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Bioaccumulation of Trace Elements and Health Risk Predictions in Edible Tissues of the Marsh Frog

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Abstract

Certain amphibian species have long served as a valuable protein source for humans, in addition to being good bioindicators for environmental pollutants. Hence, to investigate the consumption outcomes leading to potential health risks, we determined the trace element (TE) levels in the hind leg and liver tissues of marsh frogs (*Pelophylax ridibundus*), one of the delicacies of several cuisines today. The sediment, water, and frog tissue samples were collected from 15 different locations of NE Turkey and analyzed to determine the arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), vanadium (V), and zinc (Zn) concentrations. The TE concentrations in the sediment, water, and individuals were detected to show significant variations among sampling stations ($p < 0.05$). Yet, Cd and Pb concentrations of the hind legs cooked and enjoyed in the diets were determined below the European Commission's permitted levels. Furthermore, based on the TEs in edible tissues, consumption of the marsh frog did not appear to pose a risk to humans in terms of provisional tolerable weekly intake (PTWI), target hazard coefficient (THQ), and hazard index (HI).

Keywords Amphibians · Environment · Frog leg · Heavy metal · *Pelophylax ridibundus* · Pollution

Introduction

As a result of global industrialization parallel to population growth, the ecosystem is contaminated with various anthropogenic pollutants (trace elements (TEs), polycyclic aromatic hydrocarbons, etc.). One of the most important anthropogenic pollution sources in this environment is mining activities. Mining operations may pose a pollution danger to the region in which they operate and the nearby areas, and the species that live in the surrounding ecosystem. Especially in abandoned mining areas, TE pollution can also be transported to water sources, wetlands, and agricultural areas with the help of runoff, precipitation, wind, etc. [31]. The main concern with TE pollution is the metals' acute toxicity and their ability to accumulate in the biological systems [40, 42]. Therefore, perpetual monitoring of these pollutants with the help of sensitive keystone species (i.e., bioindicators that would help reveal pollutant presence and status) is essential for the ecosystem.

Since TEs are not biodegradable, they accumulate in the environment, which subsequently ends up in the food chain and poses severe threats to organisms [9]. In addition, revealing the current situation by examining the TE concentrations in the water and sediments where aquatic organisms live is vital for environmental quality assessment. It is also essential to investigate the TE levels in the tissues of the organisms consumed in terms of nutritional quality. Furthermore, the analysis of the natural frog populations might provide valuable information as robust bioindicators regarding the results of an ongoing TE accumulation process, thereby the pollution dynamics in the aquatic environment over a long period.

Amphibians are susceptible to environmental contamination [4]. Their all-life stages are sensitive to dermal absorption, inhalation, or ingestion of toxic substances present in the surrounding water [38]. Furthermore, they are food chain organisms with a high trophic level. Invertebrates are their

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primary food source, serving as an entry point into the food chain [30]. All these factors suggest that frogs are notable bioindicator candidates that could be practical models for environmental assessment and biomedical research as well as monitoring water quality and ecosystem health [15].

The literature proved that *Pelophylax ridibundus* and *Pelophylax esculentus* are great bioindicators for monitoring TEs and other pollutants [30, 37, 49]. Indeed, Vogiatzis and Loumbourdis [49] showed that these frogs meet all the criteria to be bioindicator species based on the rules listed by Lower and Kendall [24]. Furthermore, except for frog species that are potentially hazardous to humans [25], all Ranidae family species are appropriate for human consumption, such as the ones listed above. The frogs are nutritious with low-fat content and high protein and mineral values [29]. Their legs are among the most preferred recipes of traditional cuisines, especially in European countries [36]. The frog legs are served as a menu option in restaurants serving foreign tourists in several countries, including Turkey [10]. Literature highlights that the most popular culinary treats that could be cooked as a dish are the marsh frog (*Pelophylax ridibundus*. However, preference rates raise whether the consumption of this frog produced on farms or collected from nature poses human health risks due to the TEs that can be accumulated in its tissues. Indeed, several studies have found that frog consumption is a source of TE exposure for consumers [7, 12, 23]. However, risk assessment predictions of this frog in terms of human health and environmental contamination have not yet been investigated in the literature. Therefore, in this study, we aimed to determine the TE concentration in the tissues of *P. ridibundus*, a bioindicator organism, and to evaluate whether there are potential health risks that may arise from consumption.

Materials and Methods

Study Area and Sampling

The Eastern Black Sea region (Turkey) has rich copper (Cu), lead (Pb), and zinc (Zn) reserves. However, the mining activities in the long run cause TE contamination in the region's soil and aquatic ecosystem [14, 32, 51]. Marsh frogs were collected from 15 stations belonging to 5 different provinces and 3 different locations from each province (Fig. 1; Table S1). A total of 75 individuals belonging to 15 populations were sampled from 5 different regions within the distribution area of *P. ridibundus* in Turkey between June and September 2020. Marsh frogs that live in small streams or ponds were caught by hand or using a dip net and then placed in Ziploc bags. Respective water and sediments were also sampled from the same stations. Water samples were collected by hand using a polyethylene sampling bottle (500 mL). Sediments (around 1 kg) were taken using a shovel from the sediment surface (0–3 cm depths) and then transferred to Ziploc bags. All collected samples (frogs, water, and sediment) were stored in the iceboxes and transported to the laboratory. The frogs and the sediment samples were stored at − 20 °C for further experiments. Water samples (around 50 mL) were filtered through polytetrafluoroethylene (PTFE) filters (0.45 m particle size), and 5 drops of HNO_3 (Suprapur, Merck) were added for decreasing pH < 2 before being stored in the refrigerator until the TE analyses.

