

## Determination of selected elements in freshwater sponge tissue, natural water and sediments by inductively coupled plasma optical emission spectrometry

S. Rončević, L. P. Svedružić

Laboratory of Analytical Chemistry, Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102A, HR-10000 Zagreb, Croatia

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Metal content in the samples of freshwater sponge *Eunapius subterraneus* which is an endemic species of karstic underground in Central Croatia was determined by inductively coupled plasma atomic emission spectrometry (ICP–OES). Additionally, metal content was determined in the samples of water and sediment collected at the same sampling sites. Analytical procedure of sample preparation included acid digestion in open vessel. The accuracy of the applied methods tested by measurement of certified reference samples of water and sediment comprised 5–10% of declared metal concentrations. It was established that metal content (Al, Ba, Ca, Cd, Cu, Fe, Mg, Mn, Ni, Pb, Sr, and Zn) measured in sponge tissue, water and sediment samples is related to sampling site. Influence of anthropogenic pollution through sewage deposition was observed in slightly higher content of Al, Cu, and Zn in water, and also Cu, Pb and Zn in sediment sample. Analysis of two morphologically different sponge samples showed different abundance of Cu, Zn, Pb and Cd.

**Key words:** ICP-OES, freshwater sponge, metal content.

### INTRODUCTION

Determination of metal content in aquatic ecosystem is recognized as extremely important in wide range of different studies which include chemical characterization of species, routine monitoring of environmental pollution, and studies on the ecological and physiological role of toxic and essential metals. Atomic spectrometry methods based on absorption or emission of electromagnetic radiation combined with optical or mass spectrometers are firmly established in elemental analysis of environmental samples and biological tissues [1-3]. For the monitoring purposes of heavy metal pollution in marine ecosystem several species of marine sponges were selected as a potential bioindicator organism [4,5]. More profound insight on the environmental status was achieved by determination of metal content in marine sponges using multielement analytical techniques as inductively coupled plasma atomic emission (ICP–OES) or mass spectrometry (ICP–MS) [6].

Sponges are sessile organisms which have a property of filtering a large volume of water and accumulate metals and other contaminants from the environment. Recent investigations in this field describe the influence of some toxic metals and other pollutants on sponge cell aggregation [7-9].

The uptake of metals is usually influenced with type of metal species and concentration present in either water or sediment, genus of the sponge, and habitat as well [10,11].

Although sponges are wide-spread in marine environment, only a minor number of organisms inhabit groundwater. All freshwater sponges are classified in the Demosponigae, family Spongillidae. Published studies of metabolites of freshwater sponges, namely lipid compounds, included analytical methods as NMR, HPLC, CE, IR and UV [12,13]. The data on element distribution in freshwater sponges are rarely represented in the literature. One of recent examples is the study of element composition of Baikal sponge tissue by ICP–MS which showed the degree of trace elements accumulation [14].

In this work, an inductively coupled plasma atomic emission spectrometry (ICP–OES) is used for the determination of selected metal content in sponge tissue, natural water and sediment samples which were collected from karstic region in Central Croatia. The centre of the region is the city of Ogulin with geographic coordinates: latitude 45° 15' 58N and longitude 15° 13' 43E. The Dinaric karstic area of Croatia Republic is well known as the richest reserve of the subterranean water fauna with high number of endemic species with sponges as a specific example. The only specialised subterranean freshwater sponge known to date is

\* To whom all correspondence should be sent:  
E-mail: roncevic@chem.pmf.hr

the *Eunapius Subterraneus* Sket & Velikonja, 1984, which includes two morphotypes that inhabit exclusively the karstic subterranean waters of Ogulin region [15,16]. Poor natural purification ability of karstic water streams indicates that monitoring of possible pollution in such ecosystems is of crucial interest. From the first description of endemic sponge species in 1984, there was no systematically collected data on the sponge status. The recent investigations were conducted in order to analyze key morphological features, to record the distribution patterns and to examine the current state of habitat quality and population-threatening changes in the environment [17]. In order to record the present status and to provide the base for the further comparison of possible changes in the ecosystem, the content of the selected metals was determined in sponge tissue, as well as in water and sediment samples collected from habitat.

