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LAMPENFLORA AND THE ENTRANCE BIOFILM IN TWO SHOW CAVES: COMPARISON OF MICROBIAL COMMUNITY, ENVIRONMENTAL, AND BIOFILM PARAMETERS

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Abstract

Phototrophic microorganisms from two caves in Serbia (Podpeć and Stopić) were examined. Samples were taken from the entrances where natural light was present, as well as from the inside caves near artificial light (lampenflora community). *Cyanobacteria*, *Chlorophyta*, *Bacillariophyta* and *Xanthophyta* were documented, with 51 taxa in total. The highest number of taxa recorded in the *Cyanobacteria* were coccoid cyanobacteria; *Gleocapsa* and *Chroococcus* were dominant. According to the redundancy analysis (RDA), *Cyanobacteria* were dominant at cave entrances while other groups (*Chlorophyta*, *Bacillariophyta* and *Xanthophyta*) were documented in lampenflora samples. Temperature, relative humidity, and light intensity were measured, as well as chlorophyll *a* concentrations and biofilm parameters (water, organic and inorganic matter content). Ecological parameters did not show significant variation, while light intensity depended on the position of sampling sites. RDA showed that the water content was higher in biofilm samples from cave entrances, while levels of inorganic matter were increased in lampenflora samples. The concentration of chlorophyll *a* did not show significant correlations with any of the measured ecological or biofilm parameters. Although the ecological parameters inside the cave did not show significant variation, they should be monitored because of the potential influence on the development of the lampenflora community that has a negative aesthetic impact on cave formations.

Introduction

Despite their extreme conditions, caves are unusual ecosystems inhabited by unique organisms. On the inside of caves, light intensity (LI) decreases as we go further away from the entrance, while the temperature (T) and air humidity show little or no variation, but the cave's entrance is under the influence of temperature and humidity from the outside environment (Hajdu, 1977; Vinogradova et al. 1998; Pedersen, 2000). In isolated and extreme environments, such as caves, specific organisms from different groups can be found (bacteria, cyanobacteria, algae, fungi, mosses, lichens, invertebrates, and vertebrates) (Mulec et al., 2008; Mulec and Kosi, 2009; Cerwik-Marcinkovska, 2013). Due to their natural beauty, caves are often open to the public, which can lead to the disturbance of the stable conditions in these habitats. The installation of artificial light in caves is the main reason for cave substrate colonization by phototrophic organisms. The lights change the values of the temperature and air humidity, which affects the rock surface the phototrophs' development (Mulec and Kosi, 2009). This phototrophic community is called *lampenflora* or lamp flora and includes many organisms, such as bacteria, cyanobacteria, algae, mosses, fungi and lichens (Dobat, 1998; Mazina and Severin, 2007). Lampenflora may include "r-selected species" and fast-growing species often capable of tolerating lower temperature (T), relative humidity (RH) and nutrient input (Aleya, 1991; Borderieet al., 2014). Biochemical deterioration (also known as biocorrosion) of the substratum is caused by the metabolic processes of the microorganisms (Macedo et al., 2009). During the respiration process, microorganisms release CO_2, which when mixed with the surrounding water, produces the carbonic acid that causes the biodeterioration of cave structures (Aleya, 1991; Macedo et al., 2009; Borderie et al., 2014). According to Jurado et al. (2010) communities of these organisms can include potentially toxic or pathogenic microorganisms that represent a potential danger for animals and humans, and species whose physiology is still unknown.

The study of the diversity and growth control of lampenflora communities in tourist caves seems particularly important. In the Republic of Serbia, the pioneering study was performed by Popović et al. (2015a, b; 2016a, b, c; 2017a, b). Similar research endeavors in the region were conducted in the Republic of Slovenia (Klemenčić and Vrhovšek, 2005; Mulec and Kosi, 2008, 2009; Mulec et al., 2008, 2012) the Republic of Croatia (Ercegović 1925, 1932; Golubić, 1967) and the Republic of Macedonia (Tofilovska et al., 2014).

The aim of this study was to investigate the diversity of cyanobacteria and algae in two tourist caves, Podpeć and Stopić, in Serbia, and to compare the microbial communities from cave entrances with those deeper inside the caves

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from typical lampenflora. Additionally, environmental parameters were measured. Moreover, primary production and biofilm parameters (water, organic and inorganic matter content) were measured and compared between entrance biofilms and lampenflora.

Materials and Methods

Sampling Sites

Podpeć Cave is located in Western Serbia, in the village of Potpeće, 186 km from Belgrade (43°47'45.1"N 19°56'00.8"E). The cave entrance has a horseshoe shape 50 m height with a 22 m wide arch. The width at the base is 12 m. This cave was formed by the Petnica River as it sunk in Drežnička Valley and flowed through 5 km long underground streams. According to Cvijić (1914a), limestone rocks in this area were formed in the Middle Trias and are characterized by whitish colors, cracked porosity, and a mosaic structure (Marković, 1957). The cave has two levels of passages, older – the Upper Cave, and younger – the Lower Cave. The portion of the cave that is developed for visitors, with paths and artificial light, is 555 m long.