Sample Digestion

Sediment samples were kept at room temperature (RT) before being dried in the oven. *P. ridibundus* samples were defrosted and rinsed through ultrapure water at RT. The individuals' snout-vent lengths and weights were measured (Table S1). Then, the liver and the hind leg muscles of frogs were removed using a stainless-steel dissection set. Each individual was dissected separately. The 2 g fresh weight of liver and leg tissues were placed in different digesting tubes. For sediment, digestion was performed using 0.5 g (dry weight) of the samples. Five milliliters of HNO_3 (Suprapur, Merck) was added to the tubes including the tissues and sediment samples, sealed with polypropylene covers, and left overnight. The tubes were incubated for 2.5 h at 95 °C in a block heater. After cooling down to RT, the tubes were kept at 95 °C for another 2 h with 2.5 mL of H_2O_2 (Suprapur, Merck). The tubes were in the block heater with the caps removed until the solution contents were lowered to around 2 mL. The solutions were diluted with ultrapure H_2O, filtered through PTFE syringe filters (0.45 mm pore size) (US [44], and stored at + 4 °C until measurements.

Quality Control and TE Analysis

The identical procedure used on the samples was also applied to the reference material (ERM-CE278k Mussel Tissue) to check and verify the methods' digestion efficiency (Table 1). TE concentration was measured using an ICP-MS (Agilent, 7800). Furthermore, blank samples and the internal standards were also evaluated to check possible interference during either the sample preparation process or ICP-MS measurements.

Health Risk Evaluation

Estimated weekly intake (EWI mg/kg/week) is calculated by multiplying the weekly TE intake in the digestive tract of an adult person [13]. We calculated the EWI using the average portion size via determined TE concentrations in the leg tissues. The data were then compared to the provisional tolerable weekly intake (PTWI) values declared by the FAO/WHO Joint

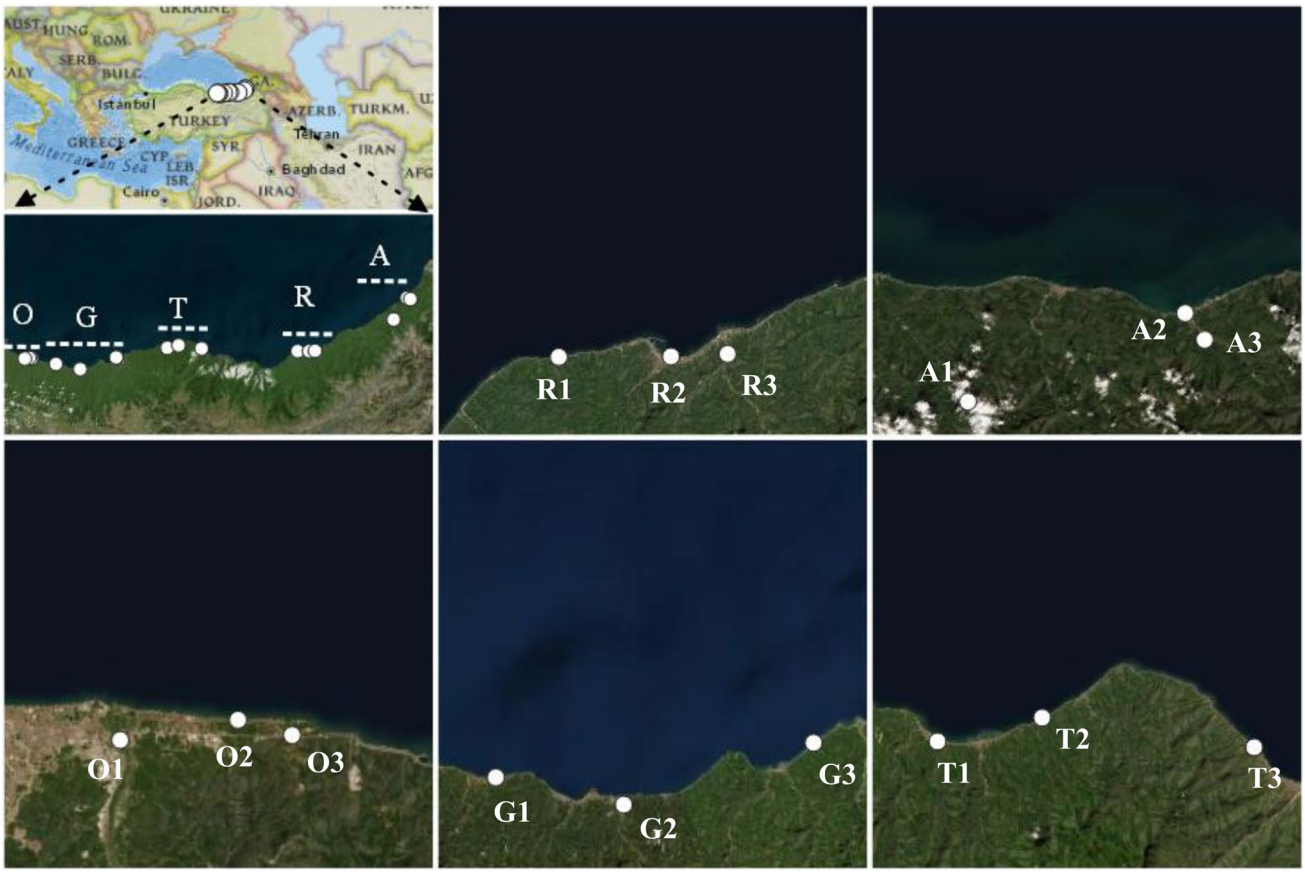


Fig. 1 Sampling area. Marsh frog (*Pelophylax ridibundus*), sediment, and water samples were collected from 15 different stations along with the NE Turkey. Letters O, G, T, R, and A indicate provinces where the frogs were collected. O: Ordu, G: Giresun, T: Trabzon, R: Rize, A: Artvin. Numbers 1, 2, and 3 indicate stations in the provinces

