

Evaluation of metal pollution related with human health risk in freshwater snail *Viviparus contectus* (Millet, 1813) as a potential bioindicator species in Lake Habitat

Filiz KUTLUYER KOCABAŞ (✉ filizkutluyer@hotmail.com)

Munzur Universitesi <https://orcid.org/0000-0001-8334-5802>

Emsiye GÖKTÜRK AKSU

Munzur University: Munzur Universitesi

Mehmet KOCABAŞ

Karadeniz Technical University: Karadeniz Teknik Universitesi

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Abstract

For the humankind, water quality of natural resources is the most important and freshwater molluscs are used as bioindicators for the evaluation of water quality in biomonitoring studies. *Viviparus contectus* (Viviparidae: Gastropoda) is a species freshwater snail and consumed by people. Herein, the concentrations of heavy metals heavy metal (Cd, Cr, Pb, As, Zn and Cu) were measured in water and *Viviparus contectus* samples. The water quality (temperature, pH, and dissolved oxygen) were seasonally determined in water samples. The present study showed that the metal accumulation in the water and soft tissues of freshwater snail were in the order of $Pb > Cr = Zn = Cu = Cd = As$ and $Zn > Cu > Pb > Cd > As = Cr$, respectively. The elevated levels of heavy metals (As, Cr, Cu, Zn and Hg) were recorded in autumn. Seasonal variations affected significantly Pb concentration in the freshwater snail samples ($P < 0.05$). EDI (estimated daily intake) values were lower than the recommended tolerable daily intake (TDI) values for adults and the values of HI (hazard index) were lower than 1 for adults. Zn and Pb values in freshwater snail samples were above the limits according to the Turkish Food Codex, FAO/WHO, JECFA and EC limit values. The water analysis indicates mean concentrations of metals below the maximum permissible limits established by the World Health Organization, Turkish Pollution Control Regulation and USEPA, except Pb. Overall, anthropogenic activities adversely affected aquatic ecosystems and this study can be provided a useful data set for metallic contamination in aquatic bodies and biomonitoring studies in freshwater ecosystems.

Introduction

Heavy metal pollution in the aquatic ecosystems is resulted by man-made and natural processes (Pandiyan et al. 2021). In particular, freshwater ecosystems are adversely influenced by heavy metal contamination through urban catchments, fertilize and paper industries, mining, landfills, pesticides, tanneries, batteries and emissions (Nriagu and Pacyna 1988; Ahluwalia and Goyal 2007; Sharley et al. 2016; Liess et al. 2017; Naveen et al. 2017; Zhang et al. 2022). The level of metals in sediment, benthic organisms and fishes is relatively high than water (Namminga and Wilhm 1976; Özmen et al. 2004; Praveena et al. 2008; Pandiyan et al. 2021). Species composition is generally affected by contamination in stream and lakes (Liess et al. 2017).

As a threat, metal pollution in wetland negatively affects invertebrates through food ingestion and dissolved phase (Fisher and Reinfelder 1995; MacFarlane and Burchett 2000; Pandiyan et al. 2021). In pollution studies, invertebrates are chosen as potential ecological indicators due to fact that various species consumes benthic organisms as the major food items (Jones and Walker 1979; Yilmaz 2005; Pandiyan and Asokan 2015; Pandiyan et al. 2021). Particularly, Gastropods have been used in biomonitoring studies owing to their ubiquitous, physiology, sedentary lifestyle and tolerance to water quality (Chukaeva and Petrov 2022).