## EXPERIMENTAL

### Instrumentation

A *Prodigy High Dispersion ICP* inductively coupled plasma optical emission spectrometer (Teledyne Leeman Labs, Hudson, NH, USA) was used for the metal content determination in all samples. The specifications of spectrometer are

given in Table 1 and operating parameters of instrument are given in Table 2.

Emission lines of elements measured in this work (Al, Ba, Ca, Cd, Cu, Fe, Mg, Mn, Ni, Pb, Sr, and Zn) were selected after collecting spectral images on L-PAD detector. Several intense and weak analytical lines of each element were measured simultaneously without blooming or saturation of detector. The best ones, *i.e.* lines without spectral and background interferences which showed maximal signal-to noise ratio were chosen for analysis. Emission lines of selected metals along with method detection limits are given in Table 3. The detection limits were calculated in accordance to IUPAC recommendation, *i.e.* as the concentration equivalent to three times standard deviation ( $3\sigma$ ) of the signal of the method blank solution.

## RESULTS AND DISCUSSION

Methanesulfonic acid (MSA) is an alkylsulfonic acid, which has numerous applications, for example, as an esterification or alkylation catalyst, as a polymer solvent, in the electroplating and electrochemistry industry, etc. MSA also is an effective reagent for the conversion of alcohols into corresponding amides [12], Fries-rearrangement [13], Beckmann rearrangement [14], hydration of

**Table 1.** Specification of the spectrometer and ICP source

Spectrometer	<i>Prodigy</i> high-dispersion echelle spectrometer with dual-viewing optics (radial and axial) , Teledyne Leeman Labs, Hudson, NH, USA
Focal length	800 mm
Grating	Echelle grating 52.13 gr/mm, format of 110 mm x 110 mm UV grade
Dual Pass Prism	fused silica prism
Wavelength range	165 nm – 800 nm
Resolution	< 0.00075 at 200 nm (standard 40 $\mu$ m x 100 $\mu$ m entrance slit)
Detector	Large Format Programmable Array Detector L-PAD (28 x 28 mm, 1026 x 1026 pixels)
Rf generator	Free-running
Frequency	40.68 MHz
Power output	600 to 2000 W
Nebulizer	Pneumatic (glass concentric - <i>SeaSpray</i> )
Spray chamber	Glass cyclonic
Plasma torch	Fassel type, three concentric quartz tubes, dual-view torch
Pump	12-roler computer-controlled four channels peristaltic pump

**Table 2.** Operating conditions

Incident power	1.1 kW
Outer argon flow rate	18 L min <sup>-1</sup>
Auxiliary gas	0.8 L min <sup>-1</sup>
Carrier gas flow rate	0.9 L min <sup>-1</sup>
Liquid uptake rate	1.5 mL min <sup>-1</sup>
Nebulizer pressure	36 psi
Integration time	10 s

**Table 3.** Line selection and detection limits

Analytes, wavelengths, $\lambda$ , nm	Detection limits in pure solvent, $c_L$ , $\mu\text{g L}^{-1}$
Al I 308.215	3.05
Ba II 493.409	0.13
Ca II 396.847	5.88
Cd I 214.441	0.61
Cu I 324.754	0.64
Cr II 267.716	2.88
Fe II 259.940	2.02
Mg II 279.553	3.95
Mn II 257.610	0.41
Ni II 231.604	2.21
Pb II 220.353	6.10
Sr II 407.771	0.06
Zn I 213.856	1.13

#### Reagents and Test Solutions

High-purity deionised water (Milli-Q Element system, Millipore, USA) was used for preparation of all the sample and standard solutions. Sample dissolution procedures were performed using analytical grade chemicals: nitric acid (65 %) hydrochloric acid (36 %), and hydrogen peroxide (30%) from Kemika, Croatia. Single element standard solutions of Al, Ba, Ca, Cd, Cu, Fe, Mg, Mn, Ni, Pb, Sr, and Zn (Plasma Pure, Leeman Labs, Hudson, NH, USA) and multielement standard ICP-Mehrelement-Standardloesung IV (Merck, Darmstadt, Germany) were used for the preparation of calibration standard solutions and control of plasma positioning. All calibration standards were prepared by appropriate dilution of standard stock solutions ( $1 \text{ g L}^{-1}$ ) to obtain the concentration range from 1 to  $100 \text{ mg L}^{-1}$ .

