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Biomineralization: From Fundamentals to Biomaterials & Environmental Issues

Edited by
Frédéric Marin
Franz Brümmer
Antonio Checa
Gabriel Furtos
Isidoro Giorgio Lesci
Lidija Šiller
Biomineralization: From Fundamentals to Biomaterials & Environmental Issues

Special topic volume with invited peer reviewed papers only.

Edited by
Frédéric Marin, Franz Brümmer, Antonio Checa, Gabriel Furtos, Isidoro Giorgio Lesci and Lidija Šiller
It is a fine thing to be honest, but it is also very important to be right (Winston Churchill).
Science never solves a problem without creating ten more (George Bernard Shaw).
If the facts don't fit the theory, change the facts (Albert Einstein).

PREFACE

The present book deals with biomineralization, i.e., the process by which living organisms - from bacteria to whale - synthesize minerals. It represents the final publication of the COST Action TD0903, more simply nicknamed Biomineralix. This Action, which was set up under the auspice and financial support of the COST organization (European COoperation in Science and Technology) in November 2009, ended its activity in May 2014, after a four-and half years of intense scientific activity.

Let us briefly remind how things were initiated: the idea of structuring a European consortium focused on biomineralization was already floating in the air, since the beginning of the years 2000 and resulted from constructive discussions between two of us (D. Medakovic, F. Marin) in the course of 2003 and 2004, and from different bilateral collaborations established by the Croatian team (D. M.) with Italian and Slovenian partners. However, the true founding act was the ‘shaping’ of the full-length proposal by the Croatian partner, an action that gave the decisive push without which no ‘nuclei would have germinated and grown’ (after all, we deal with mineral, don’t we?). Following the ‘grand oral’ exam in front of the COST commission in Brussels, the positive evaluation of the proposal in March 2009 and its subsequent acceptance by the COST Office, the Biomineralix network was constituted in the fall of 2009, with eight partner countries. To our knowledge, this was the first COST network of this type, i.e., entirely focused on biomineralization. Its main objective was to bring together research groups with very different expertise throughout Europe and elsewhere, to stimulate new, innovative and high-impact interdisciplinary research in this very peculiar field. Because of its chemistry-oriented nature and of potential applications in industry ensuing from the network’s activity, our consortium was administered by the Chemistry and Molecular Sciences and technologies domain (CMST), one of the nine scientific domains of the COST organization, covering all aspects of sciences and technologies in Europe. However, due to its multidisciplinary nature, our network belonged also to the tenth ‘Trans-Disciplinary’ (TD) domain.

This book presents a collection of papers – reviews, research articles, technical notes – that reflect some of the research directions developed during four and half years of activity. In short, it gives a ‘flavour’ of some topics, tackled by the network, in particular topics related to biological and proteomic research in biomineralization, to the advanced analysis and characterization of biomineralizations and biomaterials, to the environmental bio-monitoring by biominerals, to researches on novel biomaterials and fossil biominerals.

This network would not have worked properly without the help and contribution of key-peoples: first of all, we would like to thank Lucia Forzi, the Scientific Officer of the COST Office, in charge of many actions within the CMST domain. Lucia was, from the beginning to the end, the vigilant guardian of our consortium, reminding us some rules of functioning that we tended to forget. We also include in our acknowledgments the other members of the COST Office: Valentina Vignoli, Svetlana Voinova, Andrea Tortajada, Monica Dietl, Marie-Eve Hastir, Jacob Riambau Santacana, Kent Hung, Erwan Arzel. We also thank Dieter Schinzer,
the Chair of the COST CMST domain and all the CMST evaluators, present at the successive Annual Progress Conferences.

We also warmly thank Professor Jan Reedijk (Leiden University, The Netherlands) who was our COST rapporteur from the beginning, and carefully advised us, during our successive management committee meetings, on how to conduct properly the network. We are also grateful to Professor Maggie Cusack (University of Glasgow, UK) who took time, as external evaluator, to appraise our action.

We acknowledge all the numerous guest researchers (we cannot cite them all) that were invited to give extended talks during our six consecutive international workshops. All the local organizers of these workshops are also thanked for setting up nice conferences including social activities.

Last but not least, we thank all the colleagues who jumped into our ‘COST Action boat’ and contributed to feed its engine via fruitful discussions, posters, oral presentations and scientific interactions. Are thanked all the early-stage researchers, particularly those who were active in the network by ‘experimenting’ short-term scientific missions and by showing their results during subsequent yearly workshops.

And at the very last, we would like to thank the contributors of this book, and our editor at Trans Tech Publications, Thomas Wohlbier, and his team.

Frédéric Marin, Lidija Šiller, Franz Brümmer, Isidoro Giorgio Lesci, Antonio Checa and Gabriel Furtos
Successive meetings of the Cost Action TD0903 Biomineralix. A: kickoff meeting, Brussels (BE); B: Pula (HR); C: Newcastle (UK); D: Cluj Napoca (RO); E: Aarhus (DK); F: Bologna (IT); G: Granada (ES); H: logo of the COST Action TD0903 ‘Biomineralix.’
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COST (European Cooperation in Science and Technology) is a pan-European intergovernmental framework. Its mission is to enable break-through scientific and technological developments leading to new concepts and products and thereby contribute to strengthening Europe’s research and innovation capacities.

It allows researchers, engineers and scholars to jointly develop their own ideas and take new initiatives across all fields of science and technology, while promoting multi- and interdisciplinary approaches. COST aims at fostering a better integration of less research intensive countries to the knowledge hubs of the European Research Area. The COST Association, an International not-for-profit Association under Belgian Law, integrates all management, governing and administrative functions necessary for the operation of the framework. The COST Association has currently 36 Member Countries.

