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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Trace element bioaccumulation and health risk assessment derived from leg consumption of the marsh frog, *Pelophylax ridibundus* (Pallas, 1771)

Ova kurbağası, *Pelophylax ridibundus*'un (Pallas, 1771) bacak tüketiminden elde edilen eser element biyobirikimi ve sağlık riski değerlendirmesi

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Abstract: Amphibians, which can live in aquatic and terrestrial environments, are a good indicator of pollution in these areas. Although frog leg is not consumed frequently in some cuisines, including Turkey, it is important in terms of evaluating metal accumulation since it is preferred as human food in many European countries. In this study, the quantities of trace elements (Cd, Pb, Cu, Zn, As, Co, Cr, Ni, Mn, V) were measured in the edible tissues (muscles) of an amphibian species by sampling from two frog farms in Turkey. It was aimed to assess possible health hazards for humans by frog legs consumption comparing with the toxicological limit values, including provisional tolerable weekly intake (PTWI), target hazard quotient (THQ), and Hazard Index (HI). In general, the average values (µg kg^{-1}) of trace elements were Zn (3.437.62)> Pb (69.22)> Cu (66.72)> Mn (35.07)> As (24.24)> Cr (11.47)> Ni (6.94)> Cd (6.51)> Co (2.97)> V (<0.001). The results indicated that concentrations of the analyzed trace elements were determined below the European Commission's permitted levels and edible tissues of the marsh frog posed no carcinogenic health risk to humans.
Keywords: Amphibians, environment, human health, pollution, marsh frog

Öz: Hem sucul hem de karasal ortamlarda yaşayabilen amfibiler, bu alanlardaki kirliliği gösteren önemli biyoindikatör canlılardandır. Kurbağa bacağı, Türkiye'nin de dahil olduğu bazı mutfaklarda sıklıkla tüketilmemesine rağmen pek çok Avrupa ülkesinde insan gıdası olarak tercih edilmesi nedeniyle metal birikiminin değerlendirilmesi açısından önemlidir. Bu çalışmada, Türkiye'deki iki kurbağa çiftliğinden örnek alınarak bir amfibi türünün yenilebilir dokularında (kaslarında) eser element (Cd, Pb, Cu, Zn, As, Co, Cr, Ni, Mn and V) miktarları ölçülmüştür. Geçici tolere edilebilir haftalık alım (PTWI), hedef risk katsayısı (THQ) ve Tehlike İndeksi (HI) dahil olmak üzere birçok değer toksikolojik sınır değerlerle karşılaştırılarak kurbağa bacağı tüketiminin insanlar için olası sağlık tehlikelerinin değerlendirmesi amaçlanmıştır. Genel olarak eser elementlerin ortalama değerleri (µg kg^{-1}) Zn (3.437.62)> Pb (69.22)> Cu (66.72)> Mn (35.07)> As (24.24)> Cr (11.47)> Ni (6.94)> Cd (6.51)> Co (2.97)> V (<0.001) olarak sıralanmıştır. Sonuçlar, analiz edilen eser elementlerin konsantrasyonlarının Avrupa Komisyonu'nun izin verdiği seviyelerin altında belirlendiğini ve ova kurbağasının yenilebilir dokularının insanlar için kanserojen bir sağlık riski oluşturmadığını gösterdi.
Anahtar kelimeler: Amfibiler, çevre, insan sağlığı, kirlilik, ova kurbağası

INTRODUCTION

Amphibians are poikilothermic, vertebrate animals that develop through metamorphosis and are used in many sectors, especially food (Alpbaz, 2009, Çiçek et al., 2021). Although frog meat is not consumed frequently in some cuisines including Turkey, it is important to evaluate metal accumulation because it is preferred as human food in many European countries (Şereflişan and Alkaya, 2016). Similarly, there are studies examining the effects of heavy metal accumulation on human health in some marine species such as *Solen marginatus* (Taş and Sunlu, 2019), *Rapana venosa* (Bat et al., 2016), which are not widely consumed in Turkey. Today, meeting the demand for frogs through farms has become even more critical (Helfrich et al., 2009). Although frog muscles are used in many cultures, they are not widely used in metal accumulation assessments. Indeed, it is crucial to evaluate metal accumulation due to its use in the human diet (Prokić et al., 2016a).