Stopić Cave is located on the northeastern side of Zlatibor Mountain, 250 km from Belgrade (43°42'12.0"N 19°51'12.4"E). The cave entrance is located on the right side of the Prištavica River, 711 mabove sea level. It is 35 m wide and 18 m high.The explored part of the cave is 2000 m long and in some places the ceiling is 50 m high. The limestone rocks were formed in the Middle Trias and are over 100 m thick (Cvijić, 1914b). The unique characteristics of the cave are rimstone pools formed by deposited limestone and an underground waterfall called “The Source of Life” formed by Trnavski stream that flows through the cave (Lazarević, 2012).The portion of the cave developed for tourists is several hundred meters long, and 1658 m of the cave system has been explored, so far. Locations of Stopić and Podpeć caves are presented in Figure 1.

For algological analyses, five sampling sites in Podpeć Cave were chosen. One sampling site was at the entrance (P5) and four sampling sites were chosen inside the cave (P1, P2, P3,P4). Six sampling sites were chosen in Stopić Cave, three inside (S1, S2, S3) and three at the entrance to the cave (S4, S5, S6). Sampling sites of the two investigated caves are presented in Figures 2 and 3. The sampling sites are shown in Figures 4 and 5. Samples were collected in July 2016.

Environmental Parameters

Environmental parameters were measured *in situ* using the DMV 1300 Luxmeter (Vellemarn, Belgium) for Light Intensity, and Temperature humidity meter (Extech, USA) for those parameters. These parameters were measured five times for each site and the mean values were calculated.

Biofilm Analyses

The samples for measuring the biofilm content (water content (WC), organic matter (OM) and inorganic matter (IM), and chlorophyll *a* (Chl *a*) extraction were scraped from stone substrata using a round metal moldcovering asurface area of 3.14 cm^2.

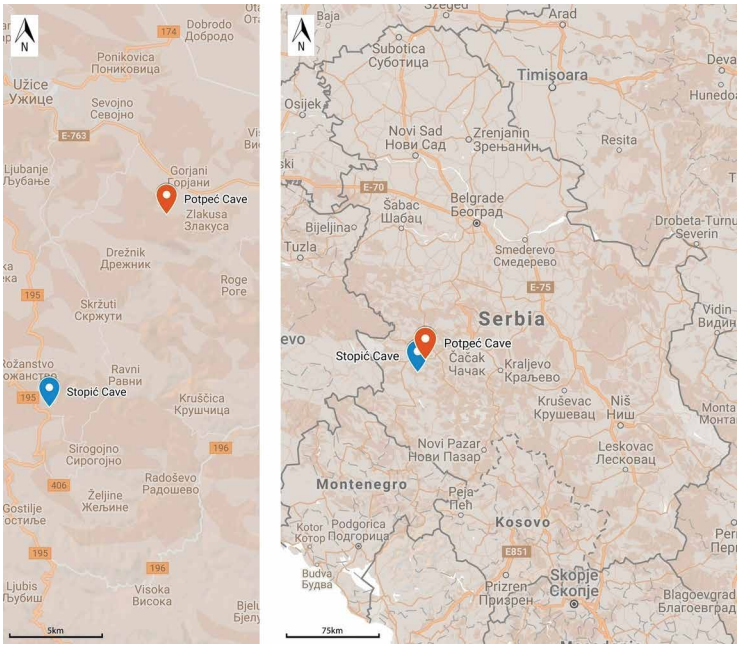

Figure 1. Maps of the two investigated caves: Stopić and Podpeć. Sources: Google Maps, http://www.clipart-library.com.

The WC, OM and IM content was calculated as the difference between the biofilm weight before and after drying at 105 ºC and after ashing at 550 ºC (Popović et al., 2017a). The WC was measured as the difference between the fresh and dried weight. OM was calculated as the weight difference between the dried and the ashed biofilm, while IM content was equal to the weight of the remains after the biofilm was ashed. All three parameters were expressed in two ways, as a quantity per surface area (mg/cm^2) and as a percentage of each constituent in a biofilm sample. The concentration of Chl *a* was determined by spectrophotometry using a formula described in Popović et al. (2015a) and expressed as µg/cm^2.

Qualitative analyses of algae were performed by using the non-destructive adhesive tape method (Urzi and de Leo, 2001) and by scraping the biofilm with a sterilized scalpel. The samples were fixed with a drop of glycerol and observed on a Zeiss Axio- Imager M1 light microscope with Axio Vision 4.8 software.

