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Various Elements Levels in Four Freshwater Mussels Shells Obtained from Gölbaşı Lake, Turkey

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ABSTRACT

The aim of this study was to determine by SEM-EDS analysis of the surface morphologies of the periostracum and nacreous layer and to determine the sodium (Na), Strontium (Sr), manganese (Mn), potassium (K), titanium (Ti), iron (Fe), magnesium (Mg), cobalt (Co), copper (Cu), chromium (Cr), zinc (Zn) and nickel (Ni) metals levels in the shells of the mussel (*Potomida semirugata, Unio terminalis, Anodonta pseudodopsis* and *Leguminaia wheatleyi*) obtained from Gölbaşı Lake, Turkey. The results of the study, the representative SEM analysis and corresponding EDS spectra of the periostracum and nacreous layer of the shells of freshwater mussels confirmed the presence of elemental compositions, including $CaCO_3$. *P. semirugata* and *U. terminalis* have the aragonite prismatic layer that shows typical polygonal organizing, regular and polygonal crystal forms, with hexagonal and coexisting rhombic shapes. However, while *A. pseudodopsis* has round aragonite crystals (Rc), *L. wheatleyi* has irregular crystal plate layers (Irc). $CaCO_3$, detected strong Ca peaks as well as C and O peaks with Mg and Si peaks. On the other hand, sodium (Na) was found in the highest concentrations ranging from 82.30±0.040 to 155.37±0.050 µg/g, and its concentrations were also higher than those of other metals in all species. The most abundant elements in shells of four freshwater mussel's species were Sr, Na, and Mn which ranged from 26.07±0.44-58.023±0.52 µg/g, 82.30±0.040-155.37±0.050 µg/g, and 6.06±0.044-9.66±0.053 µg/g respectively. To our knowledge, this is the first study in Turkey that is researched the different four freshwater mussel species in the Gölbaşı Lake, Turkey.

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INTRODUCTION

Gölbaşı Lake is a natural lake in the southeastern Mediterranean Sea of Turkey. It occupies an area of 12 km^2 including 4 km^2 of wetlands. The lake is fed by groundwater and is used for agricultural irrigation and recreational facilities. The maximum water depth after the 2.5-3 m^3/s irrigation season averages 1.5 m to 4 m in summer and 6 m to 3.5 m in winter. Lake Gölbaşı has been categorized as eutrophic (Şereflişan, 2003). The lake has an important feature due to its mollusk fauna (Şereflişan, 2001).

In recent decades, pollution of freshwater species by organic and inorganic contaminants has become a major concern (Canli et al., 1998). Trace metals are one of the main varieties of water pollutants that severely affect freshwater (DWAF, 1996). Freshwater mussels are used organisms for biomonitoring targets of aquatic areas (Fournier et al., 2001). Freshwater mussel species are in contact with the aqueous areas and are very probably to accumulate metals and other pollutants in their soft tissues (Farris & Van Hassel, 2006). They are precision to pollution,

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and their body parts such as their gill and digestive glands have nutritional functions, allowing them to filter pollutants out of their water (Chakraborty et al., 2010; Fournier et al., 2001; Królak & Zdanowski, 2001).

Generally, freshwater mussels are bivalves with two symmetrical shells, belonging to the class bivalvia of the phylum of mollusks. Freshwater mussels, with the exception of zebra mussels, generally live in the sediment part of river beds, water channels, ponds and lakes. Freshwater mussels, which also live buried in the sediment, feed by filtering plankton, bacteria, organic and mineral substances in the water thanks to their incurrent siphons (Şereflişan, 2003). Freshwater mussel is an organisms that can be used as biological indicators or bioindicators (Grabarkiewicz & Davis, 2008). A biomarker is an organism or biological response that indicates the entry of a particular substance into the environment. Both essential and non-essential trace elements are known to be highly enriched by invertebrates, especially various mollusk species (Dallinger and Rainbow, 1993). It was reported by (Koide et al., 1982) reported that mussel shells may be better biomonitors for metal contamination than soft tissue because they are lifelong integrant of metals.

Aquatic mollusks are often used as bioindicator organisms because they appear to reflect metal contamination from the environment (Ahmed et al., 2010; Dar et al., 2018; Jia et al., 2018; Manly & George, 1977; Ravera et al., 2003). Furthermore, the ability of mollusks to concentrate metallic contaminants in the marine environment has been highlighted in many publications (Chase et al., 2001; Doğan et al., 2022; Duysak et al., 2021; Duysak & Uğurlu, 2020, 2017; Elder & Collins, 1991; Gundacker, 2000; Lares & Orians, 2001; Muñoz-Barbosa et al., 2000; Ravera et al., 2003; Rosenthal & Katz, 1989).

The aim of this study was to determine by SEM analysis of the surface morphologies of the periostracum and nacreous layers at shells and to determine the metal (Na, Sr, Mn, K, Ti, Fe, Mg, Co, Cu, Cr, Zn and Ni) concentration levels in the shells of the mussel (*Potomida semirugata*, *Unio terminalis*, *Anodonta pseudodopsis* and *Leguminaia wheatleyi*) obtained from Gölbaşı Lake. To our knowledge, this is the first study in Turkey that is researched the different four freshwater mussel species in the Gölbaşı Lake.

MATERIAL AND METHODS

Study Area

Gölbaşı Lake (36º 29′ E, 36º 30′ N) is sited near the town of Kırıkhan in the Eastern Mediterranean Sea of Turkey. This natural lake insurance an area of about 400 ha (4,000,000 square meters) (Figure 1). Gölbaşı Lake, a natural lake, is fed by groundwater in various places. It is surrounded by agricultural land and used for irrigation purposes for cotton production. Therefore, spring and summer water levels are lower than at other times of the year. Four (*P. semirugata*, *U. terminalis*, *A. pseudodopsis* and *L. wheatleyi*) (Figure 2) freshwater mussel shells were collected from a location of the Gölbaşı Lake in the Eastern Mediterranean basin of Turkey in 2022. These species were chosen because their geographic range extends throughout the Gölbaşı Lake.

Mussel sampling

The freshwater mussel shells were rinsed with deionized water. Then, shell length and height of freshwater mussel species were measured with digital calipers with 0.05 mm precision. The shell length, height, habitats, and distributions of the studied species were obtained in the Table 1 (Lopes-Lima et al., 2021) (Table 1).