Heavy metals or potential harmful elements are produced by both natural, geogenic, lithogenic and anthropogenic factors (Ali et al., 2019). Determining the accumulation of trace elements in the body due to a metal combination in organisms aids in assessing its effect (Rainbow, 2018). However, the interaction between trace elements can affect accumulation and toxicity (De Medeiros et al., 2020). Therefore, it is crucial to figure out how trace elements interact with one another in tissues because trace elements have increased concentrations in the environment (Briffa et al., 2020).

Contaminated water contains many metal mixtures rather than a single metal, and aquatic organisms are influenced by mixed metals (Zeng et al., 2019; Fettweis et al., 2021). Heavy metal concentrations are toxic to mg L^{-1} levels for most organisms due to heavy metal ions irreversible suppression of particular enzymes (Henczova et al., 2008). Amphibians assimilate these metals from water and water-suspended food through adsorption by tissue and membrane surfaces with ion exchange on the skin (Birungi et al., 2007). Amphibians are more sensitive bioindicators than the other water vertebrates in showing pollution in water because they quickly absorb substances from the environment thanks to their permeable skin (Hecnar, 1995; Altunışık et al., 2021a, b).

How heavy metals in the environment affect amphibians as a bioindicator has attracted the attention of many researchers. The effects of various metals (Lee and Stuebing, 1990; Loumbourdis and Wray, 1998; Loumbourdis et al., 2007; Stolyar et al., 2008; Shaapera et al., 2013; Prokić et al., 2016a, b, 2017; Aguillón-Gutiérrez and Ramirez-Bautista, 2020), such as cadmium (Nebeker et al., 1995; Vogiatzis and Loumbourdis, 1998; Othman et al., 2009; Medina et al., 2016), lead (Kaczor et al., 2013), and copper (Papadimitriou and Loumbourdis, 2002), on amphibian tissues and surrounding waters are among the most frequently studied topics. However, heavy metal studies on amphibians and human health risk assessment studies are scarce (Thanomsangad et al., 2019). This study used the marsh frog, *Pelophylax ridibundus* (Pallas, 1771), a frog species widely distributed in Europe and Asia and frequently consumed, as a bioindicator organism since it is one of the most pollution-resistant species (Zhelev et al., 2014; Zhelev et al., 2020, Mani et al., 2021). Since it is unknown whether the edible tissues of *P. ridibundus* that supplied from frog farms pose a health risk due to potentially harmful elements, this research was undertaken.

In this regard, it was aimed to determine the concentrations of trace elements (Cd, Pb, Cu, Zn, As, Co, Cr, Ni, Mn and V) in the edible tissues (muscles) of 20 samples taken from two frog farms in Adana and Istanbul in Turkey, and to assess potential health risks for humans from frog leg consumption compared with the toxicological limit values, including provisional tolerable weekly intake (PTWI), target hazard quotient (THQ), and Hazard Index (HI).

MATERIAL AND METHODS

Sampling procedure

In this study, 20 samples of the Marsh frog, *Pelophylax ridibundus* (Pallas, 1771) was purchased from two separate frog farms (Kemal Balıkçılık, İstanbul and Sasu Su Ürünleri, Adana) in Turkey between August and September 2020. Since these examples were purchased from frog farms, there is no need for ethics committee permission in Turkey. The samples were packaged in iceboxes inside Ziploc containers, transported to the laboratory and frozen at -20 °C for subsequent laboratory experiments. After the frogs were thawed at room temperature, they were washed with pure water. Snout-vent length (SVL; range: 64-104 mm, mean: 87 mm) of the adult individuals was measured using a digital vernier caliper (500-706-11, Mitutoyo, Tokyo, Japan) with an accuracy of 0.01 mm. The body mass (range: 28-125 g, mean: 80 g) of the adult individuals was weighed with an accuracy of 0.01 g using microbalances. Then the muscle tissue, the edible part of frog's hind legs, was removed using a stainless-steel dissection set.

