NAT. CROAT. | VOL. 21 | Suppl. 1 | 36–42 | ZAGREB | September 30, 2012

SUMPORAČE – PRELIMINARY RESULTS FROM RESEARCH INTO UNIQUE ANCHIALINE SULPHUR CAVES IN CROATIA

MARIJANA CUKROV[1]*, ROMAN OZIMEC[1], NEVEN CUKROV[1,2], JANA BEDEK[1] & BRANKO JALŽIĆ[1,3]

[1]Croatian Biospeleological Society, Demetrova 1, 10000 Zagreb, Croatia
[2]Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia
[3]Croatian Natural History Museum, Demetrova 1, 10000 Zagreb, Croatia

The Sumporače are anchialine caves of the Dinaric Karst in Mokošica near Dubrovnik, and they are special due to the presence of sulphur. As a result of the sulphur, they contain ecologically very important habitats and are part of the National Ecological Network CRO NEN. Out of three known sulphur anchialine caves, two smaller (Sumporača velika and Sumporača mala) have been speleologically and biospeleologically explored, while, unfortunately, the biggest one (Mokošička špilja) was completely destroyed thirty years ago. This paper gives climate and ecological data for the cave habitats, a preliminary check list of cave fauna and the results of chemical analyses of water and surface sediments.

Key words: Dinaric Karst, cave fauna, cave ecology, cave microclimate, chemical analyses

INTRODUCTION

Three sulphur springs in Rijeka Dubrovačka (the Ombla River estuary) near Mokošica have been known since ancient times (ADAMOVIĆ, 1904), the first known records coming from 1846. CARRARA (1846) cited an unpublished manuscript of Jakov Štulić (Joakim Stulli) (1730–1817). At the beginning of the 20th century, a spa centre called Thermoterapia was built next to the two smaller springs and spring water with elevated concentrations of sulphur was used for therapeutic purposes. The entrances of those two springs (caves) were artificially expanded, probably during the construction of the spa centre. The first recorded name is garma *Smrdeč* (ADAMOVIĆ, 1904), but today these sites are known as the caves Sumporača velika (Sumporača Big Cave) and Sumporača mala (Sumporača Small Cave). The biggest known sulphur cave is Mokošička (Sumporna) špilja (the Mokošica (Sulphur) Cave), which was totally destroyed during construction works on a nearby road in the eighties. Available data suggest that Sumporače Caves were not speleologically explored until 2003, by the members of the Croatian Biospeleological Society (JALŽIĆ *et al.*, 2007).

Sumporača velika is 115 m, and Sumporača mala 37 m long and both are almost completely flooded. The water depth varies up to 2 m, and depends on the intertidal regime (Fig. 1) (JALŽIĆ *et al.*, 2007). The distance between the entrances of these two caves and the coast of the Ombla river estuary is about 20 m and there is not surface stream to the coast.