Table 1 Results for analysis of certified reference material (ERM CE278k mussel tissue)

	Detection limit*	References values, mg kg^{-1}	Analyzed values, mg kg^{-1}	Recovery (%)
As	0.039	6.70 ± 0.40	6.90 ± 0.35	102.99
Cd	0.0004	0.336 ± 0.025	0.35 ± 0.04	104.17
Cr	0.061	0.73 ± 0.22	0.68 ± 0.28	93.15
Cu	0.060	5.98 ± 0.27	5.83 ± 0.33	97.49
Ni	0.025	0.69 ± 0.15	0.63 ± 0.18	91.30
Pb	0.041	2.18 ± 0.18	2.28 ± 0.22	104.59
Zn	0.080	71.00 ± 4.00	74.08 ± 3.71	104.34

*ppb, $N = 5$

Expert Committee on Food Additives [18–20]. The following formula was used to calculate the EWI [46]:

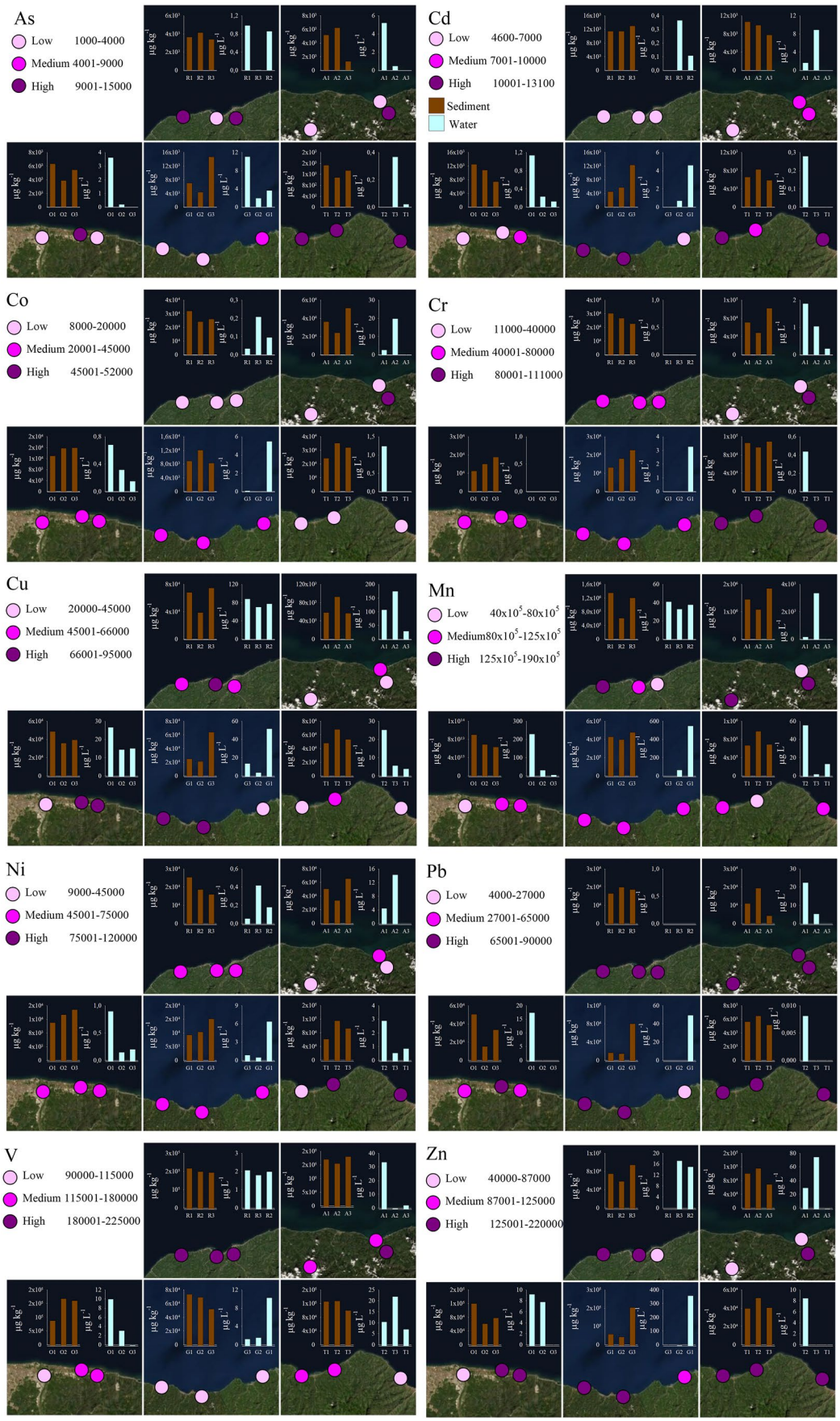
$$EWI = 7 \times \frac{C \times EF \times ED \times FCR}{BW \times ET} \times 10^{-3} \quad (1)$$

The target hazard quotient (THQ) is used to explain long-term non-carcinogenic exposure hazards as a ratio of exposure to reference dose concentrations (RfD, mg kg^{-1} day^{-1}) (US [47]. People are frequently exposed to numerous contaminants in the food they consume. Thus, the hazard index (HI) was utilized to predict the possible cumulative influence of the TEs [26] in the current study. The following formulas were used to determine THQ and HI values:

$$THQ = EDI/RfD \quad (2)$$

$$HI = \Sigma THQ_i \quad (3)$$

where EDI: estimated daily intake, RfD: reference dose (arsenic (As) = 3×10^{-4}, cobalt (Co) = 20×10^{-3}, cadmium (Cd) = 1×10^{-3}, Pb = 3.5×10^{-3}, Zn = 3×10^{-1}, Cu = 4×10^{-2}, nickel (Ni) = 2×10^{-2}, chromium (Cr) = 1.5, vanadium (V) = 5×10^{-3}: US [47]. The THQ and HI values > 1 mean that the TEs in the frogs can exert a potential non-carcinogenic health risk.