Sample digestion

Approximately 2 grams (fresh weight) of the muscle tissue were weighed and placed in glass tubes using sensitive laboratory scales (75 ml). 5 ml HNO_3 (Suprapure 65%) was added into each test tube, which was then covered with a watch glass and stored at room temperature overnight. Then, the glass tubes were then placed in the block heater, which was heated to 120 °C, and left for 8 hours. After filling the tubes with 2.5 mL H_2O_2 (Suprapure ≥ 30%) and holding them at 120 °C for another 8 hours, the watch glass was withdrawn from the tubes and placed in the heater until the quantity of solution in it was ~1.5 mL. The resulting solutions were diluted to 50 ml with the help of ultra-pure water, then passed through a 0.45 µm PTFE syringe filter and held at +4 °C until trace element analysis (Gedik, 2018).

Determination of heavy metals and quality control

ICP-MS (Agilent Technologies 7700X) was used to determine the concentrations of trace elements (As, Cd, Co, Cr, Cu, Mn, Ni, Pb, V, and Zn) in diluted and filtered solutions. It was calibrated primarily by diluting ICP-MS multi-element stock solution (1000 ppm) (0-100 ppb) (US EPA, 1994).

For the verification of ICP-MS measurements, reference solutions and metal extraction results were used as standard reference material (BCR 185R, Bovine Liver) (US EPA, 1996). Recovery values for As, Cd, Cu, Mn, Pb, and Zn from the BCR 185R ranged from 93-97% (Table 1).

Table 1. Results of the certified reference material (BCR-185R bovine liver) analysis: reference material values versus measured values (mean±SD)

	Detection Limit*	References values mg kg^{-1}	Analyzed values mg kg^{-1}	Recovery (%)
As	0.040	0.033	0.03	93.94
Cd	0.003	0.544	0.53	97.43
Cu	0.140	277.000	268.00	96.75
Mn	0.311	11.070	10.75	97.11
Pb	0.040	0.172	0.16	93.02
Zn	0.128	138.600	131.00	94.52

*ppb, N=5

Statistical analyses

We used Shapiro-Wilk and Levene test for the normality and homogeneity of the variances, respectively. The t-test was conducted to determine the differences between the areas because the data exhibited a normal distribution ($p > 0.05$). The association between trace elements was then established using the Pearson correlation test. In addition, the relationship between the concentration of trace elements and body measurements (weight and SVL) was analyzed by applying linear regression analysis. All statistical calculations were performed in R programming with a 95% confidence interval (Wickham, 2016; R Core Team, 2020).

Health risk assessment

To protect the health risk of consumers, some institutions and countries have set guidelines for maximum TE levels allowed in seafood. The European Commission (EC, 2006) and Turkish food Codex (TFC, 2011) reported that the Pb and Cd values in seafood products should not exceed 1.5 and 1.0, respectively, in terms of food safety. Therefore, the values obtained in the study were compared with these values and the suitability of the examined frog in terms of consumer health was evaluated. In addition, the effects of weekly and lifetime exposure based on average consumption were also examined in the study. For this purpose, comparisons with weekly metal intake limit values specified by JECFA (1982, 2011a,b) were made by calculating As, Cd, Cu, Pb, and Zn intakes depending on the weekly and average portion size. For this purpose, Estimated Weekly Intake (EWI, µg/kg/week) was determined by using trace elements concentrations in frog muscles to compare with Provisional tolerable weekly intake (PTWI) values (JECFA, 1982, 2011a,b), which represent the pollutant concentration present in the ingested food that would offer no health hazards if consumed weekly over the course a person's lifetime. The Target Risk Coefficient (THQs) was also utilized to quantify the hazards associated with consuming frog legs. Calculated by comparing exposed concentrations of trace elements with reference dose values, THQs are used to describe the probabilities of long-term exposure that is not carcinogenic (US EPA,2015). It means that if the value of THQs is >1, the metal can probably show negative impacts on health. EWI and THQs are calculated in the following way:

EWI: (C x FCR)/BW

THQ= (C x EF x ED xFCR)/(RfD x BW x EF x ET) x 10^{-3}

C indicates the of trace elements in frog muscle tissue (mg kg^{-1} wet weight), FCR indicates the weekly consumption of frog muscles (g). BW indicates average body weight (72.5 kg), EF specifies exposure time in one year (365 days) and ED refers to exposure time (mean life expectancy in Turkey 78 years) (Basara et al., 2016). RfD refers to the amount of reference dose (As: 0.3; Cd: 1; Co: 20; Cr:3; Cu: 37.1; Ni: 20; Pb: 3.5; Zn: 300 µg/kg/day) and finally ET is the average exposure time for non-carcinogens (365 d $year^{-1}$ x number of exposure years (average life expectancy)). Since we do not have data on the daily average consumption of the frog legs, THQ, EWI and HI values were computed according to the weekly consumption of 225 g (US EPA 2000).