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Environmental characterization of lake ecosystems located in Serbo Macedonian massif (FYRM)

Tadej Dolenec¹,a, Todor Serafimovski²,b, Sonja Lojen³,c, Matej Dolenec¹,d, Goran Tasev²,e, Sabina Kramar⁴,f, Nastja Rogan Šmuc¹,g, Petra Vrhovnik¹,h

¹Faculty of Natural Sciences and Engineering, University of Ljubljana, Department of Geology, Aškerčeva 12, 1000 Ljubljana, Slovenia
²Faculty of Natural and Technical Sciences, "Goce Delčev" University, Goce Delčev 89, MK-2000, Štip, Former Yugoslav Republic of Macedonia
³Institute Jožef Štefan, Jamova cesta 39, 1000 Ljubljana, Slovenia
⁴Slovenian National Building and Civil Engineering Institute, Dimičeva 12, 1000 Ljubljana, Slovenia

a tadej.dolenec@ntf.uni-lj.si, b todor_s2000@yahoo.co.uk, c sonja.lojen@ijs.si, d smudut@siol.net, e goran.tasev@ugd.edu.mk, f sabina.kramar@zag.si, g nastja.rogan@guest.arnes.si, h petra.vrhovnik@gmail.com

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Abstract

This paper presents the mineralogical, geochemical and isotopic characteristics of recent lacustrine sediments, of Anodonta cygnea shells and of fish species Rutilus rutilus dojranensis from the Lake Dojran (FY Republic of Macedonia, southern part of the Serbo-Macedonian Massif (SMM)), which provide indirect evidence regarding biomineralization and calcification processes as well as various geological problems. Environmental pollution can seriously impair physiological functions such as the secretion of carbonate material into the shells. For comparison, sediments and fish species Vimba melanops from the Lake Kalimanci, located in northern part of the SMM, were also included into the study.

X-ray powder diffraction (XRD), inductively coupled plasma mass spectrometry (ICP-MS) and carbon (C) and oxygen (O) stable isotope analyses were performed, and thereupon enrichment factor (EF) values were calculated. The XRD results revealed close association of sediment mineralogy with the prevailing metamorphic, volcanic and igneous rocks of the region surrounding both lakes (Dojran and Kalimanci). According to the EF value results, surficial Lake Dojran sediments are slightly enriched with Co, Cr, Cu, Pb, and Zn, moderately enriched with Au, Ni and Sb, moderately to severely enriched with Au, severely enriched with Sb and extremely enriched with As. This elemental enrichment originates from various geogenic (geological background and polymetallic mineralization) and anthropogenic (tourism, traffic, coatings, untreated wastewater discharge and agrochemicals) sources. Calculated EF revealed that surficial sediments from Lake Kalimanci are extremely enriched with Pb, Zn, and Cd, meanwhile As and Cu shows very severe enrichment. Comparing to Lake Dojran, calculated EF is much higher in Lake Kalimanci than in Dojran.

Stable isotope signature results showed that Lake Dojran sediments were strongly influenced by evaporation effect. Their $\delta^{18}O$ values range from -5.60 to +1.49 ‰ and the $\delta^{13}C_{VPDB}$ composition range from -6.45 to -1.65 ‰. Shells of A. cygnea are mainly composed of mineral aragonite and their $\delta^{18}O$ values varies between 0.43 ‰ and +1.94 ‰, and $\delta^{13}C_{VPDB}$ between -2.48 to -1.72 ‰. $\delta^{18}O$ signature in shells might be explained by precipitation of the carbonate from isotopically heavier oxygen lake water. The unusually high skeletal oxygen suggests calcification at elevated temperature and evaporation. Analyses also identify particularly high levels for Cr, Hg, Ni and Pb in fishes (V. melanops) and frog (Rana temporaria) samples from Lake Kalimanci with mean values [µgkg\(^{-1}\)]: 1090 – 6.5, 339 – 117, 567 – 5100 and 1127 – 435, respectively. Meanwhile, the
contents of Cr, Hg, Ni and Pb in fishes samples (*R. rutilus dojranensis*) from Lake Dojran are a bit lower [µgkg⁻¹]: 2320, 1690, 700 and 50.

1. Introduction

Lakes represent some of the most versatile and unique ecosystems on Earth, but unfortunately they are also readily affected by various anthropogenic activities [1]. Since lakes are mostly closed or nearly closed systems, lake sediments can be treated as the final recipients of the results of anthropogenic activity within the lake-basin area. Consequently, they operate as both carriers and storage sinks for contaminants, thus reflecting the history of long-term geogenic or anthropogenic impacts [2-5]. Contaminants, especially trace elements and metalloids, are a group of pollutants with high ecological significance in aquatic ecosystems. Trace elements do not exist in solid phases in water bodies, instead accumulating mainly in suspended particulates or in sediment material [6-8]. Metals/metalloids found in aquatic sediments may be natural or man-made in origin; weathering of soils/rocks and atmospheric deposition are known geogenic sources, while the discharge of agricultural, municipal, residential and industrial waste products from surrounding environments into water bodies has been determined as the main anthropogenic source [5-6,8-9].

One of the major environmental problem dealing with trace elements (metals and metalloids) is that they can be absorbed into living beings and, consequently, that they are toxic in even low concentrations. Not much is known about biochemical responses to trace elements by bivalves [10,11]. Trace elements in different animal species, such as bivalves and fishes, can cause difficulties in development, growth, mortality and also reproduction [12] and can be also present in shell and most likely affect biomineralization processes. Furthermore, provided data could be used for interpretations regarding alterations in biomineralization processes influenced by environmental changes.

Over the last few decades, a number of geochemical studies have focused on lake sediments with the aim of evaluating the extent of environmental contamination [13-17].

Lake Dojran, located in the SE of the FYRM, is situated on the border between FYRM and the Republic of Greece. Geothermal processes and polymetallic mineralization in the lake Dojran catchment area could potentially have conferred uniquely beneficial properties on the dark mud lake sediments, possibly such that the latter could be perceived as having healing powers (i.e. peloids) [18]. Unfortunately, information regarding the environmental status of Lake Dojran sediments is very scarce and unconfirmed [19].

Lake Kalimanci lies in NE part of FYRM, near the border with Republic of Bulgaria. Both lakes (Dojran and Klaimanci) are a part of Serbo-Macedonian Massif (SMM), which extends from northern border with Republic of Serbia to the southern border with Republic of Greece, and is mainly composed by igneous and metamorphic rocks. In the vicinity of Lake Kalimanci can be found Pb-Zn ore deposits as well as active mines with its belonging tailing dams. Lake Kalimanci environment was heavily affected by a failure of Sasa tailings dam, therefore increased amounts of potentially toxic (trace) elements are expected in lake sediments.