* marijana.cukrov@gmail.com

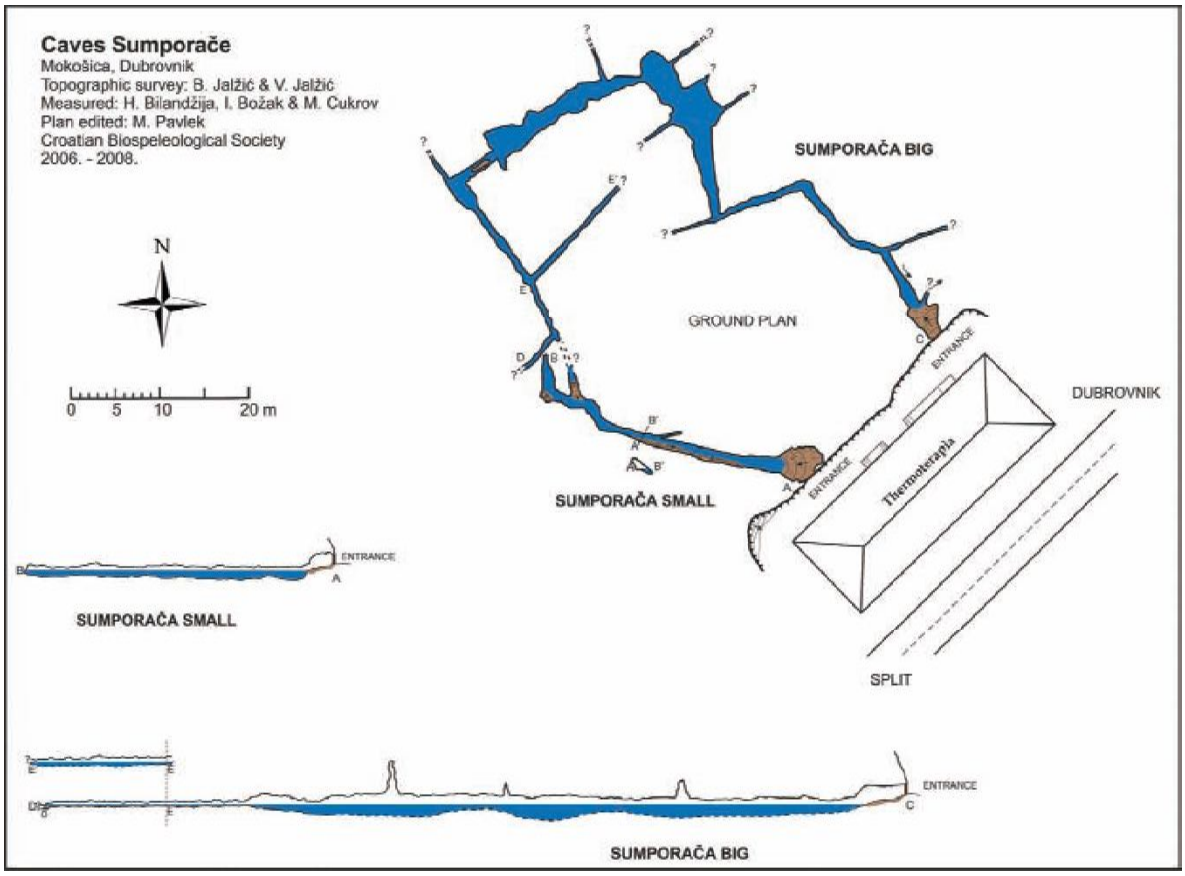

Fig. 1. Topographic plan of Sumporače Caves

Fresh water is accumulated in Cretaceous and Palaeogene limestone and efflux occurs at the contact of Eocene limestone with flysch (MARKOVIĆ, 2002).

The first chemical analyses of the spring's water were performed in 1902. The water analyses, performed in 1948, indicated mixed fresh and sea water (MARKOVIĆ, 2002).

In 1948 and 1949 bacterial colonies from the cave were investigated by academician Vale Vouk and biologist Zvonimir Devide from Zagreb, as well as by the biologists Zora Klas and Stanko Mihalić from Split and Stjepan Čanadžija from Dubrovnik. This resulted in a list of bacteria and some ecological and morphological parameters (DEVIDE, 1949; KLAS, 1949; VOUK, 1953). The sulphur bacterium *Thiophysa gigantea* Klas, 1950 was described from the springs (KLAS, 1950). The caves were biospeleologically explored by Jože Bole in the seventies and by Tonći Rađa in 1979, but the only data ever published are on terrestrial isopods (GOTTSTEIN, 2010; BEDEK *et al.*, 2011).

In Croatia, with the exception of the lake Zmajevo oko, anchialine ecosystems are represented only by caves/pits along the Adriatic coast and on the islands. To date, more than 60 anchialine caves have been recorded (CUKROV *et al.*, 2009), but only in the Sumporače Caves were elevated sulphur concentrations in the water column noted.