#### Strategy

**Study area.** The study area is located in Central Croatia: Medvedica, Zala, Gojak and Tounjcica. The karstic system of underground caves in Ogulin region of Croatia is known as habitat of several endemic species. Subterranean sponge had inhabited the Medvedica cave system in the past, precisely in 1984 and 1986. No sponge species was found during last sampling in the Medvedica cave. In earlier period some attempts of pedological investigations were made, but afterwards no sustained research of this site has been conducted [18].

#### Sampling

**Water samples:** Water samples were collected by scuba diving in winter season when water level was at lowest. Samples of natural water were taken from four caves: Medvedica, Zala, Gojak and Tounjcica and preserved according to ISO 5667- Water quality sampling [19].

**Sediment samples:** The samples of sediment were collected from the cave Medvedica. All samples were stored immediately in a cool box then transferred to a refrigerator, where they were stored at  $4 \text{ }^\circ\text{C}$ . Before the digestion, samples were ground in a Retsch BB51 (Haan, Germany) grinding mill and sieved to particle size  $< 100 \mu\text{m}$ . Powdered samples were dried in drying oven at  $105^\circ\text{C}$  [20].

**Freshwater sponge samples:** Samples of sponges were accessible for collection only in two caves: Gojak and Tounjcica. Samples of sponge from the cave Tounjcica were egg-shaped, they had irregularly wrinkled surface with a size of approximately 3 cm. Other samples from the cave Gojak were cone-shaped with smooth surface and sized approximately 2 cm. Each of sponge samples was washed several times in distilled water in order to remove the majority of viewable sediments and small pieces of impurities. Additional cleaning was performed by washing with high-purity deionised water in ultrasonic bath for 20 minutes. Sponges were dried in drying oven at  $105 \text{ }^\circ\text{C}$ . Dried residues were grinded in agate mortar.

#### Digestion procedures

**Sediment samples:** After drying of powdered sediments, a weighted amount of 100 mg was transferred into Kjeldahl flasks. The analytical digestion procedure included repeated addition of 5 mL aliquots of HCl /  $\text{HNO}_3$  mixture [21]. The remaining acidic solutions were transferred into a 50 mL volumetric flask and filled up to mark with high-purity deionised water. In order to control the accuracy of the preparation procedure, the solution of reference stream sediment sample NCS DC 73309 (*China National Analysis Center for Iron and Steel*) was prepared in the same way.

**Freshwater sponge samples:** The limited amount of endemic sponge tissue samples was the prevailing condition for the selection of digestion procedure. Despite the fact that digestion of biological samples in a closed system such as microwave oven has a notable advantage because the volatilization losses can be minimized [22], the open vessel digestion was the method of choice. An amount of 20 mg of the powdered sample of sponge tissue was weighted and transferred into Kjeldahl flasks. The analytical digestion procedure in open vessel included subsequent addition of mineral acids [23]. A volume of 2 mL of conc.  $\text{HNO}_3$  was added into flask and heated until approximately 0.5 mL of starting solution was remained. After cooling, 1 mL of conc. HCl was added and the flask was heated again. Procedure was repeated in several

steps until the clear solution without any precipitate was remained. A volume of 1 mL of hydrogen peroxide was then added into flask and solution was heated until complete peroxide was boiled. The remaining acidic sample solutions were transferred into a 50 mL volumetric flask and filled up to mark with ultra-pure deionised water. A method blank was prepared by following the same procedure.

