×10^{-6} | 0.31    | 2.09×10^{-3} | 0.01 | 1.94 |
| ST4     | 1.21     | 5.4×10^{-4} | 0.08    | 3.10×10^{-6} | 0.24    | 1.62×10^{-3} | 0.00 | 1.53 |
| Average | 1.43     | 6.4×10^{-4} | 0.085   | 3.2×10^{-6} | 0.35    | 2.35×10^{-3} | 0.002 | 1.87 |
heavy metals in zander were significantly higher than the amphipod (Table 5) confirming the principle of biomagnification. Moreover, among the four heavy metals, the mean values of lead concentrations in zander (0.84 μg/g of dry weight) and in the amphipod (0.59 μg/g of dry weight) were higher, which indicated the high sensitivity of zander to lead. The calculated THQ for arsenic exceeded the international standards and showing harm for the consumers of this fish. At all stations, the values of CR_Cd were > 1 × 10^{-3}, which showed the degree of carcinogenicity of this metal in all parts of the Aras River.

Unfortunately, no comprehensive research has been conducted on the heavy metals in the bodies of organisms living in the Aras River except for the study by Nasehi et al. (2012, 2013) who investigated the concentrations of heavy metals in carp muscles and did not detect any cadmium or lead deposition in this fish species. However, in the present research, the amounts of both of these metals at station 2 exceeded the international standards in all seasons. This indicates extremely high quantities of these two metals deposited in zander species among all our stations. The calculated value of TTHQ (> 2) for station 2 indicates the critical situation in the area, which probably resulted from the application of pesticides and chemical fertilizers.

In general, the amounts of the studied metals in zander exhibited ascending trends in spring and summer compared to the other two seasons mainly because of the extensive use of phosphorus fertilizers in agriculture and also the greater activity of industries and mines. In fall and winter of this cold region the use of phosphorus fertilizers and the activities in the industries and mines decrease leading to reduced pollution.

### Conclusions

Considering the high values of CR_Cd (> 10^{-3}) and CR_As (> 10^{-4}), especially in spring and summer, if management measures concerning entrance of pesticides and chemical fertilizers and effluent discharges from the adjacent mines into the river tributaries are not efficiently taken, irreparable damage will be inflicted on the sensitive ecosystems of this river and the human communities living close to it. In addition, periodic assessment of the various contaminants in this river, especially in the areas located at station 2, can be an effective step in becoming aware of the environmental situation. Based on the present research, it is recommended that zander and amphipods can be used as bioindicators in assessing the extent of pollution in the various parts of the Aras River and its tributaries as the introduced models are abundantly and easily available in this river.

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### Author contribution

Amir Dehghani collected the materials and analyzed and interpreted the heavy metals. Atabak Roohi Aminjan performed the analyses of heavy metals. Allahverdi Dehghani was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

### Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

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