All trace elements in the leg muscle tissue were assessed using the Hazard Index (HI) to determine their potential non-carcinogenic health hazard (Newman and Unger, 2002). The total of all THQs is HI, which is expressed as follows.

$$HI = \sum_{i}^{x} \text{THQi}$$

If the HI value is > 1, it indicates that trace elements in the frog's leg may indicate a potential non-carcinogenic health risk.

RESULTS AND DISCUSSION

Spatial distribution of trace elements detected in the muscle tissue of *P. ridibundus*

The distributions of Cd, Pb, Cu, Zn, As, Co, Cr, Ni and Mn concentrations in *P. ridibundus* edible hind leg tissues sampled from two different regions, the average and confidence range are given in Figure 1. There were no significant differences ($p<0.05$) between the two-frog farm regarding trace element concentrations. Vanadium concentrations were found to be below the detection limit on both sides. In the Adana location, average trace element concentrations were 26, 7, 0.4, 11, 70, 36, 11, 75, 2900 µg kg^{-1} for As, Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn, respectively. In İstanbul location, these concentrations were detected as 23, 6, 6, 12, 63, 34, 3, 63, and 3977 µg kg^{-1}, respectively.

The average values (µg kg^{-1}) of trace elements detected in general were found in the order of Zn (3437.62)> Pb (69.22)> Cu (66.72)> Mn > (35.07)> As (24.24)> Cr (11.47)> Ni (6.94)> Cd (6.51)> Co (2.97)> V (<0.001). According to our findings, only Ni was shown to be positively correlated with the SVL of the marsh frog, but the other elements (Zn, Pb, Cu, Mn, As, Cr, Cd, and Co) were not (Figure 2). Furthermore, the weight of marsh frogs was favorably associated with Ni and Zn, but not with the remaining elements (Figure 2). The mean Pb, Cu, As, Cr, Ni, Mn, and Cd concentrations did not differ between the sites ($p<0.05$). In contrast, the Zn and Co concentrations were significantly higher ($p<0.01$) in the İstanbul site compared to the Adana site. There was a positive relationship between Cu-As, Cr-Cd, Cr-Pb, Cu-Pb and Cd-Pb as while other elements were not found to have a significant relationship with each other (Table 2).