Viviparus contectus (river snail) is a type of freshwater snail in the Viviparidae family of the Gastropoda class and has a translucent and dark green shell with brown spiral bands on it (Chiu et al. 2002; Kutluyer Kocabaş and Kocabaş 2022). Viviparidae is widely distributed in world (Europe, Australia, America, Africa, Asia and parts of the North) between 0 and 20 m depths, but there are significant gaps about many Viviparidae species in taxonomic records (Lu et al. 2014; Kim et al. 2019). Due to contain high amounts of protein, essential amino acids, and minerals, *V. contectus* is consumed as a food supplement and processed food (sauce, tin cans or freshly cooked) in China (Cao and Yao 2005). Additionally, liver diseases and alcohol poisoning have been treated with *V. contectus* as a traditional medicine in China and Korea (Fu and Zhang 2010; Cui and Zhao 1989; Jiang et al. 2013; Kim et al. 2019). As a bioaccumulator, freshwater snail *Viviparus contectus* (Prosobranchia, Viviparidae) was selected in this study. To our knowledge, this is the first study about the seasonal variations in the heavy metal levels of *V. contectus* caught from wild, although researches have been conducted about bioaccumulation of heavy metal in *V. contectus* (Jurkiewicz-Karnkowska 2011; Felagha et al. 2020) and different freshwater snail species (*Melanoides tuberculata*, *Helisoma duryi*, *Bellamya unicolor*, *Cleopatra bulimoides*, *Helisoma duryi*, *Physa acuta*, *Nerita lineata*, *Lymnaea natalensis*, *Succinia cleopatra*, *Planorbis planorbis*, *Biomphalaria alexandrina*, *Biomphalaria glabrata*, *Bulinus truncates*, *Theodoxus niloticus*, *Bellamya unicolor*, *Lanistes carinatus*, *Gabbiella senaariensis*, *Melanoides tuberculata*, *Cleopatra bulimoides*, *Egeria radiata*, *Limicolaria flammea*, *Viviparus mamillatus*, *Viviparus viviparus*) (Mahmoud and Abu Taleb 2013; Yap and Cheng 2013; Kader et al. 2016; Vukašinović-Pešić et al. 2017; Spyra et al. 2019; Felagha et al. 2020; Orabi and Khalifa 2020; Chukaeva and Petrov 2023). Within the context, the current study is targeted to determine the level of heavy metals in water and freshwater snail (*V. contectus*) with special emphasis on human health risk.

Material and Methods

Sampling sites and snail collection

The freshwater snails (*V. contectus*) were collected from three different localities (38°39'39"N-28°20'53"E, 38°41'17"N-28°22'38"E, 38°28'07"N-28°19'59"E) (Fig. 1) of Demirköprü Dam Lake (Manisa, Turkey) between December 2021 and November 2022 using hand nets. After washing with distilled water, samples were transferred into plastic bags and kept at -20°C until analysis.

Water sampling and physico-chemical characteristics

Sterile plastic bottles (100 ml) were used for water sampling according to the analytical specifications and, stored at 4°C until transport to the laboratory. YSI-Proplus brand multi-parameter device was used for measurement physico-chemical characteristics of water [pH, electrical

conductivity (µS/cm), water temperature (°C), dissolved oxygen (mg/L) concentration, Nitrite (mg/l), Nitrate (mg/l), Ammonia (mg/l), and Phosphate (mg/l)] during sampling.

Metal Analysis

An Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES, Spectro Arcos, vertical plasma) was used for analysing the heavy metal concentrations (As, Cd, Cr, Cu, Pb, Zn, Hg) in water and freshwater snail samples. The separation of tissues was performed using a stainless steel dissection and the inner part was completely removed and samples were dried using an oven at 105°C for 24 h. An agate mortar was used by crushing for homogenization for dried samples. Dried sample (0.5 g with a sensitivity of 0.1 mg) is extracted with HNO₃:H₂O₂ (6:1 proportions) in a closed vessel microwave digestion system (Milestone Ethods D). Detection limits and correlation coefficient for water and snail samples are presented in Tables 1 and 2. Relative Standard Deviation (RSD) ≤ 5%; Correlation Coefficient ≥ 0.9962%; The mean values of detected metal concentrations in the freshwater mussel and water samples were expressed in µg/g and µg/L, respectively

Table 1
Detection limits for water and mussel samples.

Water samples							
µg/L	Cr	Cu	Zn	As	Cd	Pb	Hg
	1.4	7.4	0.94	2.5	2.4	13.2	2.0
Freshwater snail samples							
µg/g	Cr	Cu	Zn	As	Cd	Pb	Hg
	0.14	0.74	0.09	0.25	0.24	1.32	0.2

Table 2
Correlation coefficients for water and mussel samples.

Metals	Cr	Cu	Zn	As	Cd	Pb	Hg
Correlation Coefficient	0.9999	0.9999	0.9998	0.9999	0.9999	0.9962	0.9999

Human health risk assessment (HHRA)

The safety guidelines based on established maximum permissible limits (MPLs) [Food and Agriculture Organization/World Health Organization (FAO/WHO), Joint FAO/WHO Expert Committee on Food Additives (JECFA), European Commission (EC), and Turkish Food Codex (TFC)] were used for comparison the heavy metal concentrations in the freshwater snail tissues. The calculations of estimated daily intake (EDI), target hazard quotient (THQ) and hazard index (HI) were performed according to Yabanli and Alparslan (2015) and Javed and Usmani (2016) for human health risk assessment.