The main objective of the research was to characterize studied freshwater environments to reveal the growth patterns of biominerals and possible changes in metabolic pathways in biomineralizing organisms under various environmental influences. Additionally, some other objectives were determined as follows:

- to evaluate the elemental content and distribution in Lake Dojran and Lake Kalimanci sediments,
- to define the anthropogenic versus natural contribution to Lake Dorjan and Lake Kalimanci sediment trace element concentrations,
- to provide isotopic characteristic of sediments and *Anodonta cygnea* shells originating from Lake Dojran, and
- to evaluate the pollution status of Lake Dojran and Lake Kalimanci environment, to reveal the influences of trace elements on biomineralization of freshwater shells.
2. Materials and methods

2.1. Description of the study sites and geological characteristics

Lake Dojran, a natural wonder with a rich cultural heritage, is located in the SE of the FYRM, on the border between FYRM and the Republic of Greece (Fig. 1). Lying at an altitude of 148 m a.s.l., the lake has a mean depth of 6.7 m and a surface area of 43.10 km$^2$, 66% of which belongs to the territory of the FYRM. Lake catchment area is about 120 km$^2$ (mainly from the N and NE, where the Bjelasica mountains are situated). Lake Dojran is elliptical in shape, with a N-S elongation 8.9 km in length and 7 km in width. In the northern part of the lake there are a few small groundwater sources. Due to high water outflow (irrigation system) in the south, the Macedonian Government decided to build water-feeding system into the lake, which consists of wells system in river Vardar (near city Valandovo), 20 km away from the Lake Dojran. All of them are combined into one inflow with capacity of 650-800 l/s, near the city of Novi Dojran. The Dojran area was and still is an important agricultural and tourist province [19]. Agricultural and tourism activities around the lake resulted in enhanced agrochemicals, landfill leachate and municipality discharges into the lake and increased traffic effects.

Lake Kalimanci is located in the east of FYR Macedonia (Fig. 1). It is an artificial lake, with its longest length 4 km and greatest width 0.3 km, and a maximum depth of 80 meters. The surface area of the lake is 4.23 km$^2$ and it accumulates approximately 127 million m$^3$ of water. Lake Kalimanci is surrounded on the eastern side by uncultivated ground, with no industry or households. Meanwhile, in the western part a main road runs parallel to the lake and serves as the main connection between the agriculturally important Kočani Field, Sasa Pb-Zn Mine, and the Bulgarian border.

Fig. 1: Study area (Lake Dojran and Lake Kalimanci region, FYRM).

Lake Dojran is located in the Dojran graben, the tectonic border between metamorphic rocks of the SMM and volcanic/sedimentary rocks of the Vardar zone. Development of the Dojran graben is related to the neotectonic movement during the Neogene which were followed by volcanic and post-volcanic activities. Precambrian and Palaeozoic metamorphic rocks (gneisses, shales, amphibolies and marbles) of the SMM currently comprise the NE of the study site, together with intrusions of Bjelasica granites and basic intrusions of gabbro. Serpentinites can be found in the eastern part of the massif, while the border of the SMM with the Vardar zone is composed of granite porphyries, metarhyolites and metamorphic pyroclastites. The region SW of the lake is
represented by four main lithologies: Permian to Triassic metamorphic sedimentary and volcanic rocks, and Tertiary Q conglomerates and volcanic rocks (trachytes and rhyolites) (Fig. 2). Polymetallic mineralization with chalcopyrite, pyrite, bornite and gold is located in the contact between muscovite gneisses and amphibolite shales, whereas a pyrite-arsenopyrite-gold polymetallic association forms part of basic rocks [18]. While Lake Kalimanci is also a part of the SMM, the same geological setting of researched area was determined there. The main difference that should be exposed is that in the vicinity of Lake Kalimanci is located a large Pb-Zn ore deposit, as well as an active mine with accompanying tailings dam which is directly connected to the Lake Kalimanci through River Kamenica.

2.2. Sampling

Sampling of lake sediments was carried out on six sampling locations (D1, D2, D3, D4, D5 and D6) across Lake Dojran (FYRM) are shown in Fig. 3a. Samples were precisely collected with the push plastic corers (tube 20 cm long with a 10 cm internal diameter), tightly packed into pre-
cleaned plastic bags and stored in the laboratory at 4 °C. Samples were sectioned into three 5 cm depth slices: 0-5 cm (6 locations), 5-10 cm (2 B locations) and 10-15 cm (2 C locations).

The 31 surficial sediment samples from eight profiles along Lake Kalimanci (Fig. 3b) were collected using plastic corers, 10 cm long, with 10 cm internal diameter. They were then packed into plastic bags and stored in the laboratory at 4°C.

Samples from both lakes were prevailingly composed of sand, silt and clay. They were dried at 50 °C for 48 h, before being sieved through a 0.315 mm polyethylene sieve in order to remove molluscan remains and other organic debris. Finally, the samples were homogenized using a mechanical agate grinder into a fine powder (<63 µm) for subsequent analyses.

Anodonta cygnea shells were gathered by dragging from Lake Dojran clay bottom. Shells were randomly collected and stored in plastic tanks with natural lake water and temperature similar to natural conditions. Bodyweight of Anodonta cygnea samples was up to 200 g. The shell of Anodonta cygnea was selected: it represents a good model to study biomineralization processes because it possesses large volume compartments with easy access to their organic matrices. In these shells, biomineralization occurs in two different compartments, each with specific biological fluid (haemolymph and extrapallial fluid) which are involved in mineral production at the mineral concretions (microspherules) level [11,20].

To collect the fish samples, fishing was performed during late afternoon towards the end of the fishing season in November 2010 with the help of professional local fisherman, as suggested in literature [21]. Fishes were caught in both lakes (two samples at each profile), afterwards they were stored in a container, cooled on crushed ice, and brought to the laboratory for further analysis. The fishes were sectioned, and all non-edible parts (e.g., bones, intestine, gills, fins, etc.) were removed so that only soft tissues were freeze-dried (for at least 72 h) until a plateau weight was reached. Dried samples were homogenized and crushed to a fine powder by grinding in an agate mortar. The same procedure was used for frog collection around the Lake Kalimanci.