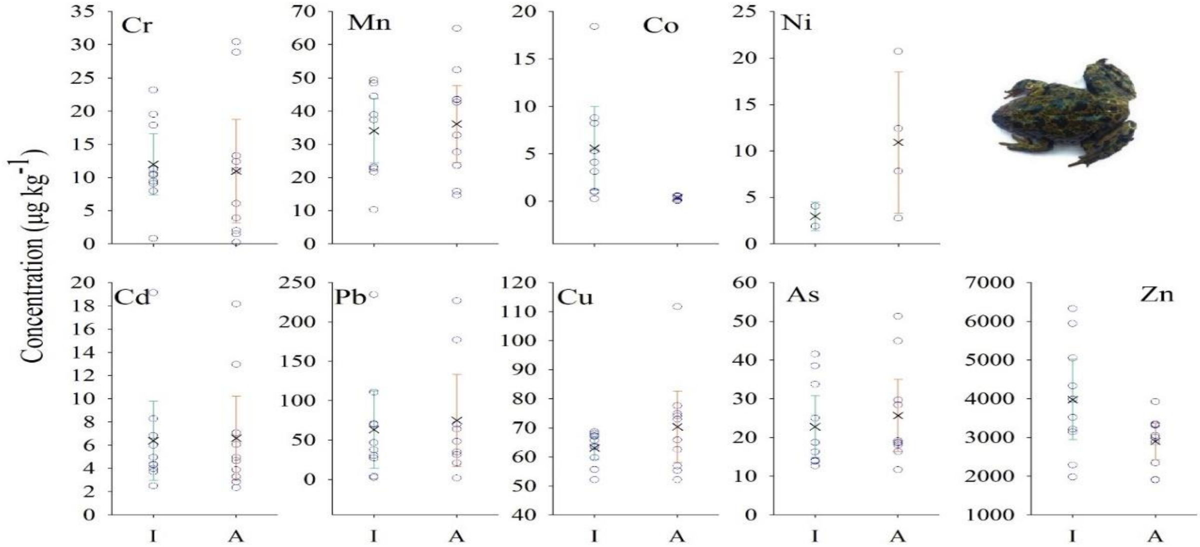

Figure 1. Distribution of trace elements in *Pelophylax ridibundus* muscle tissues. (A) refers to Adana, (İ) refers to İstanbul. Each blank circle represents one sample (n=10 per station). The negative number of empty circles are not shown, as they are below the detection limit. x shows the averages of the stations. Error bars indicate the confidence interval (95%). T-test was applied to determine the statistical differences between stations and no significant difference was detected.

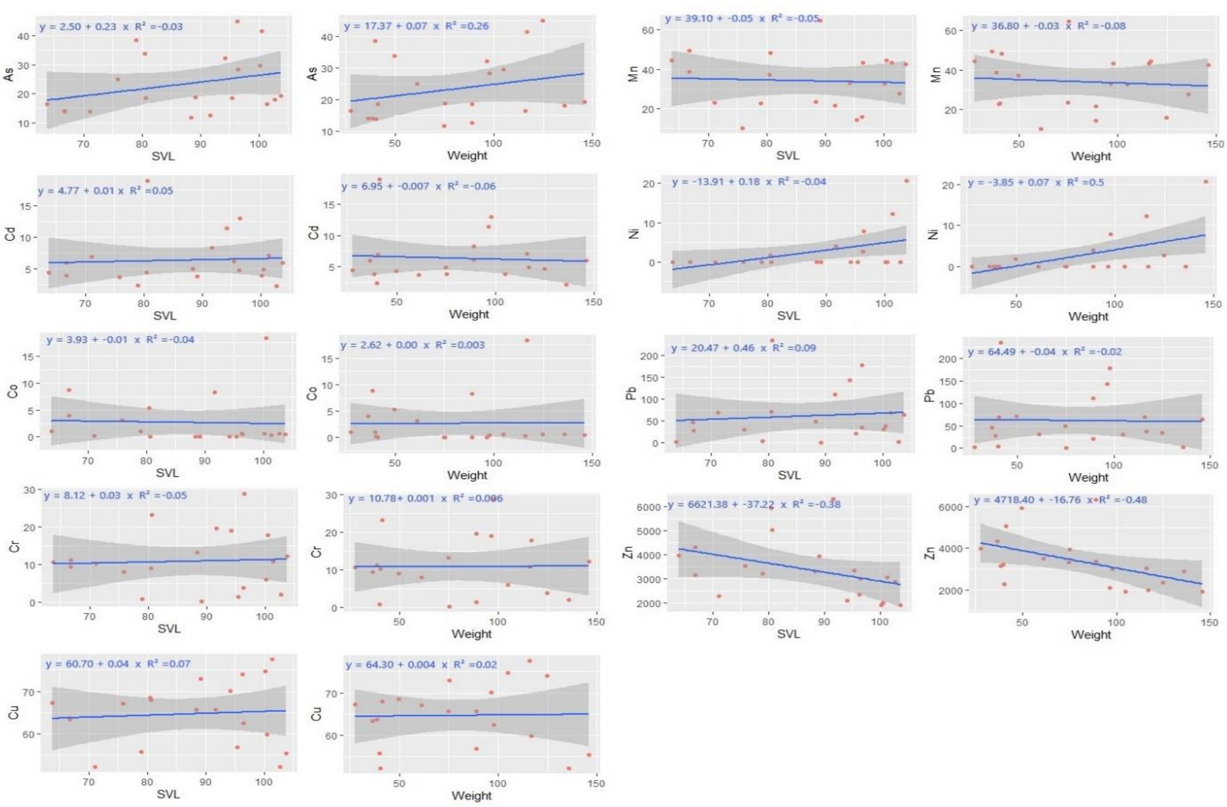

Figure 2. The relationship between the potential toxic element concentrations and SVL, and weight of the individuals belonging to the *Pelophylax ridibundus*

Table 2. The relationship among heavy metal concentrations detected in frog muscle tissue. The numbers are given in bold show the correlation coefficient.

	Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Pb
Cr	**1**	**0.352**	**0.357**	**0.078**	**0.432**	**0.168**	**0.187**	**0.859**	**0.871**
		0.128	0.16	0.883	0.056	0.48	0.43	*0.000	*0.000
Mn			**0.276**	**0.619**	**0.367**	**0.094**	**0.019**	**0.398**	**0.487**
			0.284	0.19	0.112	0.693	0.936	0.081	0.034
Co				**-0.525**	**-0.040**	**0.152**	**0.243**	**-0.002**	**0.018**
				0.285	0.876	0.56	0.348	0.994	0.944
Ni					**-0.492**	**-0.623**	**-0.508**	**0.090**	**-0.058**
					0.322	0.186	0.303	0.864	0.912
Cu						**0.11**	**0.496**	**0.527**	**0.532**
						0.643	*0.026	0.017	*0.019
Zn							**-0.244**	**0.227**	**0.305**
							0.301	0.336	0.204
As								**0.202**	**0.199**
								0.392	0.415
Cd									**0.98**
									*0.0001

Light colored numbers show the p value; * p <0.05

Comparison with literature

Despite the effects of trace elements on amphibians' tissues have been well documented (Lee and Stuebing, 1990; Nebeker et al., 1995; Papadimitriou and Loumbourdis, 2002; Loumbourdis et al., 2007; Othman et al., 2009; Kaczor et al., 2013; Medina et al., 2016; Prokić et al., 2016a, b,2017; Zhelev et al., 2020), there is limited work involving human health risk assessment via consumption of frog (Thanomsangad et al., 2019). Although Turkey is a major supplier of frogs (Çiçek et al., 2021) and Anatolian water frogs (*Pelophylax* spp.) have been gathered for more than 40 years, shipping about 700 t of frogs annually and for frog trade (Akın and Bilgin, 2010; Kürüm, 2015), the low intake of these frogs in Turkey may have slowed the estimation of the health risks associated with their consumption.

Our results for trace elements in the muscle tissue of the marsh frog are given in Table 3. Although trace elements are found in water at lower concentrations, relatively higher concentrations in frog tissues have been reported in several studies (Stolyar et al., 2008; Borković-Mitić et al., 2016; Prokić et al., 2016b;).

Table 3. Estimated Weekly Intake (EWI; µg / kg / week), target risk coefficient (THQ), Hazard Index (HI), arising from consumption of frogs sampled from Adana (A) and İstanbul (İ) regions

		As	Cd	Co	Cr	Cu	Ni	Pb	Zn	HI
Metal Concentration in frog*	A	25.66	6.62	0.36	10.95	70.35	10.91	75.05	2898.52	
	İ	22.81	6.40	5.57	11.99	63.08	2.97	63.38	3976.71	
EC (2006)*			1000					1500		
TFC (2011)*			1000					1500		
EWI	A	0.08	0.02	0.001	0.03	0.22	0.03	0.23	8.90	
	İ	0.07	0.02	0.02	0.04	0.20	0.01	0.20	12.34	
PTWI		15	7			3500		25	7000	
RfD		0.3	1	20	3	37.1	20	3.5	300	
THQs	A	$1.2x10^{-4}$	$9.2x10^{-6}$	$2.5x10^{-8}$	$5.1x10^{-6}$	$2.6x10^{-6}$	$2.1x10^{-7}$	$3.0x10^{-5}$	$1.3x10^{-5}$	1.8x10-4
	İ	$1.1x10^{-4}$	$8.9x10^{-6}$	$3.9x10^{-7}$	$5.6x10^{-6}$	$2.4x10^{-6}$	$7.6x10^{-7}$	$2.5x10^{-5}$	$1.8x10^{-5}$	1.7x10-4