Microstructure observation and characterization with the SEM and EDS

The surface morphological of the shells of the four freshwater mussels *P. semirugata*, *U.*

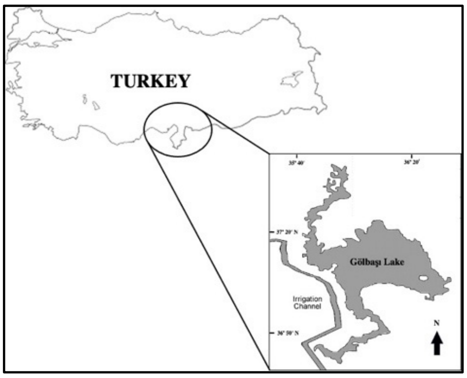

Fig. 1. Study area (Anonymous, 2023; Şereflişan et al. 2013)

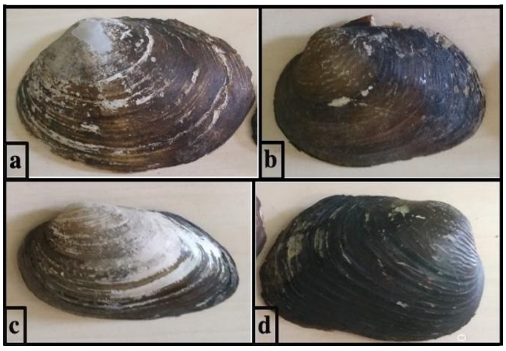

Fig. 2. a) *Anodonta pseudodopsis*, b) *Potamida semirugata*, c) *Unio terminalis* and d) *Leguminaia wheatleyi*

Table 1. Morphological characteristics (length in cm and weight in g), and distribution of four freshwater mussel shells.

Species	n	Length	Height	Distribution
P. semirugata	20	6.2±0.40	2.85±0.50	Northwestern Turkey, Greece, North Africa, France, Spain
U. terminalis	20	7.2±0.35	3.05±0.28	Eastern Mediterranean, North Africa and Europe, Turkey, Israel
A. pseudodopsis	20	10.45±0.375	4.0±0.25	Turkey and Syria
L. wheatleyi	20	7.3±0.27	2.8±0.32	Turkey, Eastern Mediterranean

terminalis, *A. pseudodopsis* and *L. wheatleyi* described two different layers with namely, the nacreous layer and the external layer. The mussel shells were cut into small pieces and flat surfaces were selected. An SEM was used to examine the surface morphology, nacreous layer and periostracum layer of freshwater mussel shells. Before shell imaging, the shells were coated with gold-palladium (Au-Pd) using a POLARON SC7620 sputter coating device. The sample was visualized under SEM (Scanning Electron Microscope) (JEOL JSM-638OLA) using 10-15 kV. Chemical composition and elemental characterization of freshwater mussel shells were carried out using the energy-dispersive X-ray spectroscopy (EDS) attached to SEM.

Metal analysis

Shells were mechanically pulverized using a grinder. 2 g shell powder samples were placed in a 20 mL digestion tube, and 5mL of high purity HCl (Merck, Hydrochloric acid fuming 37%), HNO_3 (Merck, Nitric acid 65%) and H_2O_2 (Merck, Hydrogen peroxide 30%) were added. Then, the samples were filtered throughout Whatman-Quantitative (No: 42, 110 mm £) filter paper. The digested part was added distilled water to a final volume of 20 ml. A blank digest was performed in the same procedure. Samples were studied with three replicates for each metal.

Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent, 7500ce Model) was used to determine metals. ICP-MS working conditions were as follows: radio frequency (RF), 1150 W; plasma gas flow rate, 15 L/min; auxiliary gas flow rate, 0.5 L/min; carrier gas flow rate, 1.1 L/min; spray chamber T, 2°C; sample depth, 6 mm; sample introduction flow rate, 1 mL/min; nebulizer pump, 0.1 rps; extract lens, 1.5 V.

The elements (Na, Sr, Mn, K, Ti, Fe, Mg, Co, Cu, Cr, Zn and Ni) in the samples were identified as µg metal per gram of dry weight (dw). Data quality was checked using analysis of the standard reference material DORM-2 (National Research Council of Canada; dogfish muscle and hepatopancreas MA-A-2/TM Fish Flesh). The standard solutions prepared had sodium, strontium, manganese, titanium, iron, cobalt, copper, chromium, zinc, and nickel contents ranging from 1 ppb to 100 ppm (0.001-100 mg/L) for the element.

Data Analysis

The results obtained from laboratory analysis were subjected to statistical analysis using various tools; a descriptive tool was used to determine the mean, standard deviations, and coefficient of variation, while the Pearson correlation coefficient was done to establish the dependence of metals in freshwater mussel shells. The nearer the coefficient to one (1) indicated a stronger correlation between variables, and the nearer to -1 indicated a decrease in a linear relationship (Kumari & Maiti, 2019). One-Way ANOVA was used to found if there was a significant difference between metals among freshwater mussel shells and the $p<0.05$ was noted significant (Akoto et al., 2014).

RESULTS AND DISCUSSION

Characterization of Shells

The freshwater mussel shells are tough biological material that serves as a protecting outer skeleton of the related species. A unique biomineralization method makes the shell stronger, more flexible and less crushable, giving the mussel species an advantage in preventing predators and as a scaffolding for a variety of biota. As it grows and remodels, each layer of the shell is revised so that the shell is stronger than the singular layer. The necessary shell-forming components such as crossed lamellae, laminates and prismatic structures were plentiful in each layer of the shell, regardless of the freshwater species. The basis for these diverse structures can be propertied to aragonite's inimitable crystalline properties that maintain design flexibility.

P. semirugata has the aragonite prismatic layer that presents normal multilateral organizing, with prisms of very altered sizes, resulting in part from added columnar structures demonstrated by an exaggerated image in layer (Figures 3B, 5B). *U. terminalis* has regular and hexagonal crystal shapes, and coexisting with rhombic shapes, are observed (Figure 5B). However, while *A. pseudodopsis* has different diagonal aragonite crystals (Dc), *L. wheatleyi* has irregular rounded aragonite crystalline structure plate layers (Irc) (Figures 7B, 9B). Lateral the prismatic layer (Pl) of *P. semirugata, U. terminalis, A. pseudodopsis,* have regular crystal plate (Rcp) (Figures 3C, 5C,7C). However, these plates were found to be irregular in *L. wheatley* (Figure 9C). Periostracum layer (Pl) showed a more pronounced porosity in *A. pseudodopsis* (Figure 7A).