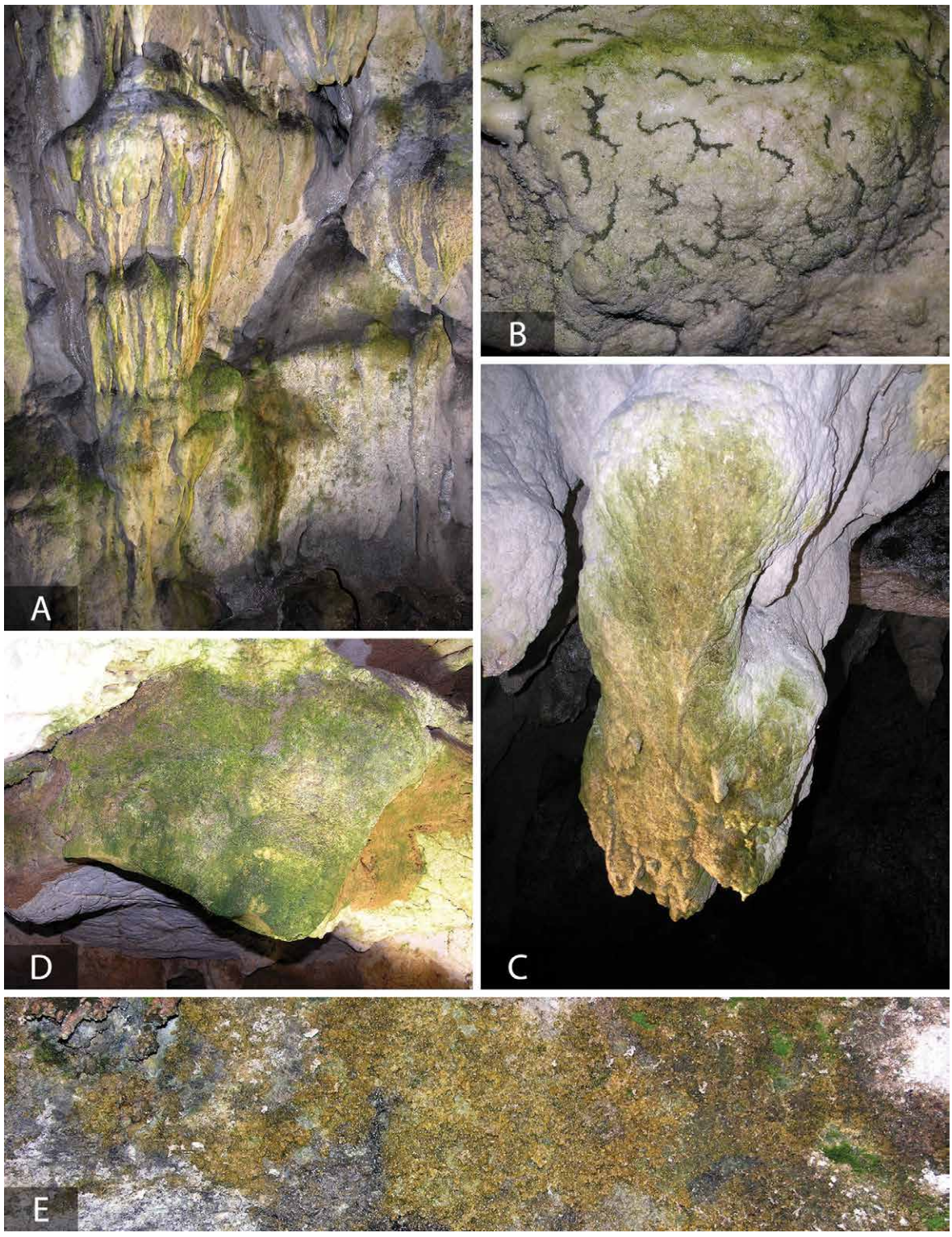

Figure 2. Podpeć cave, sampling sites: A–D – inside the cave: A – site P1; B – site P2; C – site P4; D – site P3; E – entrance of the cave, site P5.

Cyanobacteria and algae were identified using standard identification keys (Ettl, 1978; Komárek and Fott, 1983; Ettl and Gärtner, 1988; Komárek and Anagnostidis, 1998, 2005; John et al., 2003; Hoffman et al. 2013; Komárek, 2013).

Statistical Analyses

The redundancy analysis (RDA) was performed using the program CANOCO for Windows, Version 5.0 (Ter Braak and Šmilauer, 2012). The presence/absence of all recorded taxa was imported into the program and each taxon was assigned to its larger taxonomic group: *Cyanobacteria* (divided into *Chroococcales*, *Oscillatoriales* and *Nostocales*), *Chlorophyta*, *Bacillariophyta* and *Xanthophyta*. These six larger taxonomic groups were used instead of the individual genera and species that were identified. RDAwas performed to demonstrate the preference of each groupinone of two communities: lampenflora and at the cave entrances. The nominal variables, lampenflora and cave entrance, were used as explanatory variables. The WC and content of (OM) and (IM) in biofilms (expressed as percentages) were included as supplementary variables.

XLSTAT addition in Excel was used to calculatethe correlation between recorded physical parameters (T, RH, LI), Chl *a* and biofilm parameters (WC, OM and IM, expressed as μg/cm^2).