$EDI = (Mc \times Cr) / Bw_a \quad (1)$

where

Mc: the metal concentration in freshwater snail was obtained on ww basis.

Consumption rate: 6.8 g/day (TURKSTAT, 2021).

Body weight: 70 kg for adults (Yaman et al. 2014).

$THQ = (EF \times ED \times CR \times Mc) / (ORD \times ABW \times AET) \times 10^{-3} \quad (2)$

where

EF: exposure frequency (365 days/year)

ED: exposure duration (70 years), equivalent to the average lifetime

CR: consumption rate (6.8 kg/day)

Mc metal concentration in mussels (mg/kg ww)

ORD: oral reference dose as given Table 3.

ABW: average body weight (70 kg for adults)

AET averaging exposure time for non-carcinogens (365 days/year × ED)

10^{-3} unit conversion factor

THQ: <1 indicates no risk for human health.

$$HI = THQ_{\text{toxicant 1}} + THQ_{\text{toxicant 2}} + \dots + THQ_{\text{toxicant n}} \quad (3)$$

where

HI: summing all the calculated THQi values

Table 3

ORD values, mg/kg bw/day (USEPA 2000)							
	Cr	Cu	Cd	Pb	As	Zn	Hg
ORD	3×10^{-3}	4×10^{-2}	1×10^{-3}	4×10^{-3}	3×10^{-3}	3×10^{-1}	3×10^{-3}

Classification of water source

The heavy metal concentrations in the water samples were compared with those of the reference site (without discharge) and World Health Organization (WHO), National Recommended Water Quality Criteria – Aquatic Life Criteria (USEPA) and Turkish Pollution Control Regulation.

Data analysis

The software package SPSS 14.0 for Windows was used for the descriptive statistics, one-way analysis of variance (ANOVA) with Duncan post hoc tests and, Kruskal–Wallis variance analysis for non-parametric data. $p < 0.05$ was set as the statistical significance. XLSTAT software (version 2015.5) was used in principal component analysis (PCA) for amplify data visualization and interpretation.

Results

The means of physico-chemical parameters of Demirköprü Dam Lake from each of three sites recorded during the four seasons are given in Table 4. Heavy metal accumulation in freshwater snail and water samples are shown in Tables 5 and 6. During the study, the metals accumulated in the water samples and freshwater snail are as follows: $Pb > Cr = Zn = Cu = Cd = As$; $Zn > Cu > Pb > Cd > As = Cr$. In autumn, the mean amount of As, Cr, Cu, Zn and Hg was higher in freshwater snail than in spring and summer while the mean amount of Pb and Cd was lower level in freshwater snail. Pb concentrations in water samples reached their highest levels in spring (106.00 ± 1.00) ($p > 0.05$). PCA analysis provides seasonal changes in the heavy metal concentrations. Figure 2 shows that PC1 (the first main component: 55.28%) and PC2 (the second principal components: 22.92%) accounted cumulatively for 78.20% of the variance and Pb has negative scores featuring the four season in freshwater snail. PC1 positively correlated with the variables Cu, Zn and Hg and, As, Cd and Cr were negatively associated with PC1. EDI (Estimated daily intake) amounts for adults by consuming freshwater snail are presented in Table 7 along with ORD amounts. THQ and HI values for adults are given in Table 8. In freshwater snail, Cd, As, Cr values were below the limits in all seasons, and Pb, Zn and Hg values were above the limits according to the TFC, FAO/WHO, EC and JECFA limit values. Cu values were above the limits in autumn and winter according to the FAO/WHO limit values. In water samples, Pb values were above the limits in all seasons according to the TPC, WHO and USEPA limit values.

Table 4
Seasonal mean heavy metal concentrations detected in water samples collected from different stations.