Since shell mineralization is under cellular control, factors affecting cellular metabolism

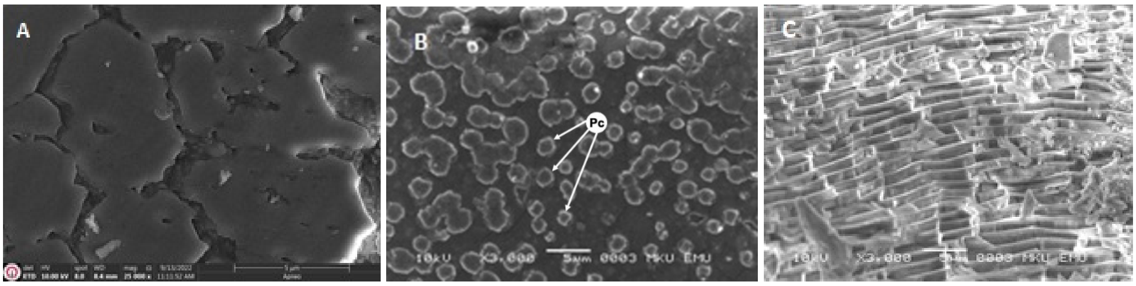

Fig. 3. A) Periostracum layers (Pl). B) Nacreous layers (Nl); Nacreous layer showing polygonal regular-forming crystals (Pc). C) Lateral observation of the prismatic layer (Pl) showing columnar convex prisms; image of regular crystal plate layers (Rcp)

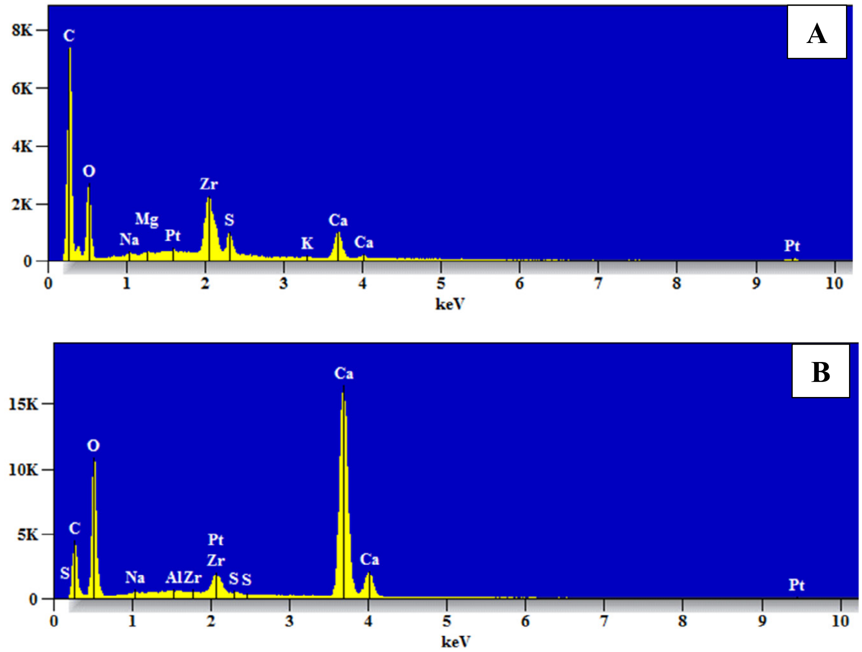

Fig. 4. SEM-EDS of different surface of *P. semirugata* shells. (A) periostracum layer, (B) nacreous layer.

in the mantle epithelium may also affect the natural structure of shell structure (Lopes-Lima et al., 2010). These factors are not only variations in water physical parameters such as pH, temperature and ionic arrangement of water, but also the presence of contaminants is known to affect biomineralization processes such as toxic metals (Machado et al., 1990; Moura et al., 2000; Okoshi & Sato-Okoshi, 1996). Generally, the demonstrative SEM analysis and corresponding EDS spectra of the periostracum and nacreous layers of the shells of freshwater mussel confirmed the presence of elemental compositions, including of $CaCO_3$. $CaCO_3$, detected strong Ca peaks as well as C and O peaks with Mg and Si peaks.

Microstructure of P. semirugata Shells

Figures 4A and 4B demonstrate the SEM-EDS images and EDS data of the periostracum and nacreous layers in *P. semirugata* shells, respectively. The semi-quantitative chemical composition demonstrated that Ca, Na, Mg, C and O as elements. Nacre layer, the chemical compositions of elements (53.04-65.80 atom%) for C, (29.30-38.53 atom%) for O, (0.16-0.27 atom%) for Na, (0.06-0.13 atom%) for Mg, (0.78-1.59 atom%) for S, and (1.52-4.48 atom%) for Ca of *P. semirugata* periostracum layer. Periostracum layer the chemical compositionf of

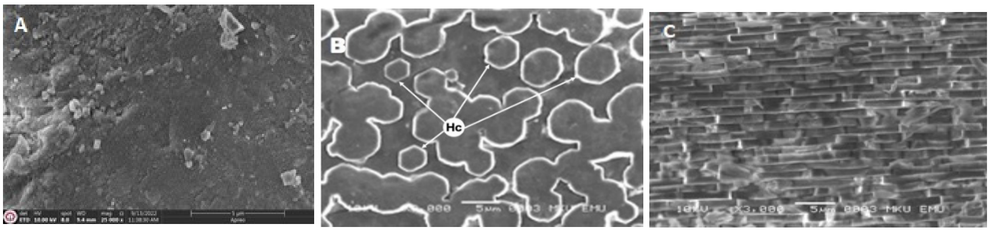

Fig. 5. A) Periostracum (Pe) layers, B) Nacreous layers (Nl); Nacreous layer showing hexagonal orderly-forming crystals (Hc). C) Lateral show of the prismatic layer (Pl) showing columnar convergent prisms; image of orderly crystal plate layers (Rcp)

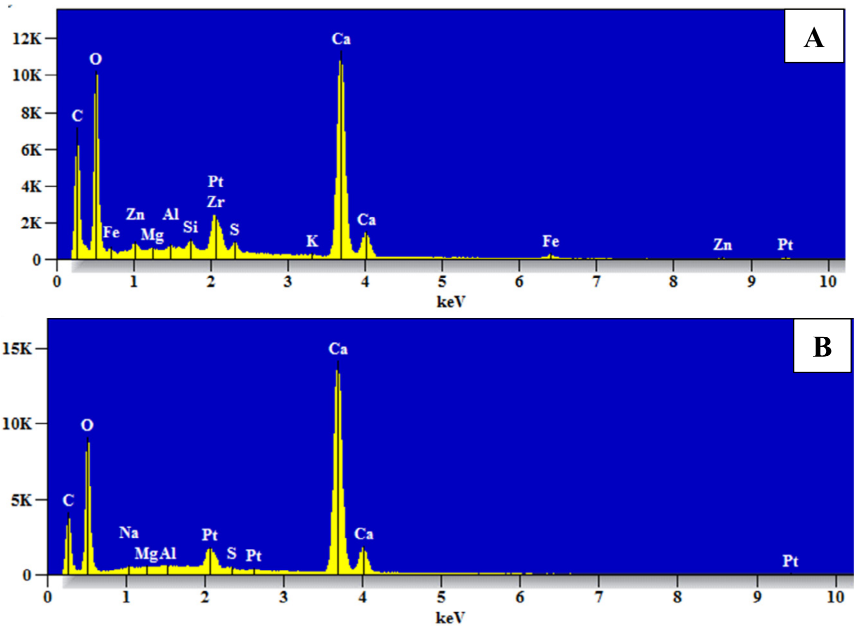

Fig. 6. SEM-EDS of different surface of *U. terminalis* shells. (A) periostracum layer, (B) nacreous layer.

elements between ranged (7.28-12.85 atom%) for C, (52.14-67.07 atom%) for O, (0.11-0.18 atom%) for NA, (0.09-0.11 atom%) for Al, (0.06-0.07 atom%) for S, (19.30-39.35 atom %) for Ca of *P. semirugata* periostracum layer.