*Water samples:* Natural water samples were filtered through PTFE filters with 45 µm pore size to remove the bulk of sediments and suspended materials. In order to achieve the better sensitivity of trace metal determination in water samples, an amount of 500 mL of collected water was preconcentrated by evaporation till dryness. Solid residues were dissolved in the same manner as sponge residues by HNO<sub>3</sub> and HCl addition. The final acidic solutions were transferred into 50 mL volumetric flasks and filled up to the mark with high-purity deionised water. For the control of possible acids impurity effects, the procedure was also repeated using high-purity deionised water to obtain the blank sample. In order to control the accuracy of the analytical procedure, the reference sample of water SLRS-4 River Water Reference Material for Trace Metals (*National Research Council of Canada*) was treated on the same way. All samples were prepared and analysed as replicates.

## RESULTS AND DISCUSSION

*Determination of Ba, Cd, Cu, Mn, Ni, Pb, Sr and Zn in the presence of Al, Ca, Fe, Mg as matrix constituents in sediments*

*Certified reference materials stream sediment sample NCS DC 73309*

Analytical procedure of sediment digestion was checked by use of certified reference material of stream sediment (NCS DC 73309) and the obtained results are shown in Table 4.

**Table 4.** Analysis of certified reference stream sediment sample NCS DC 73309

Element	w <sup>a</sup> , mg g <sup>-1</sup> found	RSD, %	w <sup>b</sup> , mg g <sup>-1</sup> certified
Al	48.298 ± 6.661	12.0	54.885 ± 0.875
Ba	0.276 ± 0.030	9.42	0.260 ± 0.017
Cu	0.086 ± 0.005	5.81	0.079 ± 0.003
Fe	2.769 ± 0.224	7.04	(3.070)
Mn	2.348 ± 0.059	2.17	2.490 ± 0.084
Pb	0.571 ± 0.064	9.80	0.636 ± 0.022
Sr	0.030 ± 0.001	3.33	0.029 ± 0.001

<sup>a</sup> w = x ± Δx (n = 6)

<sup>b</sup> w = mean ± ass.unc.

Statistical comparison of obtained data was performed by two-paired *t*-test at significance level of P = 0.05. Calculated *t*-value of 1.003 is lower than critical value of 2.47, which means that two sets of values were not significantly different. The recovery of metal content for the most of declared elements lay within 10% of certified values. By knowing that the accuracy of ICP-OES method is usually 10% depending on applied analytical procedure, the obtained results imply quite acceptable preparation and measurement conditions. Slightly negative bias was established for Al (88%) and Pb (89.8%), which might be partly denoted to incomplete digestion. The other reason of lower concentration could be explained by mode of emission measurements in plasma which is adjusted for simultaneous multi-element collection of signal, usually called compromise conditions.

### *Sediment sample from the Medvedica cave*

It was already mentioned that subterranean sponge had inhabited the Medvedica cave system in the past, but no sponge species was found during the last sampling in the Medvedica cave. In earlier period some attempts of pedological investigations were made, but meanwhile, habitat was changed under great pressure of groundwater and water pollution. The sediment samples which were collected at this site was analysed by ICP-OES method and the measured metal content is given in Table 5.

**Table 5.** Metal content in sediment samples from the Medvedica cave

Element	w <sup>a</sup> , mg g <sup>-1</sup>	RSD, %
Al	76.8 ± 10.1	11.4
Ba	0.24 ± 0.02	8.33
Ca	51.9 ± 4.7	7.90
Cd	0.0112 ± 0.0009	7.14
Cu	0.160 ± 0.009	5.00
Fe	47.8 ± 2.6	4.81
Mg	14.8 ± 0.3	2.03
Mn	11.2 ± 0.2	1.78
Ni	0.090 ± 0.010	10.0
Pb	0.070 ± 0.007	8.57
Sr	0.140 ± 0.003	2.14
Zn	0.530 ± 0.060	10.0

<sup>a</sup> w = x ± Δx (n = 6)