As
Low 1000-4000
Medium 4001-9000
High 9001-15000
Cd
Low 4600-7000
Medium 7001-10000
High 10001-13100
Sediment
Water
Co
Low 8000-20000
Medium 20001-45000
High 45001-52000
Cr
Low 11000-40000
Medium 40001-80000
High 80001-111000
Cu
Low 20000-45000
Medium 45001-66000
High 66001-95000
Mn
Low 40x10^5-80x10^5
Medium80x10^5-125x10^5
High 125x10^5-190x10^5
Ni
Low 9000-45000
Medium 45001-75000
High 75001-120000
Pb
Low 4000-27000
Medium 27001-65000
High 65001-90000
V
Low 90000-115000
Medium 115001-180000
High 180001-225000
Zn
Low 40000-87000
Medium 87001-125000
High 125001-220000

◂**Fig. 2** Trace element concentrations (TE average ± standard error) in sediment and water samples were collected from 15 different stations along with the NE Turkey ($N=3$). *X*-axis shows sampling locations; *y*-axis shows concentration (μg kg^{-1} for sediment and μg L^{-1} for water samples). Colored circles at the stations show the cluster analysis results according to the TE concentrations in the sediment sampled from the relevant station. Each classification identified (low, medium, high) indicates relative differences between the locations

Data Analysis

To determine normality and equal variance, the Shapiro–Wilk and Levene tests were performed. Data groups that were not normally distributed or homogeneous were transformed. The differences between the stations regarding the TE concentrations in the liver and soft tissues were determined using a one-way analysis of variance test (ANOVA, post hoc Tukey). Linear regression analysis was used to investigate the link between the TE concentrations in the liver and soft tissues of *P. ridibundus* along with the body lengths. The TE data in the sediments from various locations were allocated to a concentration range of high, medium, and low to establish the contamination status in the stations. Cluster (statistical) classification analysis was utilized to separate the other groups based on similarities in the TE concentrations detected in the sediments at the various stations. The relative disparities between the sampling locations were shown in each classification (low, medium, high) defined by the data collected in this study. All statistical evaluations were performed in the SigmaPlot 13 software with a 95% confidence interval.

Results and Discussion

Figure 2 shows the TE concentrations in the sediment and water collected from 15 stations across five provinces in Turkey's Northeast Black Sea region. TE concentrations in sediments ranged (min–max) as follows (mg kg^{-1}): As, 1.09–14.72; Cd, 4.61–13.07; Co, 8.47–51.74; Cr, 11.52–110.54; Cu, 22.50–94.25; Mn, 407.13–1876.23; Ni, 9.62–118.36; Pb, 4.75–82.45; V, 53.61–223.16; Zn, 41.03–211.07. TE concentrations in water samples are listed as follows (μg L^{-1}): As, under detection limits (UDL)–11.141; Cd, 0.02–8.91; Co, 0.01–20.06; Cr, 0.25–3.32; Cu, 4.37–177.40; Mn, 0.94–3402.33; Ni, 0.06–14.54; Pb, UDL–50.59; V, 0.14–34.36; Zn, 0.50–366.93. According to the mean concentration, the TEs in the sediment samples and water samples were listed as follows: Mn > V > Zn > Cu > Cr > Ni > Co > Pb > Cd > As and Mn > Zn > Cu > Pb > V > Ni > As > Co > Cd > Cr respectively.

Figure 2 shows the cluster analysis results based on TE level patterns in sediment and water samples from various stations. Three different concentration ranges for TE were determined: low, medium, and high. Cluster values do not show a universal classification or categorization for human health (low, medium, high). It solely shows the status of the stations based on TE concentrations in sediments at different sites. According to Fig. 2, 47% of the stations with the greatest As concentrations were classified as high, 47% were classified as low, and the remaining stations were classified as medium. Cu concentrations were found at low and high levels in 40% and around 33% of the stations, respectively, but medium amounts were found at only four stations (Fig. 2). Seven sites showed low Cd concentrations, four exhibited medium Cd concentrations, and the remainder showed high Cd concentrations. According to the Cr concentration data, roughly 60% of the stations were classified as medium, 27% as high, and 13% as low in terms of Cr pollution. Ni levels in the stations were categorized as medium (roughly 67%), low (approximately 20%), and high (about 13%) concentrations, according to the cluster analysis results. Based on the Pb distributions, the stations were categorized as 80% high, 13% medium, and 7% low. For V, around 33% of the locations showed low amounts, 40% medium concentrations, and 27% high values. Regarding Mn concentrations in sediment samples taken from various sites, 53% of stations had medium concentrations, 27% had low concentrations, and 20% had high concentrations. Zn's low and high values were identified in 27% and 40% of the sites, respectively, while medium levels were observed in only one station. In terms of Co content, eight sites were classified as low, six sites were classified as medium, and one site was classified as high (Fig. 2).