Microstructure of U. terminalis Shells

Figures 6A and 6B demonstrate the SEM-EDS images and EDS data of the periostracum and nacreous layers in *U. terminalis* shells, respectively. The semi-quantitative chemical composition demonstrated that Ca, Na, Mg, C and O as elements. periostracum layer, the chemical compositions of elements between ranged (15.61-60.16 atom%) for C (35.41-65.37 atom%) for O, (0.24-0.26 atom%) for Na, (0.15-0.42 atom%) for Al, (1.06-18.6 atom%) for Ca, (0.16-0.24 atom%) for Mg, (0.05-0.14 atom%) for K, (0.13-1.25 atom%) for Si, (0.17-0.71 atom%) for Zr, (0.24-0.77 atom%) for Pt and (0.17-0.93 atom%) for S of *U. terminalis* periostracum layer (Figure 6A). Nacreous layer the chemical compositionf of elements between ranged (13.4-16.77 atom%) for C, (63.69-65.99 atom%) for O, (0.15-0.23 atom%) for Na, (0.1-011 atom%) for Al, (16.01-20.76 atom%) for Ca, (0.06-0.44 atom%) for Mg, (0.05-0.07 atom%) for Si, (0.44-0.63 atom%) for Zr, (0.25-0.94 atom%) for Pt and (0.12-0.2 atom%) for S of *U. terminalis* nacreous shell (Figure 6B).

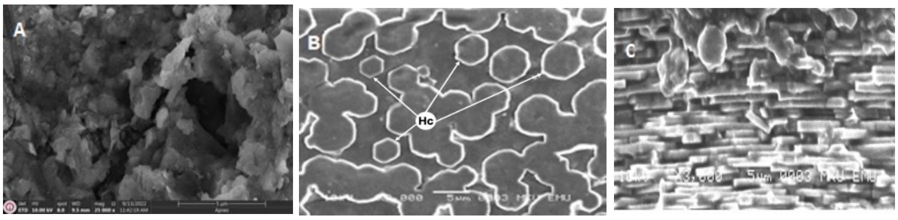

Fig. 7. A) Periostracum (Pe) layers, B) Nacreous layers (Nl); nacreous layer showing 3–4 layers of different diagonal aragonite crystals (Dc). C) Lateral view of the prismatic layer (Pl) showing columnar convergent prisms; image of regular crystal plate layers (Rcp)

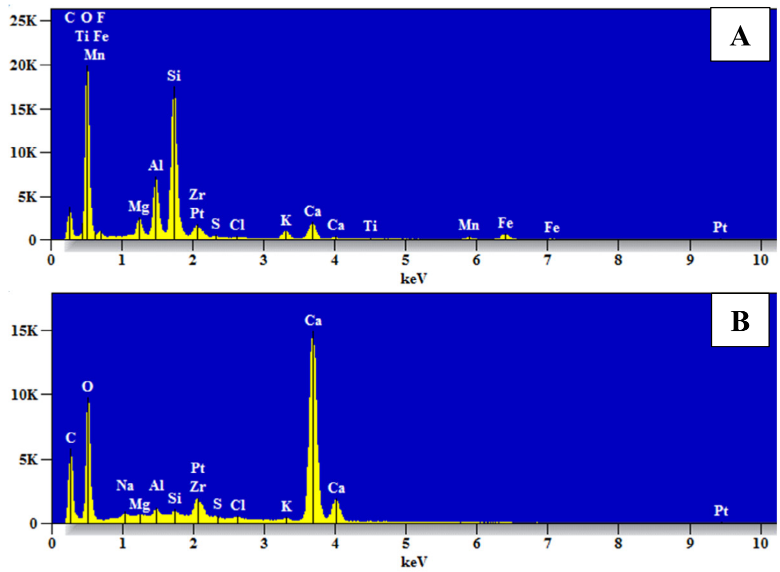

Fig. 8. SEM-EDS of different surface of *A. pseudodopsis* shells. (A) periostracum layer, (B) nacreous layer.

Microstructure of A. pseudodopsis Shells

Figures 8A and 8B demonstrate the SEM-EDS images and EDS data of the periostracum and nacreous layers in *A. pseudodopsis* shells, respectively. The semi-quantitative chemical composition observed that Ca, Na, Mg, C and O as elements. Nacreous layer, the chemical compositions of elements between ranged (13.3-22.45 atom%) for C (54.86-63.12 atom%) for O, (0.22-0.82 atom%) for Na, (0.1-0.15 atom%) for Mg, (0.35-0.66 atom%) for Al, (0.26-0.34 atom%) for Si, (0.06-0.15 atom%) for S, (0.07-0.25 atom%) for Cl, (0.04-0.39 atom%) for K, (18.30-22.2 atom%) for Ca, (0.32-0.5 atom%) for Zr and (0.31-0.39 atom%) for Pt of *A. pseudodopsis* nacreous shell (Figure 8B). Periostracum layer the chemical compositionf of elements between ranged (13,13-22.45 atom%) for C, (54.86-63.12 atom%) for O, (0.22-0.82 atom%) for Na, (0.1-011 atom%) for Al, (18.3-22.2 atom%) for Ca, (0.06-0.44 atom%) for Mg, (0.04-0.39 atom%) for K, (0.07-0.325 atom%) for Cl, (0.26-0.79 atom%) for Si, (0.32-0.5 atom%) for Zr, (0.31-0.39 atom%) for Pt and (0.06-0.15 atom%) for S of *A. pseudodopsis* periostracum layer (Figure 8A).

Microstructure of L. wheatleyi Shells

Figures 10A and 10B demonstrate the SEM images and EDS data of the periostracum and nacreous layers in *L. wheatleyi* shells, respectively. The semi-quantitative chemical composition observed that Ca, Na, Mg, C and O as elements. Nacreous layer, the chemical compositions of

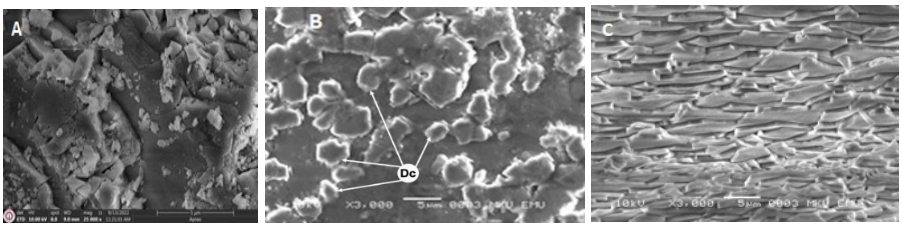

Fig. 9. A) Periostracum (Pe) layers, B) Nacreous layers (Nl); nacreous layer showing its irregular rounded aragonite crystalline structure (Irc). C) Lateral view of the prismatic layer (Pl) showing columnar convergent prisms; image of irregular crystal plate layers (Icp)