Since additional sediment sampling was disabled, the metal concentrations showed in Table 5 could be compared to the recorded data of same site which were formerly obtained by spectrophotometric measurements of Cu, Fe, Mn, Pb and Zn [18]. Measured concentration of Mn is slightly higher than content of 8.5 mg g<sup>-1</sup> published

before. Two-fold higher concentrations of Zn compare to previous 0.25 mg g<sup>-1</sup> was established in our measurements. Almost five-fold higher iron content in sediment compare to published 10.5 mg g<sup>-1</sup> of Fe was noticed here. As opposed to formerly published data for Cu and Pb of 0.020 mg g<sup>-1</sup> and 0.005 mg g<sup>-1</sup>, ten-fold higher concentrations of those metals are observed in present sediment sample. Generally, the obtained results did not exceed the maximum permissible concentrations of heavy metals which were prescribed by domestic regulations of quality of soils. However, the intention of rising heavy metals concentration was noted here. One of the probable reasons for such change in chemical composition of sediment at same sampling site is that during last decades an additional load of sewage water flew through the underground cave system.

*Determination of Al, Ba, Ca, Fe, Mg, Mn, Na and Sr in water samples*

*Determination of Al, Ba, Ca, Fe, Mg, Mn, Na and Sr in certified standard water SLRS-4.*

Analytical preparation of water samples included preconcentration step and for this reason the accuracy was checked using certified reference sample of water (SLRS-4). The obtained results showed in Table 6 impute the factor of 10 to measured values because of the 10-fold sample preconcentration.

Table 6. Analysis of certified reference water sample SLRS-4

Element	$\gamma^a$ , mg L <sup>-1</sup> found	RSD, %	$\gamma^b$ , mg L <sup>-1</sup> certified
Al	0.060 ± 0.005	6.67	0.054 ± 0.004
Ba	0.013 ± 0.001	7.69	0.012 ± 0.001
Ca	6.70 ± 0.40	5.22	6.20 ± 0.19
Fe	0.095 ± 0.005	4.21	0.103 ± 0.005
Mg	1.55 ± 0.09	5.16	1.60 ± 0.10
Mn	0.0033 ± 0.0001	3.03	0.0031 ± 0.0007
Na	2.57 ± 0.06	1.94	2.40 ± 0.19
Sr	0.026 ± 0.001	3.85	0.026 ± 0.003

<sup>a</sup>  $\gamma = x \pm \Delta x$  (n = 6) <sup>b</sup>  $\gamma = \text{mean} \pm \text{ass.unc.}$

Statistical comparison of two sets of data was performed by two-paired *t*-test at significance level of P = 0.05. Calculated *t*-value of 1.198 is lower than critical value of 2.364, which means that two sets of values were not significantly different. Compared to general ICP method accuracy of 10% it is obvious that recovery of selected metal content was satisfactory for all measured elements.

*Determination of Al, Ba, Ca, Fe, Mg, Mn, Na and Sr in natural water samples, collected from four sampling sites*

Samples of natural water collected from four sampling sites were analysed by ICP-OES. The measured content of metals in water samples collected from underground cave system is represented by Figures 1 and 2.

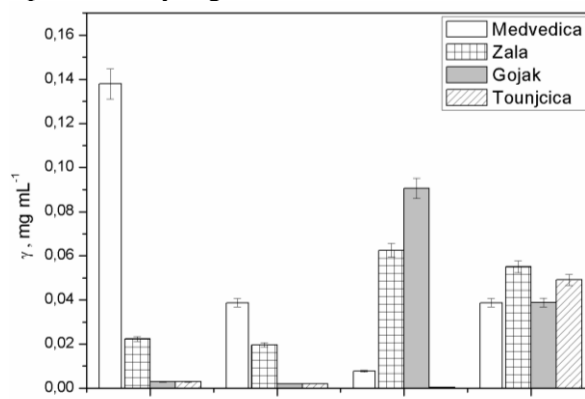


Fig. 1. Major metal constituents of water samples.