MATERIALS & METHODS

Speleological and cave diving techniques were used for measurements and sampling of water, surface sediment and fauna in Sumporača mala and Sumporača velika

Tab. 1. Concentrations of elements in mg/kg (unless otherwise indicated) in the surface sediments from Sumporače anchialine caves. The first line refers to the Sumporača velika, and the second to Sumporača mala.

Na (%)	Mg (%)	Al (%)	P (%)	S (%)	K (%)	Ca (%)	Ti (%)	Fe (%)
1.15	1.73	4.5	0.079	1.152	0.87	1.71	0.01	3.55
1.47	2.6	3.64	0.064	0.322	0.79	6.98	< 0.01	3.9
Hg	**Cd**	**Pb**	**Cu**	**Zn**	**Cr**	**Co**	**As**	**Ni**
0.293	0.44	23	77.4	133	234	14.5	19.2	221
0.316	0.23	21.9	60.2	119	194	18.2	9.8	225
Li	**Be**	**B**	**V**	**Mn**	**Ag**	**Au**	**Th**	**U**
71.5	1.6	44	126	215	0.397	0.0297	3.2	3.8
55.6	1.4	37	83	381	0.079	0.0243	3.5	1.3
Zr	**Sc**	**Pr**	**Gd**	**Dy**	**Ho**	**Er**	**Tm**	**Nb**
2.6	9	4.6	3.7	2.9	0.6	1.3	0.2	1.3
3.2	9.1	4.3	3.3	2.65	0.5	1.2	0.2	0.7
Se	**Rb**	**Sr**	**Y**	**Mo**	**Tl**	**Ga**	**Ge**	**Bi**
11.4	70.1	57.6	17.2	8.41	1.61	14.2	0.2	0.33
1.9	53.7	164	13.3	1.21	0.5	10.1	0.2	0.23
In	**Sn**	**Sb**	**Te**	**Cs**	**Ba**	**La**	**Ce**	**Nd**
0.07	9.19	0.1	0.05	12.5	37.3	16.7	29.4	17.9
0.05	7.81	0.07	0.08	5.95	44.5	15.4	30.3	16.1
Hf	**Ta**	**W**	**Re**	**Sm**	**Eu**	**Tb**	**Yb**	**Lu**
< 0.1	< 0.05	< 0.1	0.01	3.3	0.8	0.5	1.2	0.1
< 0.1	< 0.05	< 0.1	0.002	3.3	0.8	0.5	0.9	0.1

caves. For chemical analyses waters and sediments were sampled only once, in September 2010, while fauna of the caves was collected on several occasions in the period 2004 – 2010. Air temperature and humidity were measured with a Mini Thermometer TESTO H1 and a Kestrel 3000, while the water parameters (temperature, salinity, dissolved oxygen concentrations, pH and redox potential) were determined *in situ* with a Hach Lange HQ40D Multimeter. CO_2 concentrations were measured with a Telaire 7001.

Electrochemical determinations of total reduced sulphur species (RSS) were made by cathodic stripping linear sweep voltammetry (CSLSV) in unfiltered samples (BURA-NAKIĆ *et al.*, 2009), while concentrations of SAS were determined by AC voltammetry, as described elsewhere (CUKROV *et al.*, 2012). Total concentrations of trace metals in water samples were measured by stripping voltammetry, as described earlier (CUCULIĆ *et al.*, 2009; 2011). Chemical analyses of sediments were performed in the ACTLABS (www.actlabs.com), Ontario, Canada, by ICP, ICP/MS and cold vapour AAS (Hg). The sample material was digested with aqua regia. Fauna in caves were collected using traps with attractants and with tweezers, and material was preserved with 75% ethanol.