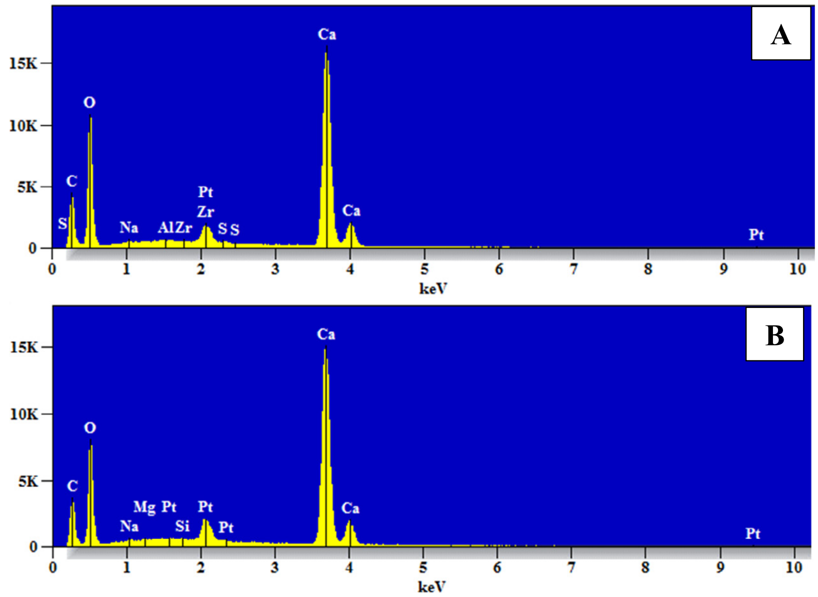

Fig. 10. SEM-EDS of different surface of *L. wheatleyi* shells. (A) periostracum layer, (B) nacreous layer.

elements between ranged (12.33-12.92 atom%) for C, (65.63-66.49 atom%) for O, (0.14-0.2 atom%) for Na, (0.07-0.09 atom%) for Mg, (0.06-0.08 atom%) for Si, (20.06-22.6 atom%) for Ca, (0.15-0.87 atom%) for Zr and (0.57-0.75 atom%) for Pt of *L. wheatleyi* nacreous shell (Figure 10B). Periostracum layer the chemical compositionf of elements between ranged (7.28-12.85 atom%) for C, (52.14-67.07 atom%) for O, (0.11-0.18 atom%) for Na, (0.09-0.11 atom%) for Al, (19.3-39.35 atom%) for Ca, (0.03-0.15 atom%) for Zr, (0.49-1.12 atom%) for Pt and (0.06-0.07 atom%) for S of *L. wheatleyi* periostracum layer (Figure 10A).

Metals in Shells

In this study, concentrations of strontium (Sr), cobalt (Co), titanium (Ti), magnesium (Mg), sodium (Na), potassium (K), cupper (Cu), manganese (Mn), iron (Fe), nickel (Ni), zinc (Zn) and chromium (Cr) were investigated 4 different freshwater mussel shells collected from the Lake Gölbaşı in the North East Mediterranean area of Turkey. The concentration of Sr, Co, Ti, Mg, Na, K, Cu, Mn, Fe, Ni, Zn and Cr in the shell of freshwater mussels *P. semirugata, U. terminalis, A. pseudodopsis* and *L. wheatleyi* are shown in Table 2. The metal levels in freshwater mussel species were determined that the similar to each other (Figure 11).

Especially Mg, Mn, and Sr are generally studied metals in mollusk shells and are often used as represents for environmental conditions (Poulain et al., 2015; Schöne et al., 2011). For

Table 2. Metal concentration (in µg g^{-1}) in the shells of *P. semirugata* (PS), *U. terminalis (UT), A. pseudodopsis (AP) and L. wheatleyi (LW) freshwater mussels* from Gölbaşı Lake.

Species	Sr	Co	Ti	Mg	Na	K	Cu	Mn	Fe	Ni	Zn	Cr
PS	26.07± 0.04a	1.07± 0.05^{a}	1.32± 0.01^{a}	1.08± 0.00^{a}	82.30±0 $.03^{a}$	3.53± 0.02^{a}	0.48± 0.02^{a}	8.06± 0.03^{a}	1.09± 0.04^{a}	0.01± 0.00^{a}	0.18± 0.01^{a}	0.22± 0.01^{a}
UT	38.06± 0.03^{b}	0.56± 0.05^{b}	1.28± 0.02^{a}	0.86± 0.00^{a}	101.65± 0.03^{b}	5.60± 0.05^{b}	0.25± 0.00^{b}	9.66± 0.04^{b}	1.29± 0.04^{b}	0.01± 0.00^{a}	0.37± 0.01^{b}	0.23± 0.00^{a}
AP	38.58± 0.04^{b}	0.29± 0.03^{c}	1.17± 0.02^{b}	1.02± 0.02^{a}	116.70± 0.05^{c}	4.59± 0.06^{c}	0.52± 0.00^{c}	7.90± 0.05^{c}	0.90± 0.04^{c}	0.01± 0.00^{b}	0.16± 0.00^{c}	0.26± 0.01^{b}
LW	58.02± 0.25^{c}	0.36± 0.00^{c}	1.22± 0.00^{c}	1.29± 0.00^{a}	155.37± 0.04^{d}	5.15± 0.06^{d}	0.18± 0.02^{d}	6.06± 0.03^{d}	0.59± 0.02^{d}	0.01± 0.01^{b}	0.33± 0.01^{d}	0.27± 0.00^{b}

Vertically letters a,b,c and d indicates differences among species ($p<0.05$).

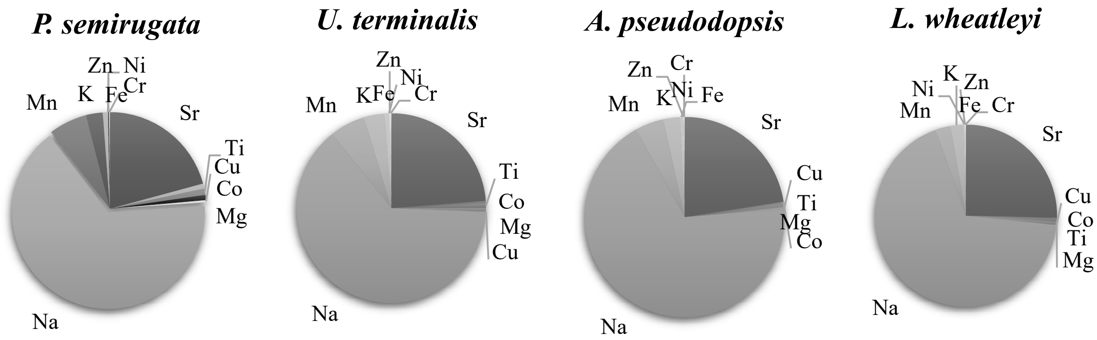

Fig. 11. Variation in the metal concentration in the shell of four freshwater mussel species sampled from Gölbaşı Lake.

that purpose, *Potomida semirugata* (Lamarck, 1819), *Unio terminalis* (Bourguignat, 1852), *Anodonta pseudodopsis* (Locard, 1893) and *Leguminaia wheatleyi* (I. Lea, 1862) samples were collected from Gölbaşı Lake regions. These four freshwater species are sediment species and filter feeders; they constantly filter food organic and inorganic particles out of the water (Allen, 1921; Watters, 1992). Table 4 represents the metal concentrations and standard deviation of various metals analyzed in four freshwater mussel species collected from Gölbaşı lake.