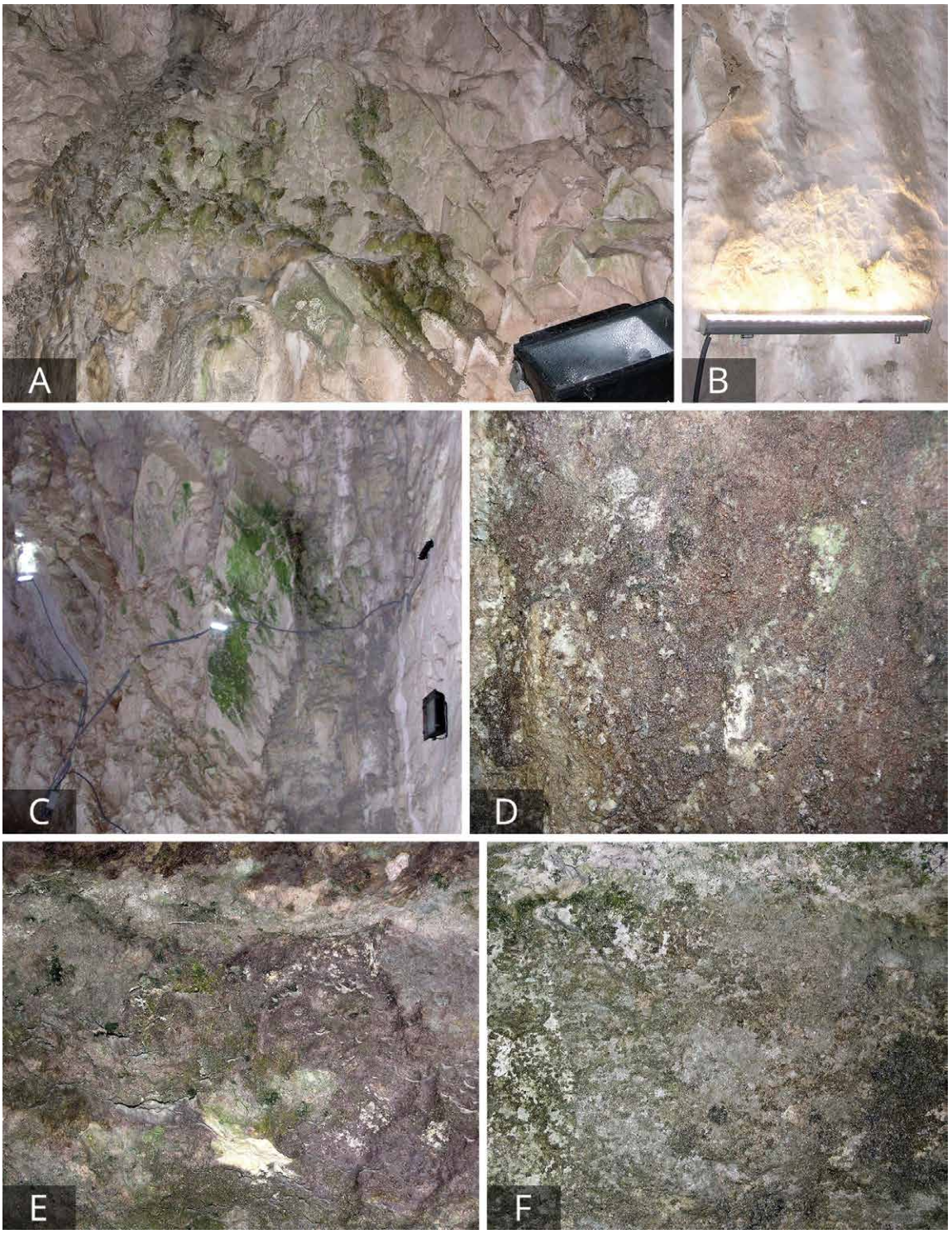

Figure 3. Stopić Cave, sampling sites: A–C – inside the cave: A – site S1; B – site S2; C – site S3; D–F – entrance of the cave: D – site S4; E – site S5; F – site S6.

Results

The ecological parameters measured in the Podpeć (P1, P2, P3 and P4 inside the cave, P5 at the entrance) and Stopić caves (S1, S2, S3 inside the cave, S4, S5, S6 at the entrance of the cave) are presented in Figure 6. For both caves, the ecological parameters (T and RH) showed a certain degree of variation, but only LI values showed notable differences among sampling sites, as well as between caves. The highest temperature was measured at P5 (22 ºC), while the lowest was at P2 (15.3 ºC) in Podpeć Cave, while in Stopić Cave the highest temperature was at S2 (19.9 ºC) and the lowest was at S3 (16.3ºC). RH varied from the lowest value of 56 % at S2 to the highest value of 78 % at sampling sites S5 and S6 in Stopić Cave. The highest value of relative humidity in Podpeć Cave was at the point P5 (85%), while the lowest was at P1 (58%). Between the sites inside Podpeć Cave, T varied up to 5 °C, RH varied up to 12 %, while between the sites inside Stopić Cave, T varied up to 3.6 °C while RH varied up to 19 %. Outside sampling points in Podpeć Cave had higher T and RH than inside the cave, while at the entrance of Stopić Cave, values of the T and RH were similar to those measured inside the cave. The lowest value of LI was 4 lux at sampling site S3 and the highest value was 3100 Lux at sampling site S2 in Stopić Cave. In Podpeć Cave, the highest value of LI was 840 Lux at point P4, while the lowest value of 265 Lux was at sampling site P2 .