Caught fishes and frog species were determined as follows: Lake Kalimanci - *Vimba melanops* (fishes) and *Rana temporaria* (frogs); Lake Dojran - *Rutilus rutilus dojranensis* (fishes)

![Fig. 3: Sampling locations across Lake Dojran (a) and Lake Kalimanci (b)](image)

### 2.3. Methods

An amount of sample was roasted at 1000 °C to detect the loss on ignition (LOI), while sediment pH was measured in deionized water with a 1:2 soil/solution ratio after equilibration for 15 min [22]. Total carbon, content were analysed by LECO Carbon-Sulfur analyser in a certified commercial Canadian laboratory (Acme Analytical Laboratories).
In order to define detailed mineralogical and elemental characteristics of sediment samples, X-ray powder diffraction (XRD) and ICP analyses were employed. Shells mineralogical composition was defined by X-ray powder diffraction (XRD) analysis, as well.

The mineral composition of the sediment samples was determined via X-ray powder diffraction using a Philips PW3710 X-ray diffractometer equipped with Cu Kα radiation and a secondary graphite monochromator. Data were collected at 40 kV and a current of 30 mA in a range from 2 to 70° 2θ, at a speed of 3.4 o/min.

All surficial sediments were measured for their detailed geochemical composition in an accredited commercial Canadian laboratory (Acme Analytical Laboratories, Vancouver, B.C., Canada). Total abundances of major elements and trace elements were determined by ICP-emission spectrometry following lithium metaborate/tetraborate fusion and dilute nitric digestion. In addition, a separate sample was digested in Aqua Regia and analyzed via ICP Mass Spectrometry to report on precious and base metal content.

Duplicate samples and blanks were also included to ensure the accuracy of all analytical methods employed. Analytical precision and accuracy were better than ± 5% for the investigated elements.

Additionally, for C and O isotopic analysis, samples were obtained as a split of powder prepared for abovementioned analysis. In order to speed up the reaction time and to ensure complete reaction of carbonates, powdered samples were prepared by overnight digestion in excess 100‰ phosphoric acid at 50°C. CO₂ gas released during acid treatment was cryogenically cleaned and analyzed for carbon and oxygen isotopic composition on a Varian MAT 250 mass spectrometer. All investigated samples were analyzed in duplicate or triplicate. The δ¹³C values were normalized assuming IAEA-CO-1 standard on the VPDB scale and δ¹⁸O values were normalized according to VPDB and SMOW standards. The analytical precision, based on multiple analysis of an internal laboratory standard, was ±0.05 ‰.

All fishes samples were analyzed for 57 elements (major, minor, trace and REEs) at an accredited commercial laboratory, Act Labs (Activation Laboratories Ltd., Ancaster, Ontario, Canada in year 2011), using inductively coupled plasma mass spectrometry (ICP/MS) and microwave digestion. Dry, unwashed samples were digested in Aqua Regia solution 3/1 (v/v) (HNO₃ + 3HCl) at 95°C for two hours. Resultant sample solutions were then diluted and analyzed on a Finnegan Mat Element 2 High Resolution ICP/MS (HR-ICP/MS). The quality of the analyses was monitored by comparison to the standard materials NIST 1575a, NIST 1643e and SLRS-5 provided by Act Labs and the measurements of four samples were repeated. Besides Act Lab quality control, also our own standard MP-STD-011, and two standards prepared and provided by the International Atomic Energy Agency, Vienna (IAEA) - IAEA-407 and by National Research Council Canada - DORM-3 were sent to Act Labs for geochemical analysis as well. Our control standard MP-STD-011 is wild dogfishes soft tissue, which was analyzed many times by different techniques (ICP/MS, HR-ICP/MS, ICP/AES and XRF). The results indicated a good agreement between the certified and observed values. For reaching the best control, measurements were repeated on all fishes samples and three standards (MP-STD-011, IAEA-407, DORM-3) using X-ray fluorescence (XRF) and inductively coupled plasma atomic emission spectroscopy (ICP-AES). The standard deviations of the means observed for the abovementioned certified materials were 1-6 %.

2.4. Statistical analysis

To explore the elemental associations and thus origins of the analyzed elements in the Lake Dojran sediments, Pearson R correlation analyses were performed to all samples. Critical values of the correlation coefficient (r) 0.81 at p ≤ 0.05 were considered highly significant. Principal component analysis (PCA) based on a covariance matrix were used to examine variation in the measured parameters between locations. The basic statistical parameters for each element and the statistical calculations mentioned above were performed using the software programs Statistica VII and Grapher 8.0.
2.5. Enrichment factor

The enrichment factor (EF) is an environmental index indicating the level of sediment environmental contamination. It was employed to estimate possible anthropogenic input of metals/metalloids to Lake Dojran sediments [23-24], calculated as:

\[ \text{EF} = \frac{(M/\text{Al})_{\text{sample}}}{(M/\text{Al})_{\text{crust}}} \]

where \(M_{\text{sample}}\) and \(M_{\text{crust}}\) are the levels of the investigated metals/metalloids (Au, As, Co, Cr, Cu, Ni, Pb, Sb and Zn) in the sediment samples and uncontaminated crust material, respectively; and \(\text{Al}_{\text{sample}}\) and \(\text{Al}_{\text{crust}}\) are the levels of Al in sediment samples and uncontaminated crust material, respectively. Metal to aluminium ratios such as these are widely adopted, presumably because the concentrations of weathering products and their parent materials are generally comparable. Aluminium is also the normalizing element, levels of which are assumed not to be enriched owing to local contamination [25]. In this study, baseline values for \(M_{\text{crust}}\) and \(\text{Al}_{\text{crust}}\) were adopted from literature [26].

3. Results and discussion

3.1. Physico-chemical characteristics of lake sediments

The sampled lake sediments were characterized by an LOI content of less than 33 %; the highest value was detected at location D4, while slight variability was observed between the northern and southern parts of the lake. A strong and statistically significant correlation was found between LOI and total C. Sediment pH values ranged between 5.8 and 7. The measured pH of surficial sediments from Lake Kalimanci ranges between 5.5 and 7.5, and redox potential ranges between -325 mV and +180 mV. The organic matter content lays in the following ranges in Lake Kalimanci surficial sediments [%]: C\text{org} 0.05 – 2.70, N\text{org} 0.00 – 0.34, H\text{org} 0.29 – 5.97 and S\text{org} 0.00 – 2.13, and has almost homogeneous concentrations of compounds through the lake.