Sodium (Na) was found in the highest concentrations ranging from 82.30±0.040 to 155.37±0.050 g/g, and its concentrations were also higher than those of other metals in all species. Following Na; Sr generally showed the second highest levels. The most abundant elements in shells of four freshwater mussel's species were Sr, Na, and Mn which ranged from 26.07±0.44-58.023±0.52 µg/g, 82.30±0.040-155.37±0.050 µg/g, and 6.06±0.044-9.66±0.053 µg/g respectively (Table 2). The concentration of Co (1.07±0.061 µg/g), was significantly higher in the shells of *P. semirugata* and Sr (58.023±0.52 µg/g) and Na (155.37±0.050 µg/g) were higher in the shells of *L. wheatleyi.*

In this study, metal concentration levels in the shells of freshwater mussels *P. semirugata, U. terminalis, A. pseudodopsis* and *L. wheatleyi* of Gölbaşı Lake were analyzed. Mean accumulation levels (in µg/g dw) with standard errors (x±s) in the shells of *P. semirugata, U. terminalis, A. pseudodopsis* and *L. wheatleyi* were given in Table 2.

Concentrations of Macroelements

Magnesium (Mg)

The magnesium content in freshwater mussel shells *P. semirugata, U. terminalis, A. pseudodopsis*, and *L. wheatleyi* from Gölbaşı Lake was 1.08±0.009 µg/g, 0.86±0.001 µg/g, 1.02±0.020 µg/g and 1.29±0.004 µg/g, respectively (Table 2). Highest Mg levels were measured

Table 3. Pearson correlation coefficient matrix of metals in four freshwater species collected from the Gölbaşı Lake in Hatay, Turkey.

	Sr	Co	Ti	Mg	Cu	Na	Mn	K	Fe	Ni	Zn	Cr
Sr	1											
Co	-.729**	1										
Ti	-.564	.881**	1									
Mg	.610*	-.121	-.269	1								
Cu	-.738**	.296	-.056	-.274	1							
Na	.983**	-.758**	-.665*	.678*	-.607*	1						
Mn	-.664*	.278	.435	-.980**	.224	-.752**	1					
K	.642*	-.695*	-.319	-.202	-.736**	.533	.143	1				
Fe	-.755**	.478	.599*	-.902**	.251	-.846**	.964**	-.022	1			
Ni	.610*	-.726**	-.863**	.562	.057	.733**	-.697*	.104	-.805**	1		
Zn	.561	-.251	.151	-.048	-.937**	.403	.095	.828**	.042	-.249	1	
Cr	.810**	-.818**	-.848**	.550	-.240	.884**	-.679*	.383	-.801**	.871**	.093	1

* Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

in *L. wheatleyi shells*. There was no significant alteration in the mean Mg concentration between the freshwater mussel species ($p>0.05$). Level of Cr reported for Anadonta sp. caught from lake at Aberffraw determined 76 ppm (Segar et al., 1971).

Sodium (Na)

The sodium content in freshwater mussel shells *P. semirugata, U. terminalis, A. pseudodopsis*, and *L. wheatleyi* from Gölbaşı Lake was 82.30±0.040 µg/g, 101.65±0.030 µg/g, 116.70±0.066 µg/g and 155.37±0.050 µg/g, respectively (Table 2). The mean Na concentrations in the shells of all freshwater mussel species varied significantly ($p<0.05$). Segar et al. (1971) and Boulanger & Glascock (2015), 1489±100 ppm also establish that the higher levels of Na in shells of mollusks which were determined in the Aberffraw Lake in Anglesey and Eastern North America.

Potassium (K)

The potassium content in freshwater mussel shells *P. semirugata, U. terminalis, A. pseudodopsis*, and *L. wheatleyi* from Gölbaşı Lake was 3.53±0.023 µg/g, 5.60±0.065 µg/g, 4.59±0.076 µg/g and 5.15±0.082 µg/g dw, respectively (Table 2). Levels of K reported for some freshwater mussel species caught from Aberffraw Lake in Anglesey as 1.06-2.07 mg/kg dw (Segar et al., 1971). This data showed that K levels in shells of freshwater mussel species were lower than the values determined from the other region before and when checked with the results of prior studies K levels showed to be decreasing in Gölbaşı Lake of Turkey.

Concentrations of Trace Elements

The concentration (µg/g dw) ranges of defined trace metals in fish and prawn species were found as Na (82.3-155.37), Sr (26.07-58.02), Mn (0.86-1.29), K (3.53-5.6), Ti (1.17-1.32), Fe (0.59-1.29), Mg (0.86-1.29), Co (0.29-1.07), Cu (0.18-0.52), Cr (0.22-0.27), Zn (0.16-0.37) and Ni (0.007-0.014). The average levels of Sr, Co, Ti, Cu, Mn, Fe, Ni, Zn and Cr in the *P. semirugata* shells were 26.07±0.04 µg/g, 1.08±0.05 µg/g, 1.32±0.01 µg/g, 0.48±0.02 µg/g, 8.06±0.03 µg/g, 1.09±0.04 µg/g, 0.01±0.00 µg/g, 0.18±0.01 µg/g and 0.22±0.01 µg/g, respectively. The leveles of metals in the shell of *P. semirugata* was in the order of Na> Sr> Mn> K> Ti> Fe> Mg> Co> Cu> Cr> Zn> Ni (Table 2).