RESULTS AND DISCUSSION

According to GOTTSTEIN (2010) Sumporače belong to hydrothermal sulphuric caves (Pal. Class. 65.44; NKS. H.1.5.1). The air temperature in them varied seasonally

Tab. 2. The list of recorded taxa in Sumporača mala and Sumporača velika caves with ecological notes: TB/SB – troglo/stygobiont; TF/SF – troglo/stygophile; TX/SX: troglo/stygoxene

CLASS (ORDER)	TAXA	Endemics	Ecology	Source
Regnum Prokaryota (Bacteria)				
Gamma Proteobacteria (Thiotrichales)	*Beggiatoa alba* (Vaucher) Trevisan 1845		Sulphur springs	Klas, 1949
	Beggiatoa arachnoidea (Agardh) Rabenhorst 1865		Sulphur springs	Klas, 1949
	Beggiatoa mirabilis Cohn, 1865		Sulphur springs	Klas, 1949
	Beggiatoa gigantea Klas, 1937	Dinaric Karst	Sulphur springs	Klas, 1949
	Thiothrix nivea (Rabenhorst) Winogradsky 1888		Sulphur springs	Klas, 1949
	Thiothrix longiarticulata Klas, 1936	Dinaric Karst	Sulphur springs	Klas, 1949
	Thiothrix voukii Klas, 1936	Dinaric Karst	Sulphur springs	Klas, 1949
	Thiophysa macrophysa Nadson, 1913		Sulphur springs	Klas, 1949
	Thiophysa volutans Hinze, 1903		Sulphur springs	Klas, 1949
	Thiophysa gigantea Klas, 1951	Sumporače caves	Sulphur springs	Klas, 1949
	Thiovulum muelleri (Warming) Lauterborn 1916		Sulphur springs	Devide, 1949
	Thiospira sp.	?	?	Klas, 1949
Regnum Protoctista (Protista)				
(Tubulinida)	*Gen/sp.*	?	?	Klas, 1949
Regnum Animalia				
Secernentea (Rhabditida)	*Gen/sp.*	?	?	Klas, 1949
Gastropoda (Pulmonata)	*Ovatella* sp.	?	SF	Jalžić *et al.*, 2007
Gastropoda (Pulmonata)	*Vitrea* sp.	?	TF/TX	CBSS collection
Gastropoda	in. det.	?	SF	CBSS collection
Arachnida (Araneae)	*Pholcus* sp. – in det.	?	TF/TX	CBSS collection (det. M. Pavlek)
Arachnida (Araneae)	*Nesticus* sp. – in det.	?	TF/TX	CBSS collection (det. M. Pavlek)
Arachnida (Pseudoscorpiones)	*Chthonius densedentatus* Beier, 1938	Dinaric Karst	TB	CBSS collection (det. R. Ozimec)

CLASS (ORDER)	TAXA	Endemics	Ecology	Source
Arachnida (Pseudoscorpiones)	*Roncus dalmatinus* Hadži, 1933	Dinaric Karst	TF	CBSS collection (det. R. Ozimec)
Arachnida (Oribatida)	*Galumna* sp.	?	TF/TX	CBSS collection (det. R. Ozimec)
Maxillopoda (Harpactoida)	in. det.	?	?	CBSS collection
Malacostraca (Isopoda)	*Chaetophiloscia* sp.	?	TF/TX	CBSS collection (det. J. Bedek)
Malacostraca (Isopoda)	*Buddelundiella cataractae* Verhoeff, 1930		TF	CBSS collection (det. J. Bedek)
Malacostraca (Isopoda)	*Trichoniscus matulici* Verhoeff, 1931		TF	CBSS collection (det. J. Bedek)
Malacostraca (Isopoda)	Haplophthalminae – in. det.		?	CBSS collection (det. J. Bedek)
Malacostraca (Isopoda)	*Halophiloscia* sp.		TX	CBSS collection (det. J. Bedek)
Malacostraca (Isopoda)	*Jaera* sp.	?	SF	CBSS collection (det. J. Bedek)
Entognatha (Collembola)	*Heteromurus nitidus* (Templeton, 1935)		TF	CBSS collection (det. M. Lukić)
Entognatha (Collembola)	*Lepidocyrtus* sp.	?	TX	CBSS collection (det. M. Lukić)
Entognatha (Collembola)	Neelidae – in. det.	?	TF/TX	CBSS collection (det. M. Lukić)
Entognatha (Collembola)	Hypogastruridae – in. det.	?	TX	CBSS collection (det. M. Lukić)
Chilopoda (Lithobiomorpha)	*Lithobius* sp. – in det.	?	TF/TX	CBSS collection (det. R. Ozimec)
Symphyla	in. det.	?	TF/TX	CBSS collection
Diplopoda	In. det.	?	TF/TX	CBSS collection
Actinopterygii (Anguilliformes)	*Anguilla anguilla* (Linnaeus, 1758)		SF/SX	CBSS collection (det. R. Ozimec)