Seasons				
Parameters	Winter	Spring	Summer	Autumn
Temperature (°C)	9.14 ± 1.15	14.30 ± 3.29	27.1 ± 1.25	22.5 ± 4.4
Dissolved oxygen (mg/L)	7.69 ± 1.23	8.05 ± 1.55	6.6 ± 1.16	7.62 ± 3.5
pH	7.87 ± 0.45	8.28 ± 0.25	8.11 ± 0.24	8.25 ± 0.1
Electrical conductivity (µS/cm)	552.00 ± 5.11	663.33 ± 4.01	601.33 ± 4.34	632.33 ± 1.05
Nitrite (mg/L)	0.021 ± 0.037	0.010 ± 0.017	0.080 ± 0.013	0.003 ± 0.006
Nitrate (mg/L)	1.743 ± 0.551	0.594 ± 0.119	2.440 ± 0.756	0.743 ± 0.250
Ammonia (mg/L)	0.023 ± 0.012	0.004 ± 0.007	0.020 ± 0.010	0.020 ± 0.017
Phosphate (mg/L)	0.598 ± 0.267	0.161 ± 0.125	0.286 ± 0.044	0.143 ± 0.042

Table 5
Seasonal mean heavy metal concentrations detected in water samples collected from different stations.

Water samples (µg/l)													
Metals	Autumn	Winter	Spring	Summer	F values	P values	WHO (2011) Water	USEPA (2017) Water (acute exposure)	USEPA (2017) Water (chronic exposure)	Turkish Pollution Control Regulation (2004)			
										Class 1	Class 1I	Class 1II	Class 1V
Cd	< 2.4	< 2.4	< 2.4	< 2.4	*	*	3	1.8	0.72	3	5	10	> 10
As	< 2.5	< 2.5	< 2.5	< 2.5	*	*	10	150	340	20	50	100	> 100
Cr	< 1.4	< 1.4	< 1.4	< 1.4	*	*	50	570	74	-	20	50	> 50
Cu	< 7.4	< 7.4	< 7.4	< 7.4	*	*	2000	13	9	20	50	200	> 200
Pb	106.00 ± 1.00 ^a	104.67 ± 3.06 ^a	125.67 ± 36.94 ^a	105.67 ± 8.33 ^a	0.852	0.504	10	2.5	65	10	20	50	> 50
Zn	< 0.94	< 0.94	< 0.94	< 0.94	*	*	10	120	120	200	500	2000	> 2000
Hg	< 2.0	< 2.0	< 2.0	< 2.0	*	*	6	1.4	0.77	0.1	0.5	2	> 2
*: n/d element was not determined. The difference between the same letters on the same line is insignificant (p > 0.05)"													

Table 6
Seasonal mean heavy metal concentrations detected in *V. contectus* collected from different stations.

Freshwater snail samples (ppm = µg/g)				Turkish Food Codex (2002)		JECFA (2011)	EC (2006)	FAO/WHO (1989)
	Autumn	Winter	Spring	Summer	F values	P values		
Cd	n/d	0.08 ± 0.14	0.12 ± 0.20	< 0.24	*	*	1	0.01–4.8
As	0.30 ± 0.12	< 0.25	0.12 ± 0.20 ^a	< 0.25	*	*	1	-
Cr	0.33 ± 0.33	< 0.14	< 0.14	< 0.14	*	*	-	-
Cu	42.27 ± 21.11 ^a	24.33 ± 14.41 ^{ab}	12.01 ± 1.51 ^b	14.10 ± 3.66 ^a	3.422	0.073	20	-
Pb	11.33 ± 1.21 ^a	13.77 ± 1.50 ^b	12.60 ± 3.64 ^b	23.57 ± 1.89 ^b	18.259	0.001	1.5	11.80
Zn	155.33 ± 44.52 ^a	106.67 ± 44.52 ^{ab}	88.67 ± 2.89 ^b	97.00 ± 7.00 ^a	3.836	0.057	50	-
Hg	0.98 ± 0.24 ^a	0.60 ± 0.19 ^a	0.32 ± 0.65 ^a	0.61 ± 0.05 ^a	2.091	0.180	0.5	0.3–0.5
*: n/d element was not determined. The difference between the same letters on the same line is insignificant (p > 0.05)"								

Table 7
Heavy metal EDI amounts (µg/day) for adults.