3.2. Mineralogy of lake sediments

The exposed and underlying lithologies of the Dojran graben region are predominantly composed of gneisses, shales, amphibolites, marbles, granite porphyries, metarhyolites, metamorphic pyroclastites and Bjelasica granites, as well as the other metamorphic, sedimentary and volcanic rocks [18]. Surficial sediment samples mineralogy of the lake is thus closely related to these prevailing rocks. The mineralogical assemblage of the lake sediments is mostly comprised of quartz, feldspars (orthoclase, microcline and albite), clay minerals (kaolinite and montmorillonite/illite), calcite and micas (biotite, muscovite and lepidolite), along with minor augite and hornblende. Traces of zircon, epidote, glaucophane and goethite were found only sporadically. No significant changes in the predominant pattern of sediment mineral composition were observed throughout the investigated area.

The Lake Kalimanci surface sediment samples represent fine-grained, predominantly clay and silt sized lake sediment (> 0.0063 mm) deposited after the construction of the artificial dam. Lake sediments generally reflect the mineralogical composition and the presence of ore in the surrounding area and bedrocks. According to XRD analysis, the lake sediments’ main mineral phases are quartz, plagioclases (albite, anorthite), phengite, clinoclore, K feldspars, illite, and muscovite. Lake Kalimanci occasionally also contains calcite, sphalerite, epidote, gypsum, clinopyroxenes, olivine and pyrite, which in some cases contains As.

3.3. Major elements in surficial lake sediments

Major element concentrations in Lake Dojran and Lake Kalimanci sediments are gathered in Table 1.
Table 1: Major element concentrations of Lake Dojran and Kalimanci surficial sediments.

<table>
<thead>
<tr>
<th>Element</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake Dojran</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>68.5</td>
<td>30.0</td>
<td>88.0</td>
<td>21.0</td>
</tr>
<tr>
<td>Fe</td>
<td>40.3</td>
<td>16.9</td>
<td>60.6</td>
<td>15.2</td>
</tr>
<tr>
<td>Mg</td>
<td>9.92</td>
<td>4.00</td>
<td>21.4</td>
<td>6.39</td>
</tr>
<tr>
<td>Ca</td>
<td>87.2</td>
<td>36.2</td>
<td>272</td>
<td>92.6</td>
</tr>
<tr>
<td>Na</td>
<td>8.82</td>
<td>3.90</td>
<td>18.6</td>
<td>5.05</td>
</tr>
<tr>
<td>K</td>
<td>17.9</td>
<td>9.00</td>
<td>22.4</td>
<td>5.23</td>
</tr>
<tr>
<td>Ti</td>
<td>4.00</td>
<td>1.60</td>
<td>6.20</td>
<td>1.51</td>
</tr>
<tr>
<td>P</td>
<td>0.68</td>
<td>0.10</td>
<td>1.20</td>
<td>0.37</td>
</tr>
<tr>
<td>Mn</td>
<td>0.95</td>
<td>0.50</td>
<td>1.60</td>
<td>0.38</td>
</tr>
<tr>
<td>Si</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake Kalimanci</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>7.95</td>
<td>5.72</td>
<td>10.1</td>
<td>0.93</td>
</tr>
<tr>
<td>Fe</td>
<td>6.61</td>
<td>4.19</td>
<td>11.3</td>
<td>1.54</td>
</tr>
<tr>
<td>Mg</td>
<td>1.35</td>
<td>0.74</td>
<td>2.21</td>
<td>0.34</td>
</tr>
<tr>
<td>Ca</td>
<td>3.82</td>
<td>1.19</td>
<td>7.5</td>
<td>1.6</td>
</tr>
<tr>
<td>Na</td>
<td>1.14</td>
<td>0.29</td>
<td>2.06</td>
<td>0.43</td>
</tr>
<tr>
<td>K</td>
<td>2.28</td>
<td>1.37</td>
<td>2.83</td>
<td>0.35</td>
</tr>
<tr>
<td>Ti</td>
<td>0.37</td>
<td>0.32</td>
<td>0.46</td>
<td>0.03</td>
</tr>
<tr>
<td>P</td>
<td>0.16</td>
<td>0.09</td>
<td>0.23</td>
<td>0.04</td>
</tr>
<tr>
<td>Mn</td>
<td>0.57</td>
<td>0.18</td>
<td>1.33</td>
<td>0.25</td>
</tr>
</tbody>
</table>

More than 65% of major elements in almost all surficial sediment samples (D1, D2, D3, D5 and D6) were accounted for Si-Al-Fe components, likely reflecting a relatively high proportion of quartz, feldspars, clay minerals and micas present in these sediments. The measured Ca and Mg concentrations (ranging approximately from 36.2 to 271.6 g kg⁻¹ Ca and 0.4 to 2.14 g kg⁻¹ Mg in samples D1, D2, D3, D5 and D6) could reflect the presence of feldspars, montmorillonite, micas, augite and/or hornblende, as well as low amounts of calcite. In contrast, Ca is the main component (271.6 g kg⁻¹) of sample D4, indicating increased levels of calcite and/or aragonite mollusc shells defining an authigenic source of Ca²⁺. Alkalis (K and Na, respectively) were found in all samples, probably bonded to feldspars, montmorillonite/illite, micas and/or augite. Mn concentrations ranged from 0.5 to 1.6 g kg⁻¹, while phosphorous (P) content varied between 0.1 and 1.2 g kg⁻¹, with no significant variation observed amongst the analyzed samples. Titanium (Ti) levels (1.6 to 6.2 g kg⁻¹) are likely connected with the presence of minerals such as micas, augite and/or hornblende.

Analysis of the spatial distribution of elements (Fig. 4) revealed the following patterns: Al and Fe abundances were highest on the NW side of the lake; the highest Si and K values were at the N and S ends of the lake; Ca distribution showed the opposite pattern to Si; Mg, Na, Mn and Ti content decreased from N to S; the highest P concentrations were dominated in the W and SW parts of Lake Dojran. These spatial distribution patterns are closely associated with the surrounding and underlying lithologies of the wider Dojran region.