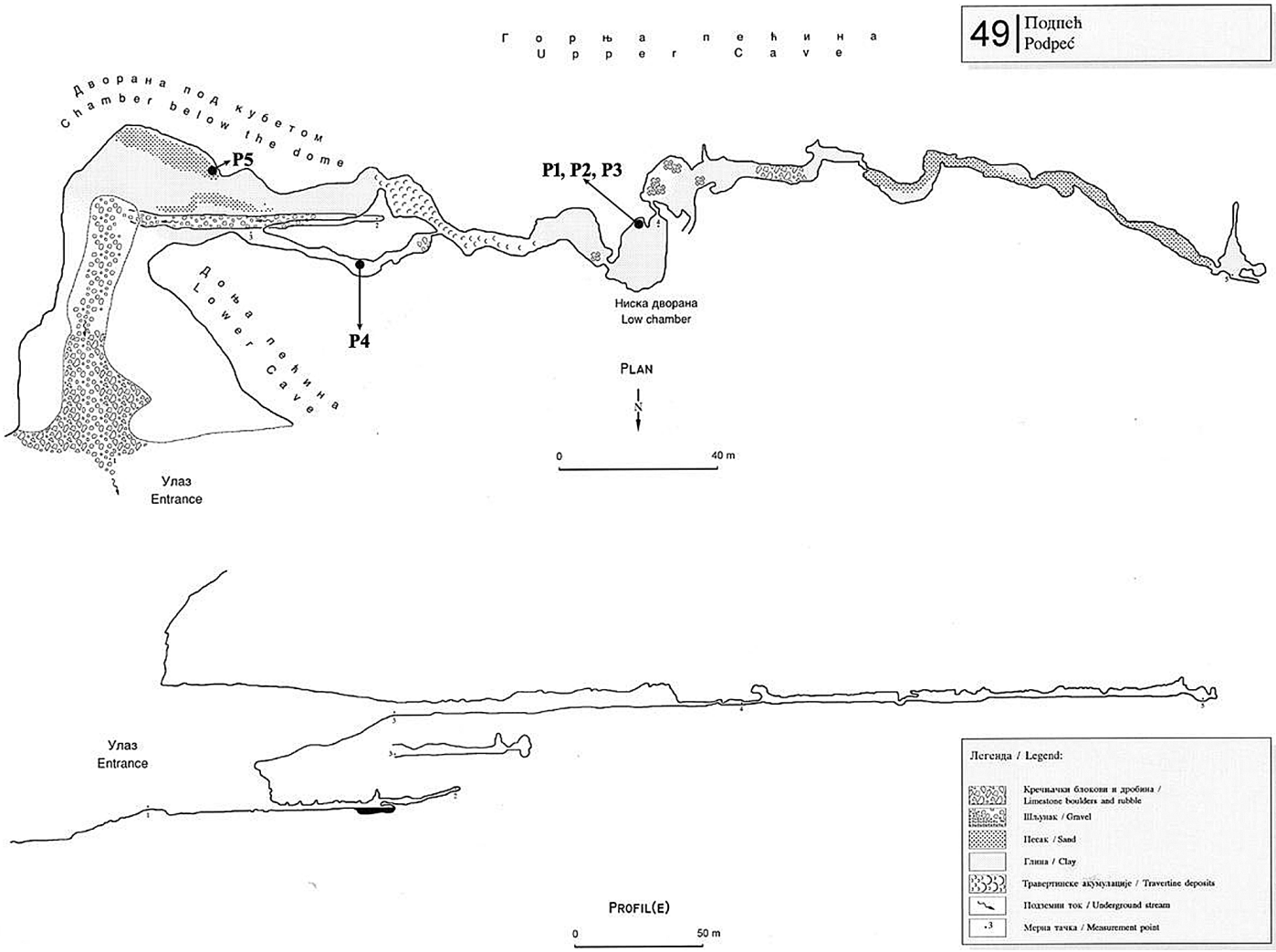

Figure 4. Map of the Podpeć Cave, sampling sites; P1−P4 – sampling sites inside the cave, P5 – sampling site at the entrance. Source: Speleological atlas of Serbia(1998).