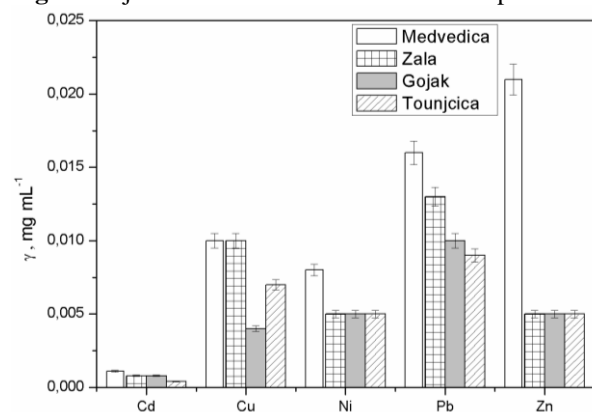


Fig. 2. Trace metal constituents of water samples.

The concentration values included preconcentration factor of 10 and precision measure expressed as relative standard deviation of three replicates. Good precision of measurement (RSD < 2.0%) was obtained on all selected lines. Metal concentrations are showed in separate figures due to different scaling of major and minor constituents. The measured content of Ba and Cr did not exceed the detection limits in all analyzed samples. The measured content of principal constituents in natural water showed slightly variations between 15–20 mg L<sup>-1</sup> of Ca and 3–5 mg L<sup>-1</sup> of Mg. The groundwaters in karstic area are moderately hard (140–215 mg L<sup>-1</sup> of CaCO<sub>3</sub>) with low content of non-carbonate ions. In typical karst water the ratio of Ca/Mg content usually ranges from 2.5 to 5 [24]. This ratio is confirmed in all samples measured in our work. However, the lower content of Ca and Mg than in typical moderately hard water was established here which classify examined samples in a class of soft water. From the measured content of other metals showed in Figures 1 and 2, a

significantly higher Al and Zn concentrations and slightly higher Fe, Cu, Ni, and Pb concentrations were measured in water from Medvedica cave than in other three caves. The lead content measured in samples from Medvedica, Zala and Gojak was slightly higher than maximum permissible concentration of  $0.01 \text{ mg mL}^{-1}$  which is prescribed by the domestic regulations of natural water quality. The observed results imply that continuous pollution through sewage and waste dumping occurs and it is especially pronounced at the site Medvedica [17]. Moreover, the fact that from the recent investigations of distribution of the sponge *Eunapius subterraneus* which showed that they are not found in the Medvedica cave where was inhabited in the past could be explained by obtained results of metal concentrations.

### 3.3. Determination of Al, Ca, Fe, Mg Ba, Cd, Cu, Mn, Ni, Pb, Sr and Zn in freshwater sponge samples

Two morphotypes of sponge *Eunapius Subterraneus* were analyzed in this work, i.e. the egg-shaped sponges with irregularly wrinkled surface from the Tounjcica cave and the cone-shaped with smooth surface from the Gojak cave. Sponge tissue analysis included complete individual organisms because they were rather small; their base was about  $1 \text{ cm}^2$  with up to 3 cm in height. Consequently, digestion procedure which started from dried tissue included weighted amounts of 20 mg of analytical sample. Although the sample mass was restricted, the advantage of ICP-OES method to show excellent linearity in trace and major constituent presence was confirmed. Precision from three replicates of signal measurement was satisfactory for all elements determined and gave the  $\text{RSD} < 1.5\%$ . Since no reference sample for sponge analysis is available, the control of analytical procedure was based on analysis of the same reference sample of stream sediment used in sediment sample analysis. The main reason for selection of this material is the fact that besides spongin the sponge skeleton is built out of silica spicules and carbonates. It means that after removal of organic substances during sample preparation, this reference material could fit as the most likely to inorganic residue. For this purpose, a smaller amount of 10–20 mg of NCS DC 73309 was digested to check the accuracy at low sample size. The obtained results matched all of measured recoveries from greater scale of 1 g of starting reference material which is presented in Table 4.

Metal concentrations in sponge samples are shown in Figures 3 and 4.