Nearly 80% of the macro-chemical components account for Si-Al-Fe assemblages in terms of the major elements presents in the surficial Lake Kalimanci sediments. This compositional relationship reflects a relatively high proportion of quartz, feldspars and chlorite in surficial lake sediments, as was also indicated by X-ray Diffraction (XRD) analysis. The aforementioned minerals are mostly associated with the acidic to intermediate igneous rocks of the Osogovo Mountains. The absence of carbonate minerals nearby is reflected in the low Ca and Mg contents (approximately 10% of all components), which could be related to the presence of feldspars,
chlorite and pyroxenes. The Na and K concentrations (~7% of all components) are most probably related to the feldspar illite-muscovite content and the presence of amphiboles (hornblende). Relatively high concentration values of Mn (1,780-13,300 mg kg\(^{-1}\) range) are possibly a result of oxic conditions during sampling, or of the low surface area of bottom sediments at some sampling points.

3.4. Trace element concentrations of surficial lake sediments

Trace element concentrations (Au, As, Co, Cr, Cu, Ni, Pb, Sb and Zn) and their mean, minimum, maximum and standard deviation values in Lake Dojran and Lake Kalimanci sediments are summarized in Table 2.

Table 2: Mean, minimum, maximum and standard deviation values of the trace elements in the Lake Dojran and Lake Kalimanci surficial sediments.

<table>
<thead>
<tr>
<th>t.e. (mg kg(^{-1}))</th>
<th>Au</th>
<th>As</th>
<th>Co</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Ni</th>
<th>Pb</th>
<th>Sb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.01</td>
<td>41.70</td>
<td>18.70</td>
<td>b.d.</td>
<td>6.10</td>
<td>34.80</td>
<td>53.80</td>
<td>26.30</td>
<td>1.90</td>
<td>8.80</td>
</tr>
<tr>
<td>Min</td>
<td>0.01</td>
<td>30</td>
<td>9</td>
<td>b.d.</td>
<td>3.40</td>
<td>18</td>
<td>27</td>
<td>16</td>
<td>1.20</td>
<td>46</td>
</tr>
<tr>
<td>Max</td>
<td>0.02</td>
<td>57</td>
<td>30</td>
<td>b.d.</td>
<td>8.10</td>
<td>54</td>
<td>75</td>
<td>46</td>
<td>2.70</td>
<td>109</td>
</tr>
<tr>
<td>Std.dev.</td>
<td>0.01</td>
<td>9.10</td>
<td>7</td>
<td>b.d.</td>
<td>1.70</td>
<td>13.70</td>
<td>18.70</td>
<td>10.60</td>
<td>0.60</td>
<td>24.20</td>
</tr>
</tbody>
</table>

Fig. 4: Principal component analysis for major elements (Lake Dojran).
Mean Lake Kalimanci
0.03 67.69 19.89 56.58 59.37 415.12 45 6176.29 1.77 8371.94
Min Lake Kalimanci
0.01 27.90 11.70 16.50 27.37 144.40 21.70 1873.70 0.60 2944
Max
0.06 128.20 27.70 136 88.94 1161.90 79.30 16300 3.60 20900
Std.dev.
0.01 16.54 4.04 27.23 14.72 233.45 12.64 3491.02 0.71 4290.95

Analysis showed that mean sediment trace element concentrations increase in the following order: Au < Sb < Cr < Co < Pb < Cu < As < Ni < Zn. The distribution of these trace elements was not uniform over the whole lake area, with concentrations higher in the W (As, Cr, Cu, Ni, Pb, Sb and Zn), N (Co) and SW (Au). This pattern reflects high values connected with the sampling points D5 and D6, which are located closest to the city of Novi Dojran (Fig. 5).

Fig. 5: Spatial distribution of trace elements (Lake Dojran).

The decreasing order of mean concentrations of the trace elements in Lake Kalimanci surficial sediments is Zn > Pb > Cu > As > Cr > Cd > Ni > Co > Sb > Au. Spatial distribution diagrams pointed out that trace elements like Cu, Pb, Zn, Ni, Cd and Sb were mostly increased between profiles two and three, which extend over the northern part of the lake. Cr, Co and As were observed to be heavily increased at the southern part of the lake.
Sediment EF values around or lower than 1.0 indicate that the element in question originates predominantly from crustal material and/or weathering processes [27], whereas EF values much greater than 1.0 display the anthropogenic origin [28]. More recently, six contamination categories have been developed on the basis of enrichment factor values [25]:

<table>
<thead>
<tr>
<th>EF</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 3</td>
<td>minor enrichment (anthropogenic impact)</td>
</tr>
<tr>
<td>3-5</td>
<td>moderate enrichment</td>
</tr>
<tr>
<td>5-10</td>
<td>moderately severe enrichment</td>
</tr>
<tr>
<td>10-25</td>
<td>severe enrichment</td>
</tr>
<tr>
<td>25-50</td>
<td>very severe enrichment</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>extremely severe enrichment</td>
</tr>
</tbody>
</table>

Enrichment factor calculations revealed Cr, Zn, Pb, Cu and Co to exhibit the lowest EF values among the trace elements (mean values 0.2, 1.33, 1.53 and 1.63, respectively); these five elements are thus only slightly enriched in surficial sediments of Lake Dojran. Calculated EF values for Ni, Au and Sb signified moderate enrichment (mean value 3.13) with Ni, moderately severe enrichment (mean value 5.5) with Au and severe enrichment (mean value 11.2) with Sb. As had the highest average EF value (32.5) among the trace elements representing very severe enrichment in surficial sediments. The greatest overall EF values were determined in samples from locations D3 (Au), D5 (As, Cr, Cu, Ni, Pb, Sb and Zn) and D6 (Co, Cu). Calculated EF revealed that surficial sediments from Lake Kalimanci are extremely severely enriched with Pb, Zn, and Cd, meanwhile As and Cu shows very severe enrichment. Comparing to Lake Dojran, calculated EF is much higher in Lake Kalimanci than in Dojran.

Particle size and density play important roles in determining the chemistry and ecology of sediments. The clay/silt fraction (<63 µm) has a high specific surface area per unit quantity of material and, due to surface coatings of Fe and Mn elements and natural organic material, is more likely to adsorb organic and trace elements contaminants [29-30]. Thus, most anthropogenic contaminants in lake Dojran are associated with the confirmed clay (24.40 %) and silt (71.50%) fractions [18]. It can be concluded that the trace elements of Lake Dojran surficial sediments is derived from a number of different geogenic and anthropogenic sources. The geological background of the lake is generally dominated by metamorphic rocks [18] which are composed of minerals that can contain trace elements in their structure, as well as polymetallic mineralization in basic rocks [18] with arsenopyrite (FeAsS) and gold; these minerals all contribute to the enhanced levels of trace elements (especially As and Au) found in lake sediments. Anthropogenic sources of trace elements include tourism activities taking place around the lake, resulting in increased traffic (Pb) and coatings (Sb), as well as urban inputs from the lake shore which discharge a large volume of wastewater, agrochemicals and landfill leachates from the nearest settlements. Solid and liquid wastes originating from urban areas are enriched with Cu, Pb, Zn and Ni, while Cu, Pb, Zn, Cr, Ni and Co are also common constituents of many agrochemicals [31]. The spatial distribution of enrichment reflects the fact that sampling points D5 and D6 are located near the city of Novi Dojran and are in the vicinity of local hotel complexes.