	Cr	Cu	Cd	Pb	As	Zn	Hg
Autumn	0.032	4.10	n/d	1.10	0.029	15.08	0.09
Winter	n/d	2.36	0.007	1.33	n/d	10.36	0.05
Spring	n/d	1.16	0.011	1.22	0.011	8.61	0.06
Summer	n/d	1.37	n/d	2.28	n/d	9.42	0.05
Total	0.008	1.66	0.005	1.48	0.01	10.86	0.06
TDIs (µg/day)	250	10,000	70	240	n/d	n/d	49
ORD (µg/kg/day)	3	40	1	0.40	0.3	300	0.30
*n/d element was not determined.							

Table 8
THQ and HI values for adults

	Cr	Cu	Cd	Pb	As	Zn	Hg	HI
Autumn	0.01	0.10	n/d	0.3	0.009	0.05	0.3	0.769
Winter	n/d	0.06	0.007	0.3	n/d	0.03	0.17	0.567
Spring	n/d	0.03	0.011	0.4	0.004	0.03	0.2	0.675
Summer	n/d	0.03	n/d	0.6	n/d	0.03	0.17	0.830
Total	0.003	0.06	0.005	0.005	0.003	0.03	0.21	0.316
*n/d element was not determined.								

Discussion

Freshwater snails have filtration ability the large volumes of water and accumulate greater amounts of metals in their tissues owing to the lack of movement (Pruei et al. 1987; Rouane-Hacene et al. 2015; Azizi et al. 2018) and therefore, they are chosen as bioindicator organisms for biomonitoring studies. The pattern of accumulation in water and freshwater snail was $Pb > Cr = Zn = Cu = Cd = As$; $Zn > Cu > Pb > Cd > As = Cr$. Yap and Cheng (2013) detected bioaccumulation of Zn and Cu at higher levels in different tissues of the mangrove snail *Nerita lineata*. Kader et al. (2016) studied bioaccumulation of heavy metal in 14 freshwater snail species and determined highest heavy metal accumulation in *Helisoma duryi* ($8787.10 \mu g.g^{-1}$ DW for Cu) and *Physa acuta* ($7626.95 \mu g.g^{-1}$ DW for Fe). Orabi and Khalifa (2020) reported that the mean concentrations of Fe, Cu, and Zn were higher levels in freshwater snail soft tissues of *M. tuberculata*. The high presence of Zn and Cu could be explained with their roles on biological and physiological processes (Catsiki et al. 1994; Depledge et al. 1994; Rainbow 1997; Langston et al. 1998). Additionally, in our study, the mean concentrations in freshwater snail followed the sequence: $Cu < Zn$ in agreement with previous study reported by Spyra et al. (2019) in the body of *P. acuta*. Season affected metal accumulation in water and freshwater snail ($p < 0.05$). In autumn, the presence of heavy metals in higher levels (As, Cr, Cu, Zn and Hg) might be result from discharge waters of industrial establishments and the urban inputs of Manisa city.

The environmental concentrations and factors affect metal bioaccumulation in shellfish (Mubiana and Blust 2007). In addition to this, the metal chemistry is influenced by temperature through changing physical kinetics, pH, solubility, speciation of chemical or reaction rates (Blust et al. 1994; Byrne et al. 1988; Rouane-Hacene et al. 2015). The activities and concentrations of bioavailable metal forms are correlated with temperature (Rouane-Hacene et al. 2015). In this study, Pb concentrations in freshwater snail samples reached their highest levels in summer and it could be explained by increase in temperature. In addition, atmospheric Pb levels, intense traffic, wastewater discharges and urban population might be affect Pb accumulation in freshwater snail.

In molluscs, the response to various environmental stressors, homeostasis, food uptake and growth rates are influenced by abiotic factors (e.g. temperature, chemical contaminants) and biotic (e.g. reproductive cycle) (Cossa et al. 1979; Davies and Pirie 1980; Phillips 1976; Rouane-Hacene et al. 2015). Previous studies have been emphasised that reproduction period affects tendency of the metal bioavailability and bioaccumulation related with devoting more energy (Blaise et al. 2002; Bodin et al. 2004; Guerlet et al. 2007; Rouane-Hacene et al. 2015). In agreement with previous reports, the reproduction period of *V. contectus* is spring and summer and the mean amount of As, Cr, Cu, Zn and Hg was lower in freshwater snail in summer than in autumn and winter.