between 16 to 21°C, which is higher than in other caves from this area. Humidity was also high and varied between 97 and 100%. A high concentration of CO_2 (> 8000 ppm) was recorded in Sumporače caves.

Salinity measurements in cave waters confirm their subterranean connection with the sea, with no tidal delay. Cave water temperatures varied seasonally from 16 to 20° C. The salinity (PSS-78) within the water column ranged from 16.8 at the surface to 26.9 at the bottom layer, while concentrations of dissolved oxygen varied from 0.99 mg/L at the surface to 0.11 mg/L at the bottom. The pH varied from 7.22 at the surface to 7.33 at the bottom, while redox potential from 25.6 mV at the surface to –1.9 mV at the bottom.

The concentration of total reduced sulphur species (RSS) measured in the Sumporača cave samples was $2.2x10^{-7}$ mol/L, while concentration of surface active substances was 0.08 mg/L (expressed in T-X-100 equivalent). Concentrations of sulphur of 11.52 g/kg were found in the surface sediment layer of Sumporača velika, while in Sumporača mala these concentrations were 3.22 g/kg. Concentrations of 62 other elements are presented in Table 1. Sediments from Sumporača mala have more Ca, Mg and less Al indicating higher content of carbonates. However, ecotoxic metal concentrations (Hg, Cd, Pb, Cu, Zn, Ni and Cr) were similar in both caves (Tab. 1). The concentrations of trace metals in the cave waters ranged between: cadmium (0.011–0.037 μg/L), lead (0.11–2.21 μg/L), copper (0.19–11.7 μg/L) and zinc (0.42–7.71 μg/L). Elevated concentrations of some ecotoxic metals (Hg, Cu, Cr and As) in the sediments of the Sumporače anchialine caves may present some threat to cave organisms.

The collected specimens of cave fauna are still in the process of identification. The majority of the recorded fauna are troglophiles and trogloxenes, due to the very limited terrestrial habitats and unfavourable environmental conditions. The only feeble efforts made to collect fauna in these aquatic habitats might possibly contribute to this. A full list of collected organisms is presented in Tab. 2.

CONCLUSIONS

In Croatia, with the exception of the lake Zmajevo oko, anchialine ecosystems are present only in caves along the Adriatic coast and on the islands. To date, more than 60 anchialine caves have been recorded, but only in the three of them, Sumporače Caves, were elevated sulphur concentrations registered within the water column. This makes them a unique anchialine ecosystem in Croatia. The connection with the estuarine waters of the Ombla River was confirmed by salinity measurements, and is also supported by the observed intertidal regime. These caves are situated in an urban zone, and the anthropogenic influence was confirmed by elevated concentrations of metals in the water columns and surface sediments. The biggest cave is already destroyed and even small action can cause damage to or even destruction of the rest of these unique ecosystems. More than 20 taxa were recorded, with several troglobitic and endemic species.