The distribution of TEs in the liver and the hind leg muscles of frogs sampled from different locations is given in Fig. 3. TE concentrations in leg tissues of frogs collected from all different stations were as follows: As, 0.24–77.82 μg kg^{-1}, Cd, 18.76–176.70 μg kg^{-1}, Co, 1.39–101.65 μg kg^{-1}, Cr, 15.60–300.34 μg kg^{-1}, Cu, 183.66–905.06 μg kg^{-1}, Mn, 12.15–104.67 μg kg^{-1}, Ni, 19.92–298.49 μg kg^{-1}, Pb, 25.52–975.54 μg kg^{-1}, V, 0.15–19.12 μg kg^{-1}, and Zn 1889–11,450 μg kg^{-1} (Table 2; Fig. 3). The mean concentrations (μg kg^{-1}) of TE were determined in the order of Zn (7392.55) > Cu (554.78) > Pb (267.97) > Mn (222.38) > Ni (109.65) > Cr (108.56) > Cd (67.92) > As (31.40) > Co (25.63) > V (8.89). The TE concentrations in liver tissues were determined as follows: As, 457–9392.65 μg kg^{-1}; Cd, 3751.56–10,145.21 μg kg^{-1}; Co, 148.07–1807.04 μg kg^{-1}; Cr, 14.33–7774.24 μg kg^{-1}; Cu, 16.52–479.79 mg kg^{-1}; Mn, 1613.74–5401.04 μg kg^{-1}; Ni, 31.24–442.67 μg kg^{-1}; Pb, 5.32–1683.40 μg kg^{-1}; V, 378.45–4779.41 μg kg^{-1}; and Zn, 19.12–48.08 mg kg^{-1} (Table 2; Fig. 3). The mean concentrations of TEs in the liver were listed as follows (μg kg^{-1}): Cu (99,652.06) > Zn (29,691.05) > Cd (6527.77) > As (3343.68) > Mn

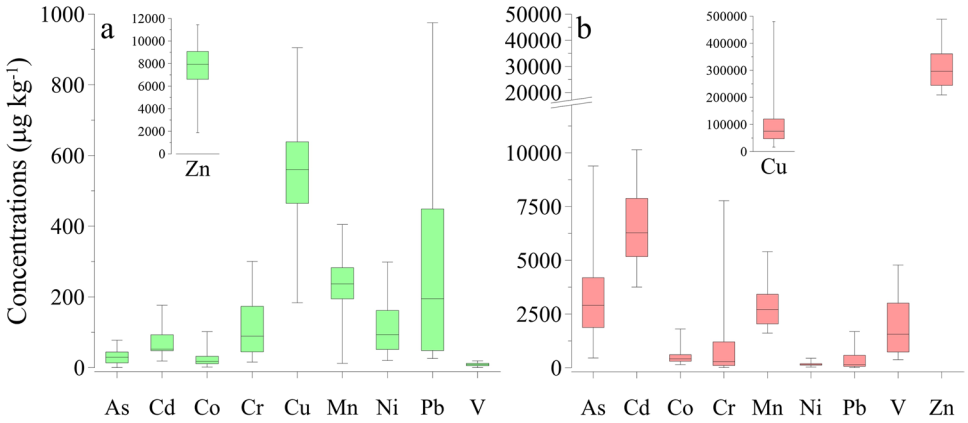


Fig. 3 Box plots showing the distribution of trace elements (µg kg^{-1} fresh weight) in **a** the hind leg muscles and **b** liver tissues of marsh frog (*Pelophylax ridibundus*) samples collected from 15 different stations along the NE Turkey ($N = 75$)

Table 2 Comparison of trace element concentrations (mg kg^{-1}) in marsh frog (*Pelophylax ridibundus*) hind leg muscle from different countries

		Location					
		NE Turkey	Southern Bulgaria	Northern Greece	Ibadan, Nigeria	Kalasin, Thailand	Sialkot, Pakistan
		Species					
		P. ridibundus	*P. ridibundus*	*P. ridibundus**	*X. laevis*, *R. esculentus*	*H. rugulosus*, *F. limnocharis*, *O. lima*	*R. tigrina*, *E. cyanophlyctis*
		Tissues					
As	Leg	0.0002–0.077	0.05–0.80		0.18–0.78	0.176–0.274	
	Liver	0.46–42.31	0.13–1.89		0.84–0.88		
Cd	Leg	0.018–0.18	0.001–0.05	0.7 ± 0.1	0.33–0.92	0.014–0.024	6.75–8.31
	Liver	3.75–42.31	0.06–9.64	2 ± 0.2	3.82–4.31		6.28–9.02
Co	Leg	0.0013–0.10		3	1.12–2.43		1.11–5.33
	Liver	0.15–1.81		6 ± 0.1	6–23–9.43		1.44–5.17
Cr	Leg	0.015–0.30		21 ± 5		2.283–2.782	0.89–2.64
	Liver	0.014–7.774		41 ± 11			1.02–1.18
Cu	Leg	0.183–0.90	0.23–1.29	27 ± 15	0.97–5.12		2.84–6.72
	Liver	16.519–479.79	34.67–81.09	1041 ± 153	10.97–67.45		6.56–27.56
Mn	Leg	0.012–0.104		164 ± 38	1.28–9.30		0.81–1.64
	Liver	1.613–5.401		64 ± 7	8.64–32.85		0.98–2.10
Ni	Leg	0.019–0.298		27 ± 3	3.4811.68		5.02–7.76
	Liver	0.031–0.442		4 ± 4	1.05–3.61		4.79–6.12
Pb	Leg	0.025–0.975	0.007–0.61	93 ± 2	0.15–5.12	0.370–0.613	11.44–37.00
	Liver	0.005–1.683	0.01–13.77	9 ± 4	4.98–11.02		9.76–21.87
V	Leg	0.0001–0.0019			3.29–102.76		
	Liver	0.378–4.779			2.93–17.93		
Zn	Leg	1.889–11.450	3.62–7.57	840 ± 136	6.93–7.82		10.30–21.09
	Liver	19.117–48.083	14.88–25.73	681 ± 88	11.35–52.96		17.25–29.98
References		Present study	[53]	Loumbourdis and Wray [23]	Tyokumbur and Okorie [43]	Thanomsangad et al. [41]	Qureshi et al. [34]

(2781.01) > V (1848.67) > Cr (1259.62) > Co (548.19) > Pb (335.72) > Ni (164.83).