Biofilm parameters (WC, OM and IM) expressed as mg/cm^2 depended on the position of the sampling site (Fig.7a). In Podpeć Cave, the (WC) was lowest at sampling site P4 and highest at P5. The OM content was also highest at P5 and very low at P1 and P2. P2 was also characterized by the lowest content of IM, while the highest value for this parameter in this cave was at sampling sites P3 and P5. In Stopić Cave, WC was highest at the site positioned at the cave entrance (S6) and lowest at S1 and S2 (lampenflora samples). The OM content was the highest at S6 and lowest at sampling sites S2 and S3. The IM content had the lowest measured value at sampling site S1 and the highest at site S5.

Figure 7b presents WC, OM and IM expressed in percentages for all biofilm samples. In Podpeć Cave, the highest WC percentage was found in the biofilms from sampling sites P2 and P5, while the lowest was found at sampling site P4. The highest percentage of OM was also recorded at P5 and the lowest at sampling site P1. On the other hand, IM had the highest value at sampling site P1, while the lowest was at P5 in Podpeć Cave. In Stopić Cave, the highest value of WC and IM was measured at sampling sites S6 and S3, respectively, while OM was highest at point S6. The lowest values of WC, OM and IM in Stopić Cave were found at sampling sites S2, S5 and S6, respectively.

The lowest concentration of Chl *a* expressed as µg/cm^2 was documented at sampling sites S3 and S1 in Stopić Cave and P2 in Podpeć Cave, while the highest values of Chl *a* were at P3 and S6 (Fig.7a).

Correlations between T, RH, LI, Chl *a*, WC, OM and IM were performedusing Pearson's coefficient (Table 1). It appears that Chl *a* does not show significant correlation, positive or negative, with any of the listed parameters. T and RH were significantly positively correlated with both WC and OM.

In total, 51 taxa were documented from the two caves (Table 2). The highest number of taxa belonged to *Cyanobacteria* (44) while the remaining taxa belonged to *Bacillariophyta* (4), *Chlorophyta* (2) and *Xanthophyta* (1). Considering the caves separately, 39 taxa were recorded in the*Cyanobacteria* division in Stopić Cave and 22 taxa were found in