The average concentrations of Sr, Co, Ti, Cu, Mn, Fe, Ni, Zn and Cr in the *U. terminalis* shells were 38.06±0.03, 0.56±0.05 µg/g, 1.28±0.02 µg/g, 0.86±0.00 µg/g, 0.25±0.00 µg/g, 101.65±0.03 µg/g, 9.66±0.04 µg/g, 5.60±0.05 µg/g, 1.29±0.04 µg/g, 0.01±0.00 µg/g, 0.37±0.01 µg/g and 0.23±0.00 µg/g, respectively. The concentration of metals in the shell of *U. terminalis* was in order of Na> Sr> Mn> K> Fe> Ti> Mg> Co> Zn> Cu> Cr> Ni. The average concentrations of Sr, Co, Ti, Cu, Mn, Fe, Ni, Zn and Cr in the *A. pseudodopsis* shells were 38.58±0.04 µg/g, 0.29±0.03 µg/g, 1.17±0.02 µg/g, 1.02±0.02 µg/g, 0.52±0.00 µg/g, 116.69±0.05 µg/g, 7.90±0.05 µg/g, 4.59±0.06 µg/g, 0.90±0.04 µg/g, 0.01±0.00 µg/g, 0.16±0.00 µg/g and 0.26±0.01 µg/g, respectively. The concentration of metals in the shell of *A. pseudodopsis* was in order of Na> Sr> Mn> K> Ti> Mg> Fe> Cu> Co> Cr> Zn> Ni (Table 2).

The average levels of Sr, Co, Ti, Cu, Mn, Fe, Ni, Zn and Cr in the *L. wheatleyi* shells were 58.02±0.25 µg/g, 0.36±0.00 µg/g, 1.22±0.00 µg/g, 1.29±0.00 µg/g, 0.18±0.02 µg/g, 155.37±0.04 µg/g, 6.06±0.03 µg/g, 5.15±0.06 µg/g, 0.59±0.02 µg/g, 0.01±0.00 µg/g, 0.34±0.01µg/g and 0.27±0.02 µg/g, respectively. The concentration of metals in the shell of *L. wheatleyi* was in order of Na> Sr> Mn> K> Mg> Ti> Fe> Co> Zn> Cr> Cu> Ni (Table 2).

Strontium (Sr)

Strontium is one of the rarest elements in the environment and is an important trace element for the formation of calcium carbonate shells. In this study. mean Sr concentration in the shells of *P. semirugata. U. terminalis. A. pseudodopsis* and *L. wheatleyi* was found as 26.07±0.4 µg/g. 38.06±0.40µg/g, 38.58±0.50 µg/g, 58.023±0.52 µg/g, respectively (Table 2). There was no significant difference in the mean Mg concentration between the all-freshwater mussel species (p>0.05). Lazareth et al. (2003) also found that the higher levels (between 767 to 785 ppm) of Sr in *Isognomon ephippium* shells of mollusks which were observed in the Tudor, Gazi, Mida and Kenyan sites. Sr concentration in the freshwater mussel shell was lower compared to previous studies reported in the River Vramsn, Southern Sweden (Nystrom et al., 1996), Rhine-Meuse delta (Verdegaal, 2002), River Ehen, UK (Bailey & Lear, 2006), Eastern North America (Boulanger & Glascock, 2015), Energy's Savannah River Site in South Carolina (Carroll & Romanek, 2008). Consequently, all the shells showed markedly lower levels of Sr compared to aragonitic other species (Brand et al., 1986; Chukaeva & Petrov, 2022; Klishko et al., 2022; Segar et al., 1971).

Cobalt (Co)

Co also poses a risk of lung cancer from inhalation of dust including Co, although this is not a major factor in this status (Leyssens et al., 2017). In this study, mean Co concentration in the shells of *P. semirugata, U. terminalis, A. pseudodopsis* and *L. wheatleyi* was found as 1.07±0.061 µg/g, 0.56±0.061 µg/g, 0.29±0.040 µg/g, 0.36±0.010 µg/g, respectively (Table 2). Average shell concentrations of Co were of similar orders of magnitude to previous study of aragonitic species such as of *Unio pictorum mancus*, *Anodonta cygnea*, *Dreissena polymorpha*, *Margaritiferira dahurica*, Anodonta sp. and *Velesunio angasi* (Chukaeva & Petrov, 2022; Klishko et al., 2022; Markich et al., 2002; Ravera et al., 2007, 2003; Segar et al., 1971); while higher concentrations were reported from Eastern North America (0.13 ± 0.05) (Boulanger & Glascock, 2015).

Titanium (Ti)

In this study, mean Ti level in the shells of *P. semirugata, U. terminalis, A. pseudodopsis* and *L. wheatleyi* was found as 1.32±0.01µg/g, 1.28±0.02µg/g, 1.17±0.02 µg/g and 1.22±0.00 µg/g, respectively (Table 2). There was no significant difference in the mean Mg concentration between the *P. semirugata* and *U. terminalis* (p>0.05). Ti concentration in the freshwater mussel shell was lower compared to previous studies reported in the rivers of Upper Amur Basin, Transbaikalia (Klishko et al., 2022), Eastern North America (Boulanger & Glascock, 2015).

Cupper (Cu)

In this study, mean Mn concentration in the shells of *P. semirugata, U. terminalis, A. pseudodopsis* and *L. wheatleyi* was found as 0.48±0.020 µg/g, 0.25±0.001 µg/g, 0.52±0.011

µg/g and 0.18±0.019 µg/g, respectively (Table 2). There was significant difference in the mean Cu concentration between the all-freshwater mussel species ($p<0.05$). In previous reports, Cu was found in the ranges of 7.6 mg/kg in Aberffraw Lake Anglesey (Segar et al., 1971), 4.9 mg/kg in Finnis River (Markich et al., 2002). In addition, freshwater mussel shell was lower compared to previous studies reported in the rivers of Upper Amur Basin, Transbaikalia (Klishko et al., 2022), Cauvery River (Hameed & Raj, 1990), Saint Petersburg in Russia (Chukaeva & Petrov, 2022), Lake Maggiore and Lake Candia, Italy (Ravera et al., 2007, 2003), Energy's Savannah River Site in South Carolina (Carroll & Romanek, 2008).

Manganese (Mn)

In this study, mean Mn concentration in the shells of *P. semirugata, U. terminalis, A. pseudodopsis* and *L. wheatleyi* was found as 8.06±0.036 µg/g, 9.66±0.053µg/g, 7.90±0.065 µg/g and 6.06±0.044 µg/g, respectively (Table 2). On the other hand, the results revealed an important difference ($p<0.05$) freshwater mussel species. Egborge (1991) and Oguzie (2000) also found that the higher levels of Mn in shells of mollusks which were determined in the Niger Delta, Egypt. Hameed & Raj (1990) reported higher accumulation level (141.6±3.9 to 152.9±6.1) in the shell of *Lamellidens marginalis* from Cauvery River. Mn concentration in the freshwater mussel shell was lower compared to previous studies reported in the rivers of Upper Amur Basin, Saint Petersburg in Russia, Transbaikalia, Aberffraw Lake in Anglesey, Lake Maggiore and Lake Candia Italy, Energy's Savannah River Site in South Carolina, Eastern North America (Boulanger & Glascock, 2015; Carroll & Romanek, 2008; Chukaeva & Petrov, 2022; Klishko et al., 2022; Ravera et al., 2007, 2003; Segar et al., 1971).