Analysis via Pearson correlation matrix presented the following significantly positive correlations: As with Pb; Co with Zn and Ni; Cr with Zn; Cu with Pb, Zn and Ni; Ni with Zn; and finally Sb with Cr and Ni. PCA (Fig. 6) accounted for 85.88 % of data variance in the first two ordination axes, revealing highly significant positive correlations between As, Cr, Pb and Sb and between Co, Cu, Ni and Zn at locations D5 and D6, and highly negative correlation was again confirmed between Au (location D3) and other trace elements. These relationships likely demonstrate possible co-contamination from the aforementioned geogenic and anthropogenic sources. Conversely, no correlations were noted between Au and any other element, suggesting that the nature of Au contamination might be from a different source (e.g. polymetallic mineralization) [32].
3.5. Mineralogy assemblage of the *Anodonta cygnea* and isotopic composition of inorganic C and O in biogenic carbonate from Dojran lake

*Anodonta cygnea* shells are composed of pure aragonite, together with a small amount of organic constituents (about 1%). The XRD analyses were confirmed with SEM-EDS system analysis, as well.

Isotopic values results are represented in the Table 3. An average value of $\delta^{13}\text{C}_{VPDB}$ is -2.17‰ and the values range from -1.72‰ to -2.48‰. Deviations in $\delta^{13}\text{C}_{VPDB}$ values in molluscs shells are 0.76‰. The values of $\delta^{13}\text{C}_{VPDB}$ decrease with depth. $\delta^{13}\text{C}_{VPDB}$ values are very negative, indicating shells enrichment with light C isotope. Furthermore, these negative values of $\delta^{13}\text{C}_{VPDB}$ show that CO$_2$ arising from decomposition of organic matter and by enhanced temperatures affects the carbon isotopic composition. CO$_2$ is enriched with light C isotope, which lowers the values of $\delta^{13}\text{C}_{VPDB}$ [33].
Table 3: Inorganic C and O isotopic composition in *Anodonta cygnea* shells (‰).

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\delta^{13}\text{C}_{\text{VPDB}}$</th>
<th>$\delta^{13}\text{O}_{\text{VPDB}}$</th>
<th>$\delta^{13}\text{O}_{\text{SMOW}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>-2.38</td>
<td>1.00</td>
<td>31.40</td>
</tr>
<tr>
<td>D2</td>
<td>-2.15</td>
<td>0.98</td>
<td>31.38</td>
</tr>
<tr>
<td>D3</td>
<td>-2.09</td>
<td>0.90</td>
<td>31.30</td>
</tr>
<tr>
<td>D4</td>
<td>-2.38</td>
<td>1.34</td>
<td>31.75</td>
</tr>
<tr>
<td>D5</td>
<td>-1.72</td>
<td>1.94</td>
<td>32.37</td>
</tr>
<tr>
<td>D6</td>
<td>-2.04</td>
<td>1.80</td>
<td>32.22</td>
</tr>
</tbody>
</table>

An average value of $\delta^{18}\text{O}_{\text{SMOW}}$ is 31.57 ‰ and $\delta^{18}\text{O}_{\text{SMOW}}$ values vary between +30.81 and +31.57 ‰. Deviations in $\delta^{18}\text{O}_{\text{SMOW}}$ values are estimated with 1.56 ‰. The values of $\delta^{18}\text{O}_{\text{VPDB}}$ decrease with depth, also. $\delta^{18}\text{O}$ signature in shells might be explained by precipitation of the carbonate from isotopically heavier oxygen lake water. The unusually heavy skeletal oxygen suggested calcification at elevated temperature and evaporation [33]. Evaporation influences the concentrations of dissolved compounds in water and the evaporation of light H$_2$O. This leads to water enrichment with heavy oxygen and consequently defines the isotopic composition of molluscs shells.

### 3.6. Isotopic composition of inorganic C and O in surficial sediments from Dojran lake

From Table 4, it is evident that $\delta^{13}\text{C}_{\text{VPDB}}$ are slightly negative indicating raised presence of light C isotope. The $\delta^{13}\text{C}_{\text{VPDB}}$ composition ranges from -6.45 to -1.65 ‰ with an average value of -4.31 ‰. The values of $\delta^{13}\text{C}_{\text{VPDB}}$ increase with depth, where the presence of heavy C isotope prevails. This enrichment originates from methane production in sediment, which is depleted in heavy C isotope [34].

Table 4: Inorganic C and O isotopic composition in Lake Dojran sediments (‰).

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\delta^{13}\text{C}_{\text{VPDB}}$</th>
<th>$\delta^{13}\text{O}_{\text{VPDB}}$</th>
<th>$\delta^{13}\text{O}_{\text{SMOW}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>-4.98</td>
<td>-4.79</td>
<td>25.43</td>
</tr>
<tr>
<td>D2</td>
<td>-5.06</td>
<td>-5.84</td>
<td>24.35</td>
</tr>
<tr>
<td>D3</td>
<td>-4.50</td>
<td>-1.79</td>
<td>28.53</td>
</tr>
<tr>
<td>D4</td>
<td>-2.02</td>
<td>1.49</td>
<td>31.90</td>
</tr>
<tr>
<td>D5</td>
<td>-1.65</td>
<td>1.02</td>
<td>31.42</td>
</tr>
<tr>
<td>D6</td>
<td>-6.45</td>
<td>-5.60</td>
<td>24.60</td>
</tr>
</tbody>
</table>

$\delta^{13}\text{O}_{\text{SMOW}}$ deviations in sediments are very high (7.55 ‰) and they vary from +24.34 ‰ to +31.90 ‰. An average value $\delta^{13}\text{O}_{\text{SMOW}}$ is +27.99 ‰. $\delta^{13}\text{O}_{\text{VPDB}}$ results increase with depth. Lake Dojran sediments are strongly affected by evaporation effect, revealing that the values of $\delta^{13}\text{O}_{\text{SMOW}}$ are much closer to $\delta^{13}\text{O}_{\text{SMOW}}$ values of marine sediment than $\delta^{13}\text{O}_{\text{SMOW}}$ values of freshwater sediment. $\delta^{13}\text{O}_{\text{SMOW}}$ results for marine sediments range from +26 to +32 ‰ (after [35]).