* µg kg^{-1}. EC (2006): European commission regulation "determination of maximum levels for certain contaminants in foodstuffs", TFC (2011): Turkish food codex contaminants regulation, PTWI (µg/kg/weeks): provisional tolerable weekly intake, Cu and Zn (JECFA, 1982), As (JECFA, 2011a), Cd and Pb (JECFA, 2011b). RfD (µg/kg/day): reference dose, from USEPA THQ: Target Risk Coefficient <1 is unlikely to cause any adverse health effects, if THQ> 1 may cause adverse health effects. EWI, THQ and HI values were calculated according to the weekly consumption of 225 g.

The values of Cd, Pb, Cu, Zn, and As obtained in the present study were lower than those of another study (Zhelev et al., 2020) performed on *P. ridibundus* from two sites in Bulgaria. Trace element bioaccumulations in the muscles were determined in the order of Zn > Se > As > Cu > Pb > Cd in those Bulgarian populations (Zhelev et al., 2020). Similar results (since Zn was in the first and Cd in the last order) were also observed in this study in terms of the order of trace elements. In Ibadan (Nigeria), researchers investigated the major and minor element accumulation in *Rana esculentus's* some organs, including muscle tissue and found trace element concentrations in the order of Ni> Zn > Pb> Co> Cu> Cd> As (Tyokumbur and Okorie, 2011), which was different as compared to this study.

Moreover, they found relatively higher mean trace element (Cd, Pb, Cu, Zn, As, Co, Cr, Ni and Mn) concentrations than our study. Besides, the concentrations of eight elements identified in Quereshi et al. (2015)'s experiment for the species *R. tigrina* and *E. cyanophlyctis* were much higher than ours (Table 4).

Table 4. Comparison of trace element concentrations (mg kg^{-1}) in the marsh frog (*Pelophylax ridibundus*)' leg muscle from different countries

	Location	West and South Turkey	NE Turkey	Southern Bulgaria	Ibadan, Nigeria	Sialkot, Pakistan
	Species	*P. ridibundus*	*P. ridibundus*	*P. ridibundus*	*X. laevis, R. esculentus*	*R. tigrina E. cyanophlyctis*
As	Leg	0.012-0.051	0.0002-0.077	0.05-0.80	0.18-0.78	
Cd	Leg	0.002-0.019	0.018-0.18	0.001-0.05	0.33-0.92	6.75-8.31
Co	Leg	0.000018-0.018	0.0013-0.10		1.12-2.43	1.11-5.33
Cr	Leg	0.0002-0.03	0.015-0.30			0.89-2.64
Cu	Leg	0.052-0.111	0.183-0.90	0.23-1.29	0.97-5.12	2.84-6.72
Mn	Leg	0.010-0.065	0.012-0.104		1.28-9.30	0.81-1.64
Ni	Leg	0.000023-0.027	0.019-0.298		3.4811.68	5.02-7.76
Pb	Leg	0.00003-0.235	0.025-0.975	0.007-0.61	0.15-5.12	11.44-37.00
V	Leg	0.0004-0.017	0.0001-0.0019		3.29-102.76	
Zn	Leg	1.901-6.326	1.889-11.450	3.62-7.57	6.93-7.82	10.30-21.09
References		Present study	Mani et al., 2021	Zhelev et al., 2020	Tyokumbur and Okorie, 2011	Qureshi et al., 2015

In another study conducted around an electronic-waste dump, the relative order of some trace elements was Cr>Pb>As>Cd, which was overlapped the data noticed in our study except Cr (Thanomsangad et al., 2019). However, all concentrations (As, Cu, Pb, and Zn) investigated by Thanomsangad et al., (2019) for *H. rugulosus*, *F. limnocharis*, and *O. lima* was higher than the present study. A recent study (Mani et al., 2021) conducted on wild populations of *P. ridibundus* in Turkey, all concentrations (As, Cd, Co, Cr, Cu, Ni, Pb, and Zn) in leg tissue were similar to our study (Table 4).