Iron (Fe)

Fe concentration in the shells of *P. semirugata, U. terminalis, A. pseudodopsis* and *L. wheatleyi* were found as 1.09±0.046 µg/g, 1.29±0.045 µg/g, 0.90±0.046 µg/g and 0.59±0.043 µg/g, respectively (Table 2). There was a significant difference in the mean Fe concentration between the all freshwater mussel species ($p<0.05$). Fe concentration in the freshwater mussel shell was lower compared to previous studies reported in the three lakes on Canada, Southeastern Transbaikalia, Saint Petersburg in Russia, Aberffraw Lake in Anglesey and Finniss River, Lake Maggiore and Lake Candia Italy, Eastern North America (Boulanger & Glascock, 2015; Chukaeva & Petrov, 2022; Klishko et al., 2022; Markich et al., 2002; Ravera et al., 2007; Segar et al., 1971; Vetrov & Kuznetsova, 1997).

Nickel (Ni)

Ni concentration in the shells of *P. semirugata, U. terminalis, A. pseudodopsis* and *L. wheatleyi* were found as 0.008±0.001 µg/g 0.007±0.001 µg/g, 0.014±0.001 µg/g and 0.013±0.001 µg/g, respectively (Table 2). Ni concentration in the freshwater mussel shell was lower compared to previous studies reported in the rivers of Upper Amur Basin Transbaikalia, Aberffraw Lake in Anglesey, Cauvery River, Saint Petersburg in Russia, Lake Maggiore and Lake Candia Italy, Eastern North America (Boulanger & Glascock, 2015; Chukaeva & Petrov, 2022; Hameed & Raj, 1990; Klishko et al., 2022; Ravera et al., 2007, 2003; Segar et al., 1971).

Zinc (Zn)

Zinc is one of the necessary metals in living organisms (Kouba et al., 2010). In this study, mean Zn levels in the shells of *P. semirugata, U. terminalis, A. pseudodopsis* and *L. wheatleyi* was found as 0.18±0.017 µg/g, 0.37±0.016 µg/g, 0.16±0.002 µg/g and 0.33±0.014 µg/g, respectively (Table 2). There was significant difference in the mean Zn concentration between the all freshwater mussel species ($p<0.05$). Accumulation level in the shell of four freshwater mussel species were lower linked to the previous studies showed in Fox River (Anderson, 1977), Southeastern Transbaikalia (Klishko et al., 2022), Canary Islands (Escánez et al., 2021), three lakes in Canada (Dermott & Lum, 1986), Saint Petersburg in Russia (Chukaeva & Petrov, 2022), Lake Maggiore and Lake Candia Italy (Ravera et al., 2007, 2003), Dhanmondi Lake, Bangladesh (Sultana et al., 2016), Finniss River (Markich et al., 2002), Aberffraw Lake in Anglesey (Segar et al., 1971), Cauvery River (Hameed & Raj, 1990), Eastern North America (Boulanger & Glascock, 2015).

Chromium (Cr)

In this study, mean Zn concentration in the shells of *P. semirugata, U. terminalis, A. pseudodopsis* and *L. wheatleyi* was found as 0.22±0.010 µg/g, 0.23±0.003 µg/g, 0.26±0.007 µg/g and 0.27±0.004 µg/g, respectively (Table 2). There was significant difference in the mean Cu concentration between the *P. semirugata* and *A. pseudodopsis* ($p<0.05$). Cr concentration in the freshwater mussel shell was lower compared to previous studies reported in the rivers of Upper Amur Basin, Transbaikalia (Klishko et al., 2022), Saint Petersburg in Russia (Chukaeva & Petrov, 2022), Lake Maggiore and Lake Candia Italy (Ravera et al., 2007, 2003) and Energy's Savannah River Site in South Carolina (Carroll & Romanek, 2008). Average shell concentrations of Cr were of similar orders of magnitude to previous study of aragonitic species (Boulanger & Glascock, 2015; Segar et al., 1971).

An analysis of Pearson's correlation coefficients that attempted to find significant relationships between shell metals of different freshwater mussel species collected from Lake Gölbaşı is shown in Table 3. Significant positive and negative correlations were observed between metals. A significant correlation exists between Co, Cu, Na, Fe, and Cr, each at the 0.01 level (2-tailed). Significant linear, positive and negative correlations were determined between the metals. Significant correlations exist between Co, Cr, Na, Mn, K, Ni and Sr respectively, at 0.01 levels (2-tailed). Other metals also confirmed positive correlations like, Ti-Co ($r=0.881$), Na-Ni ($r=0.733$), K-Zn ($r=0.828$), Cr-Na ($r=0.884$), Cr-Ni ($r=0.871$), Cr-Sr ($r=0.810$), Na-Sr ($r=0.983$), and Fe-Mn ($r=0.964$). In addition, at 0.05 levels (2-tailed) the Sr showed negative correlation with Fe, and Cu respectively. As clearly seen from the table, the negative correlations were found between the duos Co-Sr ($r=0.729$), Cu-Sr ($r=0.738$), Na-Co ($r=0.758$), Mn-Mg ($r=0.980$), Fe-Mg ($r=0.902$), Fe-Na ($r=0.846$), Zn-Cu ($r=0.937$), Cr-Ti ($r=0.818$), Ti-Ni ($r=0.863$) and Ni-Co ($r=0.726$) (Table 3). These results shows that the correlated metals interest a common accumulation process in the shells of freshwater mussels. Overall, this study showed diverse correlations between different metals for many reasons, because the relationship between metals depends on several factors, such as body metabolism, exposure time, health status, trophic level, and feeding habits.

CONCLUSION

The results of this study show that freshwater mussel shells of *P. semirugata*, *U. terminalis*, *A. pseudodopsis* and *L. wheatleyi* give a good indication of the bioavailability of metals in lakes and rivers. The results will be beneficial for appreciating the ecological reaction of freshwater mussels to a rise in the metal content of their bioaccumulation rates, as well as for foretelling the results for the situation of their populations with an upwards altered, up to serious, pollution of the Gölbaşı Lake.

Unlike fish, freshwater mussels are all exposed to chemical pollutants in the water; direct contamination could have the same meaning as trophic contamination. Heavy metals are associated with various pollution events and the continuous leaching of industrial, mining and industrial wastes. The strips of these shells show growth and are a great bioindicator for monitoring water pollution. Since freshwater mussels are long-lived animals, they are species that reveal the pollution aspect of the habitat they live in. Thus, it is necessary to understand the regular and normal crystal morphology and microstructure of the shells layer, for it is the final to be deposited. This report is maintained as structural, morphological, and chemical alterations within the shell layers, and may be respectable indicators of varies in environmental conditions.

In conclusion, the results of this study show the continuous deterioration of Gölbaşı Lake due to discharges from industry, mining, agriculture and households, which would increase if no strategy to protect this environment was developed and implemented by the competent authorities. The common structure of the shell layer of four mussels species was founded and may be used in upcoming findings on the influence of metals and other environmental surroundings on the shell formation process.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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