Despite low TE concentration in the aquatic environment, it is not unusual to observe relatively high concentrations of certain TEs in frog tissues [7, 33, 39]. Our data corroborate similar research in the literature. The TE concentrations in the water samples were determined up to 5300-fold lower than those measured in the frog edible tissues. This difference was more dramatic in the liver tissues of frogs. Differences in the TE

concentrations of the water and frog tissues might be attributed to the perception that the frogs accumulate TEs either from another anthropogenic or natural source or have been intermittently exposed to cumulative TE pollution in the past [28, 30]. Besides direct TE accumulation from water through the skin, frogs can accumulate TEs in their bodies through diet or accidental ingestion of the polluted sediment [30]. Nummelin et al. [27] stated that the frogs accumulate TEs in their tissues at much higher concentrations than in the sediments by feeding on the aquatic plants or other organisms in the food web growing in the wetlands. In a study on the same species living in another niche, Stolyar et al. [39] compared the TE levels in urban and rural individuals of *P. ridibundus* population in western Ukraine. Higher concentrations were detected in the frog tissues they sampled despite low TE concentrations in the water samples [39].

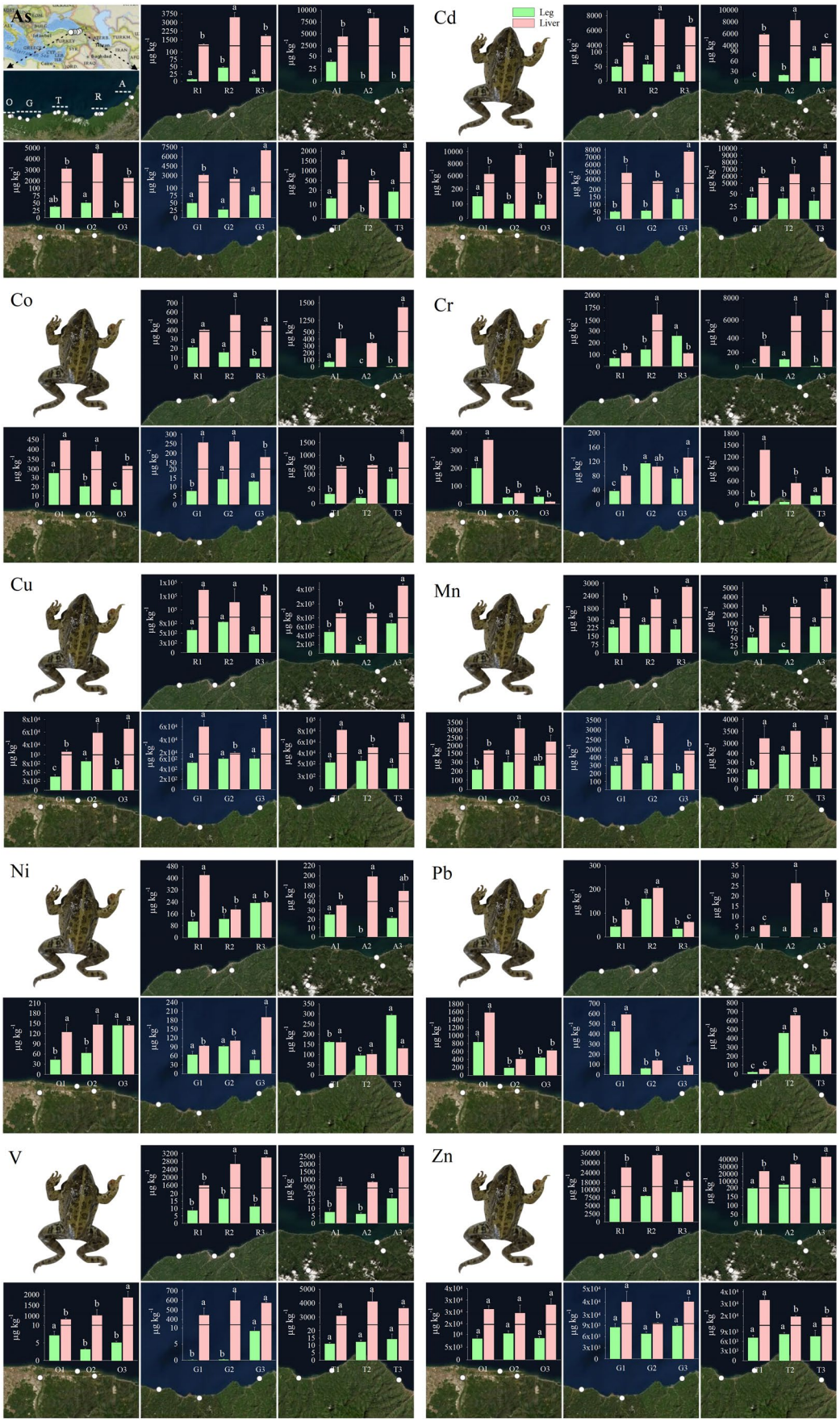
Since organisms are not able to always develop effective mechanisms to efflux TEs out of their bodies, e.g., resulting in accumulation in their tissues, the phenomenon of bioaccumulation occurs and continues throughout the food chain (biomagnification process) [8, 22, 39]. The Zn and Cu concentrations in particular, in the water, sediment, and the frog tissues, were found to be significantly higher than other metals, which is possibly related to the significant abundance of Zn and Cu in the earth's crust and their widespread use in industry [17]. These two elements may also result from ongoing mining activities in the Eastern Black Sea region [1, 52] where the samples were taken [32].

Amphibians have a life cycle spending a certain part of their lives in aquatic environments and terrestrial habitats [35, 50]. They generally spend a large part of their life dependent on water [3]. In this context, they will probably be affected by TE concentrations in aquatic environments. The TE distributions in the tissues of frogs sampled from different stations are given in Fig. 4. The data showed statistically significant differences between the sampling locations in the target provinces and different sampling stations in that province (ANOVA, post hoc Tukey; Fig. 4). The variation in TE levels across sample sites might be attributed to pollution levels in the maturation habitats. The variation in TE concentrations in the tissues of frogs collected from various sites is typically proportional to pollution in the area [16, 48]. When Fig. 2 is examined with this background, the differences in TE concentrations in sediment and water samples seem critical. Considering that frogs are good bioindicators being in close contact with their environment and reflecting the environment's conditions in terms of pollution [28, 30], TE concentration differences found in the tissues might be necessary signals showing the overall environmental quality.