Risk estimations

As a bioindicator on monitoring water pollution (Rohman et al., 2020), amphibians absorb heavy metals from the aquatic environment and accumulate them in their bodies which can pose a health risk to humans if ingested. The maximum permissible limits for Cd and Pb had been set by some international organizations such as the European Commission (EC, 2006) and Turkish Food Codex (TFC, 2011). Since the maximum permissible limits of Cd and Pb for human consumption in frog have not been set by these organizations, we used the muscle meat of fish instead of frog muscle. For example, the European Commission (EC) and TFC (2011) declared that the Pb and Cd levels in the muscle meat of fish should not exceed 1500 and 1000 μg kg^{-1}, respectively. When we compared our data to these parameters, we noticed that neither Adana nor Istanbul samples exceed the maximum risk limit set by TFC (2011) and EC (2006)/European commission regulation "determination of maximum levels for certain pollutants in foodstuffs" and TFC (2011)/ Turkish food codex contaminants regulation) in terms of Cd and Pb.

THQ, EWI and HI were computed using the estimated weekly intake amount and trace element reference doses (RfD) specified by US EPA (2015) and given in Table 3. EWI values (μg/kg/week) were calculated as 0.08, 0.02, 0.001, 0.03, 0.22, 0.03, 0.23, 8.90 and 0.07, 0.02, 0.02, 0.04, 0.20, 0.01, 0.20, 12.34 for As, Cd, Co, Cr, Cu, Ni, Pb, Zn for samples obtained from Adana and İstanbul, respectively (Table 3). The computed EWI values in our study were much lower than the PTWI values indicated by JECFA (1982, 2011a, b) (Table 3). When the contributions of calculated EWI values to the PTWI were analyzed, these values for Cu, Pb, As, Zn, and Cd were calculated to be 0.006%, 0.8%, 0.53%, 0.17%, and 0.28% for frog samples.

Similarly, THQs were computed to evaluate the hazards that may result from the non-carcinogenic exposure over a lengthy period of time by consuming the *P. ridibundus* leg from two farms in Turkey. Table 1 shows the THQ values derived using trace element concentrations, reference dosage, and daily average frog leg eating. THQ levels detected were below the limit value of 1, indicating that trace elements from *P.*

ridibundus' leg may not have harmful health impacts on humans when consumed regularly. These risk calculations were made entirely according to the raw material results. It is thought that the TE concentration will decrease due to leaching during cooking processes such as boiling and frying. Studies have shown that trace elements in seafood lose their concentration after cooking (Maulvault et al., 2013; Afonso et al., 2015). In addition, it has been determined by modeling that TE intakes were lost throughout the digestive system in humans (Amiard et al., 2008; Afonso et al., 2015; Gedik, 2018;). When such factors are considered, it can be concluded that the TEs obtained from frog consumption will remain at a very limited level.

CONCLUSION

The concentrations of trace elements were determined on edible muscle tissues of the marsh frog samples obtained from two different farms or suppliers in Turkey, and a risk assessment (according to the weekly consumption of 225 g) was evaluated. It was determined that the concentrations of Zn, Pb, Cu, As, Cr, Ni, Cd, and Co detected in the edible tissues of *P. ridibundus* varied spatially, but they were all within the safe consumption limit levels. Comparisons (EWI and PTWI values) could only be made for As, Cd, Cu, Pb and Zn and EWI values were found to be lower than the reported PTWI values. Because the computed THQ and HI values for all of the examined trace elements were less than 1, it is possible to conclude that the trace element concentrations found in *P. ridibundus* samples purchased from two suppliers in Turkey will not pose a health risk to consumers.

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AUTHORSHIP CONTRIBUTIONS

Abdullah Altunışık, Kenan Gedik: Conceptualization, methodology, software. Abdullah Altunışık, Hale Tatlı: Data curation, writing- original draft preparation. Hale Tatlı, Abdullah Altunışık, Kenan Gedik: Visualization, investigation. Abdullah Altunışık, Kenan Gedik: Supervision. Abdullah Altunışık: Software, validation. Hale Tatlı: Project administration, resources, funding acquisition. Abdullah Altunışık, Kenan Gedik: Writing- reviewing and editing.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest or competing interests.

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

For questions regarding datasets, the corresponding author should be contacted.

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