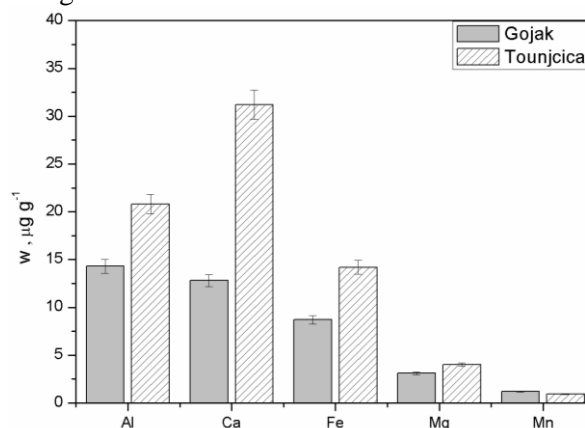


Fig. 3. Major metal constituents of sponge samples.

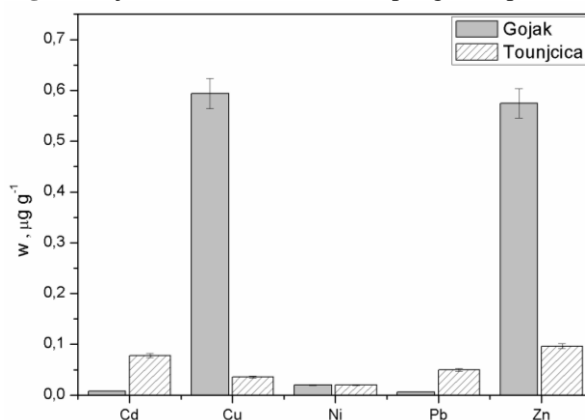


Fig. 4. Trace metal constituents of sponge samples.

The results are expressed as weight ratio, i.e. microgram of element mass per gram of dried sample mass and they include the precision of measured replicates. Ba and Sr were not detected. The concentrations of Ba and Sr in the real samples are below the detection limits. The obtained results mutually show some notable differences regarding to sponge morphotype. Among the major constituents, the different weight ratio is evident for Al, Ca and Fe content (Fig 3). The slightly different content of Cd and Pb is also noted in two kinds of sponge samples (Fig 4). The remarkable higher concentration of Cu and Zn in cone-shaped sponges from Gojak cave compared to egg-shaped sponges from Tounjcica cave was established by measurements. By general comparison of element concentrations present in sponge samples with the metal content measured in water from the same sampling sites, the greater element concentrations in sponge samples was established. Chemical composition of sponge is mostly affected by the water environment, but sponges of different classes and habitats have different capacities of metal accumulation.

The majority of published data concerning the determination of metal content deal with marine sponge organisms. Usually, investigations are conducted in order to examine the influence of heavy metal pollution on estuarine systems [4,5,11]. A higher level of Cd, Cu, and Zn content than samples from surrounding marine area was noted. The accumulation of bivalent metals by sponges at different stages of growth was studied on examples of marine sponges, too [7-9]. It was found that bioaccumulation of some toxic metals, *i.e.* Cu, Pb and V, can alter the behaviour and the physiological response, such as change in shape and growth rate. It was shown that moderate concentrations of Cu and Cd can have positive effects on sponge by enhancing cell aggregation, but long term exposition may alter cell functions. The mechanism of Cd accumulation in sponge tissue is favoured at low pH and low salinity of marine system. Generally, most of the studies confirmed the potential of marine sponges as biomarker organisms in the monitoring of marine pollution.

Unfortunately, there are only a minor number of published data on metal content in freshwater sponge species. Considering the class of Demospongiae, family of Spongillidae, the investigations are primarily focused on isolation of lipid compounds and on phosphatic metabolite variation with changes of habitat [12,13]. The representative work in metal content analysis of endemic family Lubomirskiidae of freshwater sponges is undoubtedly the paper of Paradina et al. [14]. This investigation presents the ICP-MS analysis of nineteen elements in Lake Baikal sponges where authors observed lower metal content than in family of Spongillidae. The accumulation of biologically active elements such Mn, Cu and Co, but also the considerable Cd quantities were established in their work.