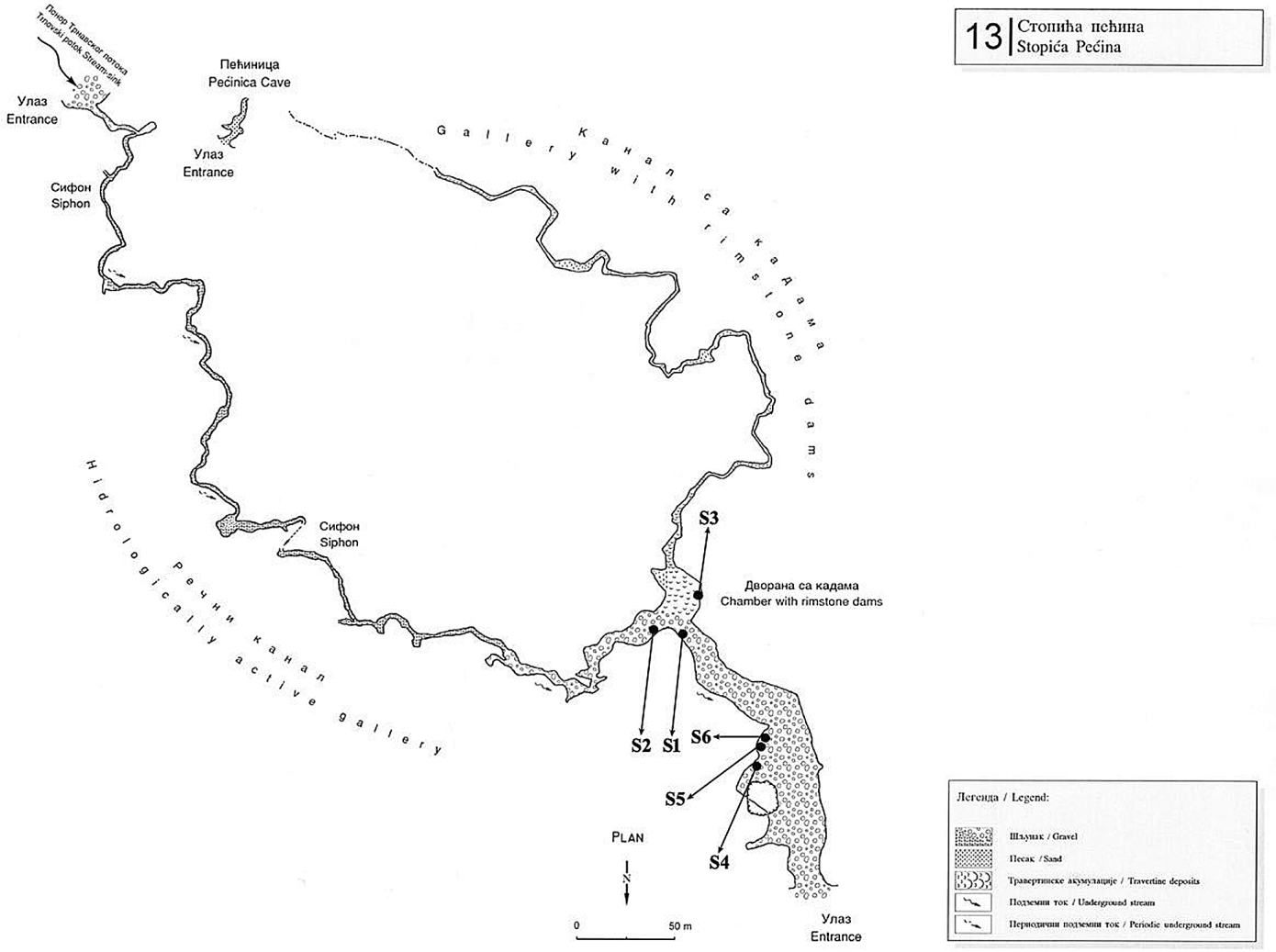

Figure 5. Map of Stopić Cave, sampling sites; S1−S3 – sampling sites inside of cave, S4−S6 – sampling sites at the entrance. Source: Speleological atlas of Serbia (1998).

Podpeć Cave. Stopić Cave was characterized by the presence of all four recorded *Bacillariophyta* taxa, while only one was documented in Podpeć Cave. Representatives of *Chlorophyta* and *Xanthophyta* were recorded in both caves. The most diverse cyanobacterial group was *Chroococcales* in both caves, where taxa of the genus *Gleocapsa* and *Chroococcus* dominate.The other two cyanobacterial groups, *Oscillatoriales* and *Nostocales*, were more numerous in Stopić Cave than in Podpeć Cave. *Asterocapsa* spp., *Chrococcus ercegovicii*, *Leptolyngbia foveolarum*, *Leptolyngbia* sp1, *Leptolyngbia* sp2, *Nostoc punctiforme* and an unknown taxon that belonged to *Xanthophyta* were present in all three sampling sites at the entrance of Stopić Cave, while inside the cave, the green algae cf. *Chlorella* sp. was dominant. *Leptolyngbia foveolarum* was the only cyanobacterial taxon found inside Stopić Cave. In Podpeć Cave, the green algae cf. *Chlorella* sp. and *Humidophila* sp. were found at the majority of the sampling sites inside the cave. At the cave entrance, *Cyanobacteria* were dominant. *Gleocapsa atrata* and *Leptolyngbia foveolarum* were the only taxa found inside Podpeć Cave. Two representatives of *Chlorophyta* have been recorded (*Chlorella* sp. and *Trochiscia* sp.) at every sampling site inside the caves (Table 2).

RDA analysis included nominal variables, lampenflora and communities at cave entrances, as the explanatory variables, and algal groups (*Cyanobacteria*– *Chroococcales*, *Oscillatoriales* and *Nostocales*, *Chlorophyta*, *Bacillariophyta* and *Xanthophyta*) as response data (Fig.8). The first RDA axis explained 58.8 % of the variability in our data. Nominal variable, Lampenflora, was placed on the left side of the ordination diagram (R = −0.9492) and the nominal variable, Cave entrance,on the right (R = 0.9492). The first axis represents the variation in microorganism assemblages between the two nominal variables. *Bacillariophyta* and *Chlorophyta*, as well as *Xanthophyta*, were dominant in lampenflora samples, while all three cyanobacterial groups were mostly documented in the biofilm samples taken at cave entrances. Supplementary variables show that the levels of IM in biofilms were higher in lampenflora samples, while the WC was higher in the biofilm samples at cave entrances (Fig.8).