The regulation of many biological processes is provided by Zn as an essential element in invertebrates (Danabaş et al. 2022). Zn accumulated the large amount in tissues of freshwater snail and reached their highest levels in autumn. The source of Zn in freshwater snail and water samples could be several factors such as the domestic and industrial growth (Yap et al. 2010,2016). The concentrations of the metal in freshwater snail were above the limits according to the TFC and FAO/WHO limit values. According to Turkish Water Pollution Control legislation (2004), Demirköprü Dam Lake is in Class I water resources according to Zn levels.

The multiple enzymatic reactions, cells physiology, functions and structure of proteins and hemoglobin synthesis are provided by Cu as an essential element in invertebrates (Saha et al. 2016; Danabaş et al. 2018; Pandiyan et al. 2021). Cu concentration exceeded a certain threshold in autumn and winter according to the TFC and FAO/WHO limit values while its concentrations were below the limits in spring and summer. In consistent with our results, the presence of high levels of Cu has been reported by Orabi and Khalifa (2020) in soft tissues of *M. tuberculata*. The high presence of Cu has been reported by Mahmoud and Abu Taleb (2013) in tissues of five freshwater snails (*Bellamya unicolor*, *Cleopatra bulimoides*, *Helisoma duryi*, *Physa acuta* and *Theodoxus niloticus*). The source of Cu in freshwater snail and water samples could be several factors such as intensive inputs of urban, using fungicides and paints, aquaculture and food processing and the anthropogenic activities (e.g. mining and industries producing wire and cables, electronic devices) (Bilandzic et al. 2012, Simonato et al. 2016, Blewett et al. 2017; Danabaş et al. 2018).

Cd concentration accumulated the highest levels in tissues of freshwater snail in spring and summer according to the JECFA (2011) permissible limit values unlike previous report by Chukaeva and Petrov (2023) in *Viviparus* sp. The binding to the protein and the long half-life might be affect Cd accumulation in freshwater snail (FDA 2001; Suprapti et al., 2016; Riani et al. 2018).

The relationships among variables were assessed using Principal component analysis (PCA). The data for freshwater snail in this study showed that Cu was strongly related to Zn and Hg and, As, Cr and Cd were negatively correlated with Pb. The noted difference in correlation among variables can be explained with seasonal differences.

The amounts of shellfish intakes per week affect the health risks to the consumers (Yap et al. 2016). Felagha et al. (2020) reported the values of EDI, THQ and CR in *V. contectus* from Yenagoa in Bayelsa State within acceptable limits for heavy metals (Cd, Mn, Pb and Zn). Similarly, the EDI values for adults were well below the values of oral reference dose and tolerable daily intake. The calculated values of THQ and HI values for all metals (As, Zn, Cd, Cr, Pb, Cu and Hg) much less than 1. Overall, our data showed no health risk for consumer of freshwater populations in Demirköprü Dam Lake.

From the obtained results, it is clear that concentrations of heavy metal in freshwater snail tissues were higher levels than in water. In this sense, freshwater snail (*V. contectus*) could be the potential bio-indicator organisms in terms of reflect ambient metal bioavailability. The discharge waters of industrial establishments may be elevating the heavy metals concentrations. Additionally, tendency to accumulation of heavy metals increased in

autumn. Aquaculture facilities and governmental monitoring programmes are required for regularly biomonitoring studies and crucial for the future of freshwater ecosystems.

Declarations

Author contributions Conceptualization, supervision and writing were performed by Filiz KUTLUYER KOCABAŞ, investigation were performed by Emsiye GÖKTÜRK AKSU, sampling and analysis were performed by Mehmet KOCABAŞ.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests

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Figures

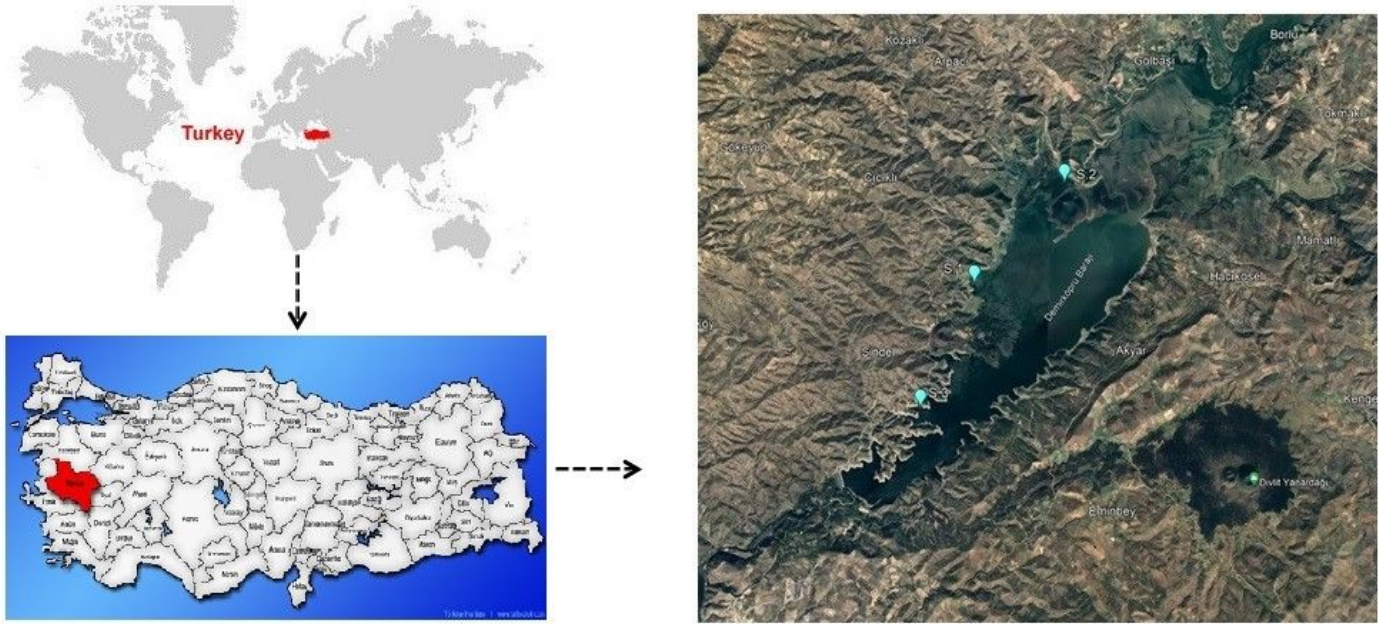


Figure 1

Sampling locations of the freshwater snail *Viviparus contectus* from the Demirköprü Dam Lake from 2021 to 2022.

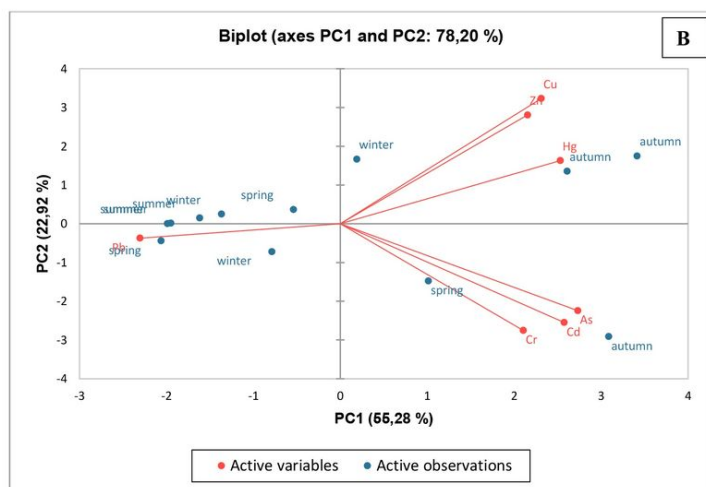
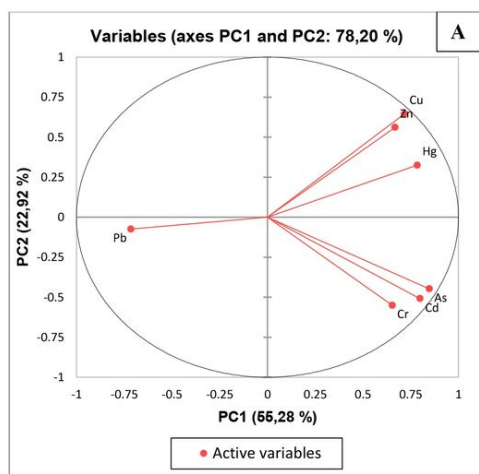


Figure 2

Illustration of scores (A) and loadings (B) from the Main Component Analysis of seasonal heavy metals in freshwater snail.