### 3.7. Trace element composition of fish and frog species selected from Lake Kalimanci and fish species from Lake Dojran

Analyses identified particularly high levels for Cr, Hg, Ni and Pb in fishes (*Vimba melanops*) and frog (*Rana temporaria*) samples from Lake Kalimanci with mean values [µgkg$^{-1}$]: 1090 – 6.5, 339 – 117, 567 – 5100 and 1127 – 435, respectively. Meanwhile, the contents of Cr, Hg, Ni and Pb in fishes samples (*Rutilus rutilus dojranensis* from Lake Dojran are a bit lower [µgkg$^{-1}$]: 2320, 1690, 700 and 50. Complete results are presented in Table 5.
Table 5: Mean trace element contents in freshwater fishes and frog samples (Lake Dojran and Lake Kalimanci).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cd</th>
<th>Co</th>
<th>Cu</th>
<th>Cr</th>
<th>Hg</th>
<th>Mo</th>
<th>Ni</th>
<th>Pb</th>
<th>Sb</th>
<th>Se</th>
<th>Zn</th>
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<td>Detection Limit</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>0.5</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>0.1</td>
<td>10</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Vimba melanops</td>
<td>µg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
</tr>
<tr>
<td>Lake Kalimanci</td>
<td>7</td>
<td>30.9</td>
<td>1653</td>
<td>1090</td>
<td>338.7</td>
<td>58.3</td>
<td>567</td>
<td>1127</td>
<td>3.1</td>
<td>1433</td>
<td>46500</td>
<td>295</td>
</tr>
<tr>
<td>Rana temporaria</td>
<td>µg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
</tr>
<tr>
<td>Lake Kalimanci</td>
<td>29.9</td>
<td>112</td>
<td>1520</td>
<td>6.51</td>
<td>117</td>
<td>268</td>
<td>5100</td>
<td>435</td>
<td>8.5</td>
<td>650</td>
<td>80300</td>
<td>183</td>
</tr>
<tr>
<td>Rutillus rutillus dojranensis</td>
<td>µg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
</tr>
<tr>
<td>Lake Dojran</td>
<td>27.3</td>
<td>33.9</td>
<td>1820</td>
<td>2320</td>
<td>1690</td>
<td>63</td>
<td>7000</td>
<td>50</td>
<td>5.9</td>
<td>13000</td>
<td>942000</td>
<td>738</td>
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</table>

4. Conclusion

In the present study the mineralogical, geochemical and isotopic properties of of recent surficial sediments from the Lake Dojran and Lake Kalimanci (FYRM) and shells of *Anodonta cygnea* from Lake Dojran were examined in detail. Mineralogical analysis revealed that the lake’s surficial sediment mineralogy is closely related to the prevailing metamorphic, volcanic and igneous rocks of the Dojran region. The mineralogical assemblage of lake sediments is mostly composed of quartz, feldspars, clay minerals, calcite and micas, along with minor amounts of augite and hornblende.

More than 65% of major elements in almost all samples were accounted for Si-Al-Fe components, reflecting abovementioned mineral association. Mean trace element concentrations in Lake Dojran sediments increase in the following order: Au < Sb < Cr < Co < Pb < Cu < As < Ni < Zn. According to calculated EF values, surficial Lake Dojran sediments (0-5 cm) are little enriched with Cr, Zn, Pb, Cu and Co, moderately enriched with Ni, Au and Sb, moderately severely enriched with Au, severely enriched with Sb and very severely enriched with As. The abovementioned enrichment originates from a number of different geogenic (geological background and polymetallic mineralization with arsenopyrite (FeAsS) and gold (Au)) and anthropogenic sources. The latter include various tourism activities which take place around the lake - and the associated increase in traffic (Pb) and coatings (Sb) – as well as urban inputs from the lake shore which discharge a considerable volume of wastewater, agrochemicals and landfill leachates.

The mineralogy of *Anodonta cygnea* shells is aragonitic. $\delta^{13}C_{VPDB}$ values in *Anodonta cygnea* shells are ranging from -1.72 ‰ to -2.48 ‰, showing shells enrichment with light C isotope. The values of $\delta^{13}C_{VPDB}$ decrease with depth indicating that CO₂ arising from decomposition of organic matter and by enhanced temperatures, affects the carbon isotopic composition. $\delta^{18}O_{SMOW}$ shell values vary between +30.81 and +31.57 ‰. Stable isotope signatures showed that the biogenic carbonate fraction from the Lake Dojran was precipitated in water where the isotopic ratios were controlled by the precipitation evaporation balance.

The $\delta^{13}C_{VPDB}$ signature in Lake Dojran sediments range from -6.45 to -1.65 ‰ and $\delta^{13}C_{VPDB}$ values are increasing with depth. Thus, heavy C isotope dominates in depth. This enrichment originates from methane production in sediment, which is decreased with heavy C isotope. Measured $\delta^{13}O_{SMOW}$ sediment values vary from 24.34 ‰ to 31.90 ‰. Lake Dojran sediments are strongly affected by evaporation effect, defining $\delta^{13}O_{SMOW}$ values in sediments closer to marine sediments.
When both lakes are compared, it can be concluded, that Lake Kalimanci is much more polluted with potentially toxic elements, such as metals and metalloids, than Lake Dojran. Major cause for such heavy pollution in lake environment is related to nearby active mining. The Sasa mine, where Pb and Zn concentrates are produced, has major influence on entire lake ecosystem. Furthermore, after Sasa tailings dam failure in year 2003, all elements are heavily increased. For this reason also fish samples from Lake Kamenica contain higher contents of toxic elements than those from Lake Dojran. At the end, our intention was to compare shells from both lakes, but regarding to such heavy pollution in Lake Kalimanci, no shells could be found.

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REFERENCES