ACKNOWLEDGEMENTS

Our thanks are due to Helena Bilandžija, Elvira Bura Nakić, Hrvoje Cvitanović, Ivan Grabar, Vedran Jalžić, Ana Komeričcki, Petra Kovač Konrad, Kazimir Miculinić

and Predrag Rade for help in these researches. We are grateful to Marko Lukić for Collembola and Martina Pavlek for the Aranea identified material. We gratefully acknowledge the financial support of the city of Dubrovnik.

REFERENCES

ADAMOVIĆ, V., 1904: Rijeka – topografično-povjesničke bilješke, Srpska Dubrovačka Biblioteka br. 6, 1–40, Dubrovnik. (In Croatian)

BEDEK, J., S. TAITI, & S. GOTTSTEIN, 2011: Catalogue and atlas of cave-dwelling terrestrial isopods (Crustacea: Oniscidea) from Croatia. Nat. Croat. **20**, 237–354.

BURA-NAKIĆ, E., HELZ, G. R., CIGLENEČKI, I. & B. ĆOSOVIĆ, 2009: Reduced sulfur species in a stratified seawater lake (Rogoznica Lake, Croatia); seasonal variations and argument for organic carriers of reactive sulfur. Geochim. et Cosmochim. Acta, **73** (13), 3738–3751.

CARRARA, F., 1846: La Dalmazia descritta. Reprint Etnografski muzej Split, 2006, pp. 192 (in Croatian)

CUCULIĆ, V., CUKROV, N., KWOKAL, Ž. & M. MLAKAR, 2009: Natural and anthropogenic sources of Hg, Cd, Pb, Cu and Zn in seawater and sediment of Mljet National Park, Croatia. Estuarine, Coastal and Shelf Science, 81, 311–320.

CUCULIĆ, V., CUKROV, N., KWOKAL, Ž. & M. MLAKAR, 2011: Distribution of trace metals in anchialine caves of Adriatic Sea, Croatia. Estuarine, Coastal and Shelf Science, 95, 253–263.

CUKROV, N., JALŽIĆ, B., BILANDŽIJA, H. & M. CUKROV, 2009: Research history and anchialine cave characteristic in Croatia. Anchialine ecosystems: Reflection and prospects, Abstracts, Palma de Mallorca, 19–20.

CUKROV, N., TEPIĆ, N., OMANOVIĆ, D., LOJEN, S., BURA-NAKIĆ, E., VOJVODIĆ, V. & I. PIŽETA, 2012: Qualitative interpretation of physico-chemical and isotopic parameters in the Krka River (Croatia) assessed by multivariate statistical analysis. International Journal of Environmental Analytical Chemistry, DOI: 10.1080/03067319.2010.550003.

DEVIDE, Z., 1949: Izvještaj o citološkim istraživanjima leukobakterija, Ljetopis JAZU, 55, 77–85, Zagreb. (In Croatian)

GOTTSTEIN, S., 2010: Priručnik za određivanje podzemnih staništa u Hrvatskoj prema direktivi o staništima EU. Državni zavod za zaštitu prirode, Zagreb, pp. 99. (In Croatian)

JALŽIĆ, B., CUKROV, M., JALŽIĆ, V. & H. BILANDŽIJA, 2007: Špilje Sumporače kod Dubrovnika, Speleolog, 60–67. (In Croatian)

KLAS, Z., 1949: Izvještaj o istraživanju sastava vegetacije sumpornog vela Mokošice kod Dubrovnika, Ljetopis JAZU, 55, 145–148, Zagreb. (In Croatian)

KLAS, Z., 1950: *Thiophysa gigantea*, ein neues thiobacterium der Adriatischen küste. JAZU 280, 5–15. (In German)

MARKOVIĆ, S., 2002: Hrvatske mineralne sirovine, Zavod za geologiju, Zagreb, pp. 544. (In Croatian)

VOUK, V., 1953: Izvještaj o balneobiološkim istraživanjima u Ulcinju, Igalu i Mokošici, Ljetopis JAZU, 57, 233–234, Zagreb. (In Croatian)