Another aspect that may influence TE accumulation in the tissues is that frog sizes differ at sample locations, depending on the specific variables. To that purpose, linear regression analysis was used to examine the association between frog length–weight and TE levels in the liver and flesh in the hind leg muscles (Fig. 5). Our data showed that while there was a negative correlation between the frog length and weight and As, Cd, Co, and Pb concentrations of the tissues in the hind leg muscles, a positive correlation was found between the length–weight and Cr, Cu, Mn, Ni, V, and Zn concentrations. In addition, a negative relationship was found between the length–weight and As, Cd, Pb, Cr, Ni, and Zn levels in liver tissues. In contrast, a positive relationship existed between the length–weight and Co, Cu, Mn, and V concentrations (Fig. 5). However, these relationships overall were not statistically significant ($p > 0.05$; $r^2 < 0.3$).

TE concentrations of the frog's tissues have been reported in several kinds of research given in Table 2. Indeed, in a study conducted in a river in Northern Greece [23], the levels for Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn in both liver and leg tissues were higher than those found in our study. The two frog species examined in Pakistan's industrial areas had high amounts of Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn in their liver and leg tissue [34]. The results for *P. ridibundus* from Southern Bulgaria [53] matched the As, Zn, Cd, Cu, and Pb data in this study. In another research from Thailand [41], while the levels of Cd and Pb were comparable to those found in our study, the levels of As and Cr were lower. The values for *X. laevis* and *R. esculentus* from Nigeria [43] were similar in the TE data gathered throughout our research.

Turkey is an important frog supplier and trader [10]. Anatolian water frogs (*Pelophylax* spp.) have been collected for over 40 years, with approximately 700 tons of annual transport rate [2, 21]. Several agencies have set guidelines for maximum allowable TE levels in the food organisms to protect the consumers' health. Among these TEs, Cu, Zn, Cr, Co, Mn, V, and Ni are involved in critical metabolic activities in the organisms. However, high doses can readily be toxic. Furthermore, As, Cd, and Pb can be toxic to humans even at very low doses [6]. Therefore, these three elements are listed in the top 10 of the "Substance Priority List" announced by ASTDR [5]. Specific international organizations have also set the maximum allowable limits for As, Cd, and Pb. For instance, the European Commission (EC) stated that the Pb and Cd values in fishery products should not exceed 1.5 and 1 mg kg^{-1},


AS
O G T R A
Cd
Leg
Liver
Co
Cr
Cu
Mn
Ni
Pb
V
Zn
R1 R2 R3
A1 A2 A3
O1 O2 O3
G1 G2 G3
T1 T2 T3

◂**Fig. 4** Trace element concentrations (TE average ± standard error) in the hind leg muscles and liver tissues of marsh frog (*Pelophylax ridibundus*) samples collected from 15 different stations along the NE Turkey ($N=5$). *X*-axis shows sampling locations; *y*-axis shows concentration (μg kg^{-1}). Different letters (a, b, c) show significant differences among sampling stations (ANOVA, post hoc Tukey; $p<0.05$)

respectively [11]. When these limits were compared with our data, the Cd and Pb values did not exceed the specified threshold values (Table 3). For an average serving size (225 g), we computed the EWI levels using the measured value for As, Cu, Cd, Pb, and Zn. We also evaluated it according to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) PTWI norms (Table 3). We also revealed that the EWI rates computed for adults were below the JECFA thresholds (Table 3).

THQ was then computed and is shown in Table 3 to assess the non-carcinogenic health risk to humans posed by each TE when hind leg muscles of frogs collected from the sample locations were consumed. The average portion size of frog consumption may not necessarily have adverse health effects on adult consumers. All THQ calculated values for TE were observed to be < 1, indicating that the average portion size of frog consumption size may not necessarily have adverse health effects on adult consumers. When we looked at the HI levels, we noticed that those lower than 1 (the limit value) ranged from 6.2×10^{-5} to 1.2×10^{-3}, depending on the average portion size. Both THQ and HI levels appear to be below the limit value of 1, indicating that they are unlikely to impact human health negatively.