Each sponge species has its own spectrum of accumulation of elements which disables mutual comparison of metal content accumulated by different classes of sponge. Metal content in endemic species of *Eunapius Subterraneus* studied in our work emphasise this statement. The relationship between established Cu and Zn content of two morphotypes of sponge and the same elements content in water should not only be attributed to accumulation from water environment, but also to characteristics of sole specie. In addition, a possible pollution impact could hardly be driven based on those first results of metal distribution patterns in sponge and water from

underground habitats. Therefore, the obtained results could serve as the base for the future investigations in aspect of environmental monitoring or study of biological response of the endemic *Eunapius subterraneus* species on changes of chemical parameters in water and sediments.

## CONCLUSION

Inductively coupled plasma atomic emission spectrometry (ICP-OES) was used in determination of metal content in samples of freshwater sponge *Eunapius subterraneus* which is the endemic organism of Central Croatian karstic caves. Analysis of two morphologically different sponge samples collected at two different locations, shows mutual variation in content of trace metals (Cu, Zn, Pb and Cd). Although, the causal connections between trace metal accumulation and morphotypes appearance was not targeted in this stage, the obtained data, especially for Cu and Zn, shows that indicators of different metal accumulation are inherent to studied subspecies. The metal content in water collected from habitats and also from the neighbouring sites, where sponges had been spread in the past, was also determined. Greater concentrations of Al, Cu, and Zn in waters exposed to anthropogenic pollution were measured. Also, the greater concentrations of Cu, Pb and Zn in sediment sample from Medvedica cave in comparison with previous investigations were established here. The obtained results coincide with the fact that sponges do not inhabit this cave system as it was in the past. Present study comprises the determination of metal distribution patterns in freshwater sponge tissue for the first time. Consequently, the collected data provides the base for the further projects in active conservation of threatened *Eunapius subterraneus* species especially when very high risk of extinction is already known.

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## ОПРЕДЕЛЯНЕ НА ИЗБРАНИ ЕЛЕМЕНТИ В ТЪКАН ОТ СЛАДКОВОДНИ ГЪБИ, ПРИРОДНИ ВОДИ И УТАЙКИ ЧРЕЗ ОПТИЧНА ЕМИСИОННА СПЕКТРОМЕТРИЯ С ИНДУКТИВНО СВЪРЗАНА ПЛАЗМА.

С. Рончевич, Л. П. Сведружич

Лаборатория по аналитична химия, Департамент по химия, Факултет по науки, Загребски Университет, Хорватовак 102А, HR – 10000 – Загреб, Хърватия

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(Резюме)

Металното съдържание на проби от сладководни гъби *Eunapius Subterraneus*, които са ендемичен щам от карстовите подземни растения в Централна Хърватия, е определяно чрез атомно-емисионна спектрометрия с индуктивно свързана плазма ( ICP – OES ). В допълнение, съдържанието на метали е определяно във водни проби и утайки, събирани от някои пунктове за пробонабиране. Аналитичната процедура за обработка на пробата включва киселинна обработка в отворен съд. Точността на приложените методи, тествани чрез измерване на сертифицирани референтни проби на вода и утайка, е от порядъка на 5 – 10 % отклонение от декларираното съдържание на метали. Доказано е, че съдържанието на метали (Al, Ba, Ca, Cd, Cu, Fe, Mg, Mn, Ni, Pb, Sr и Zn), измерено в гъбните тъкани, водата и утаечните проби, съответства на това от пункта за пробонабиране. Забелязва се влияние на антропогенното замърсяване от канални отпадъци в лекото завишаване на съдържанието на Al, Cu и Zn във водата, а също и на Cu, Pb и Zn в пробите от утайка. Анализите на две морфологично различни гъбични проби показват разлика в съдържанието на Cu, Zn, Pb и Cd.