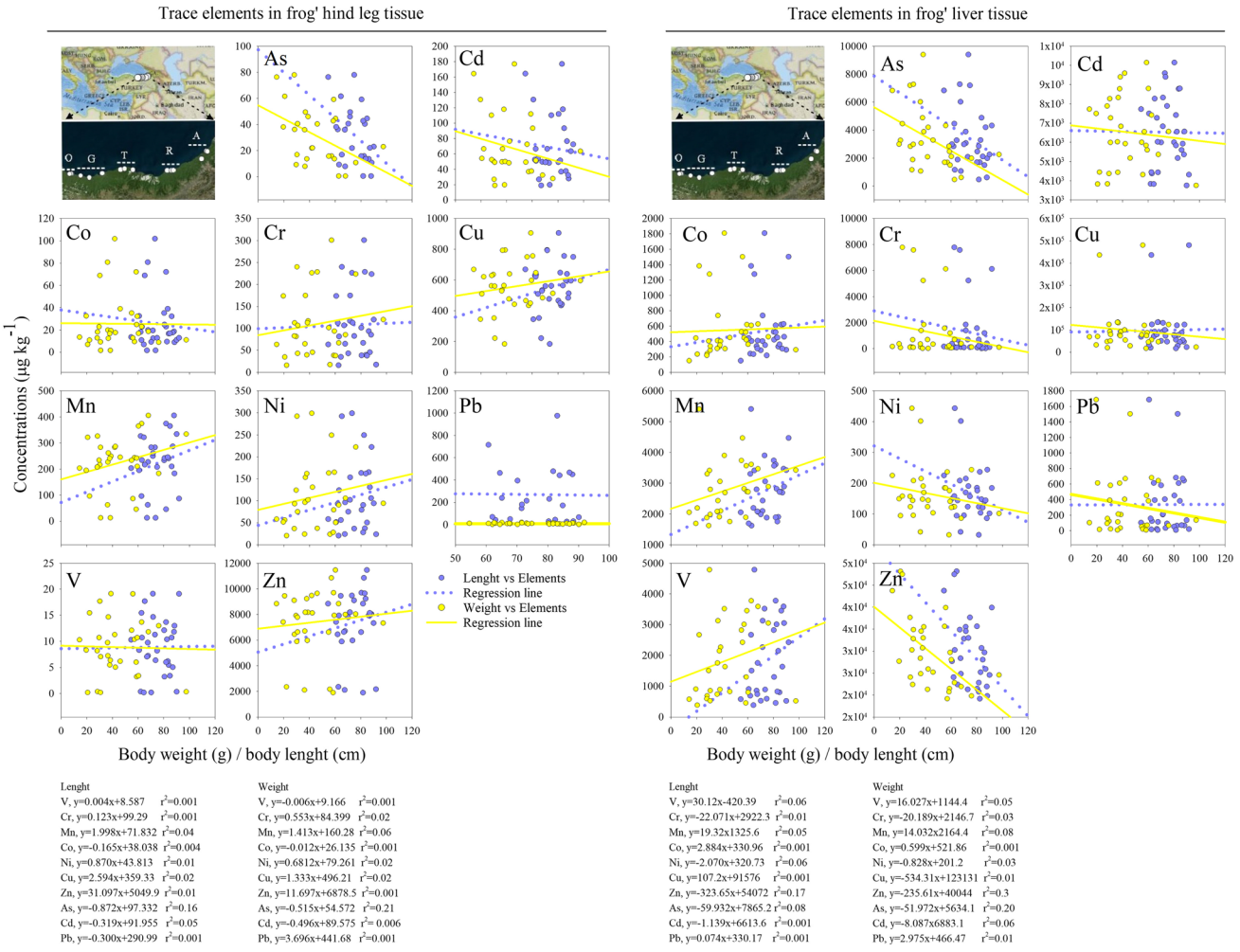


Fig. 5 The relationship between size and the trace element concentrations in the tissues of the marsh frog (*Pelophylax ridibundus*) samples collected from 15 different stations along the NE Turkey. Each dot shows the result of different samples. *X*-axis shows length or weight of marsh frog; *y*-axis shows concentration (μg kg^{-1})

Table 3 Estimated weekly intake (EWI), target hazard quotients (THQs), and hazard index (HI), through consumption of marsh frog (*Pelophylax ridibundus)* hind leg samples collected from 15 different stations along the NE Turkey

		As	Cd	Co	Cr	Cu	Ni	Pb	Zn	HI
Trace elements concentration in the frogs*	Min	0.243	18.759	1.392	15.607	183.661	19.92	25.516	1888.997	
	Max	77.822	176.697	101.65	300.34	905.063	298.49	975.538	11,450.859	
	Mean	31.399	67.922	25.627	108.557	554.781	109.649	267.973	7392.55	
EC [11]*			1000					1500		
EWI		0.000754	0.058218	0.00432	0.048436	0.569982	0.061821	0.058218	5.862404	
		0.241517	0.54837	0.315466	0.93209	2.808816	0.926349	3.027532	35.537149	
		0.097445	0.210792	0.079532	0.336901	1.721734	0.34029	0.83164	22.942397	
PTWI		15	7			3500		25	7000	
RfD		0.3	1	20	3	37.1	20	3.5	300	
THQs		1.10E − 06	2.60E − 05	9.70E − 08	7.20E − 06	6.90E − 06	1.40E − 06	1.00E − 05	8.80E − 06	6.20E − 05
		3.60E − 04	2.50E − 04	7.10E − 06	1.40E − 04	3.40E − 05	2.10E − 05	3.90E − 04	5.30E − 05	1.20E − 03
		1.50E − 04	9.40E − 05	1.80E − 06	5.00E − 05	2.10E − 05	7.60E − 06	1.10E − 04	3.40E − 05	4.60E − 04

*μg/kg EC [11]: European commission regulation on the determination of maximum levels for certain contaminants in foodstuffs. *EWI* (μg/kg/hafta), estimated weekly intake; *PTWI* (μg/kg/week), provisional tolerable weekly intake; *RfD* (μg/kg/day), reference dose; *THQ*, target hazard quotient < 1 unlikely to cause any adverse health effects; *THQ* > *1*, might cause adverse health effects. EWI, THQ, and HI values were calculated based on weekly consumption amount of 225 g. Cu and Zn [18], As [19], Cd and Pb [20]

Conclusion

This study aimed to quantify the TE bioaccumulation in frog tissues obtained from NE Turkey and determine the hazards of marsh frog (*Pelophylax ridibundus*) consumption on human health. TE concentrations in frog tissues showed significant variation according to the sampling locations. However, Cd and Pb concentrations in edible tissues were below the allowable limits specified by the EC. Furthermore, the EWI values were lower than the PTWI levels derived for adult consumers based on the average portion size. In addition, the THQ (non-carcinogenic risk) values calculated for TEs less than one confirmed that the frog intake of the collection area would not cause possible adverse health effects in humans.

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

Declarations

Ethics Approval All specimens used in this study were treated humanely and ethically following permission to perform the study by Recep Tayyip Erdogan University's Local Ethics Committee (decision number: RTEÜ.2020/06).

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