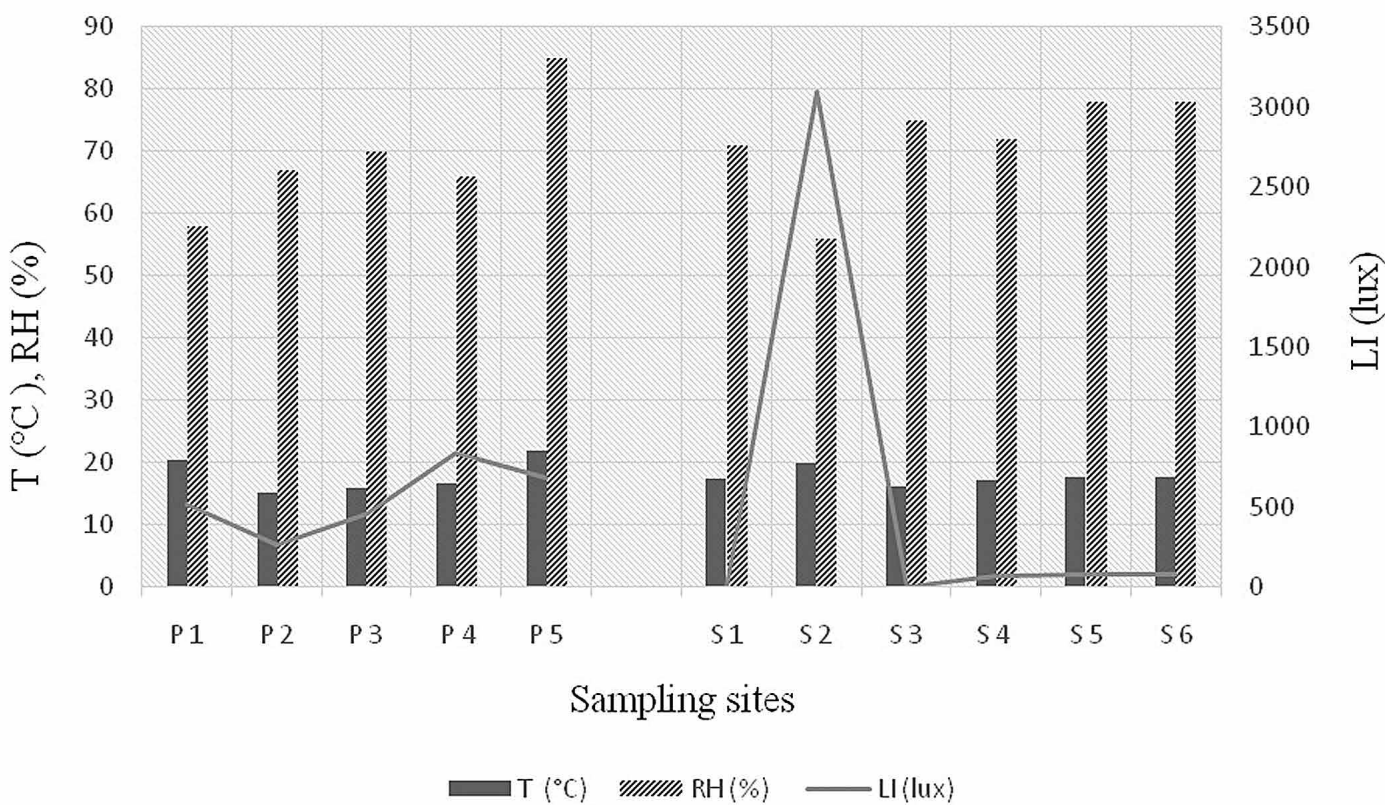

Figure 6. Values of ecological parameters: temperature (T in ºC), relative humidity (RH in %), light intesity (LI in Lux) at sampling sites from Podpeć (P1–P5) and Stopić (S1–S6) caves.

Discussion

At the entrance of caves, the influence of the outside climate (T fluctuation, LI, water regime, and UV radiation) is evident, especially when T and RH are considered (Pentecost and Whitton, 2012). In Podpeć Cave, T and RH values differed at P5 compared to the rest of the sampling sites. At this site, located at the cave entrance, the highest value of T was measured, which coincides with the results by Popović et al. (2015a) and Cennamo et al. (2012). It should also be mentioned that the season in which the sampling was conducted also played a role. At the entrance of Stopić Cave, T and RH did not vary much from site to site and their values were similar to those measured inside the cave, probably due to the morphology of the cave and the big entrance zone which is influenced more by external conditions compared to Podpeć Cave. The LI at the entrance varied among sampling sites of both caves and depended on many factors, such as the size of the cave entrance, the presence/absence of vegetation, and exposure of the sampling sites. (Popović et al, 2017a).

According to Czerwik-Marcinkowska and Mrozińska (2011), T and RH are relatively stable inside caves; the temperature inside the caves of Central Europe ranges between 5 ºC and 8 ºC, while RH is between 85 % and 95 %. However, other authors mentioned (Smith and Olson, 2007; Mulec and Kosi, 2009) that the introduction and installation of artificial light (especially warm light) and the presence of cave tourists, can have a negative impact on the microclimate and can influence changes in T and RH. According to the information provided by guides in caves, the T in Stopić Cave vary from 9.5 °C to 18 °C and the lowest RH measured was 87 % and which becomes higher depending on the season. In Podpeć Cave, T values were between 9 °C and 10.2 °C, and had RH values of 94 % and higher.

Our samples were collected in the summer; however, in both caves, the measured T was higher, while RH was lower, due to the proximity of the sampling sites to artificial light sources, especially in Podpeć Cave. The type of lamps used differs between the caves: at the time of sampling, Podpeć Cave had lamps that emitted warmer light (these lamps have been changed since) compared to those in Stopić Cave, where LED lights had been installed. Cigna and Burri (2000) state that unsuitable lamps can lead to changes in environmental parameters, (e.g., Castellana Caves, South Italy where T increased from 15 °C to 25 °C while RH decreased from 95 % to 100 % to 55 % to 60% near the light source). It is interesting to note that point S2 was found to have lower RH despite the presence of LED lamps, probably because the sampling point is very close to the light (very high LI was measured compared to other sampling sites). As seen from Table 1, increases in T and RH lead to increases in WC and OM (a significant positive correlation was observed) and higher values of WC and OM mean that better developed biofilms are present.

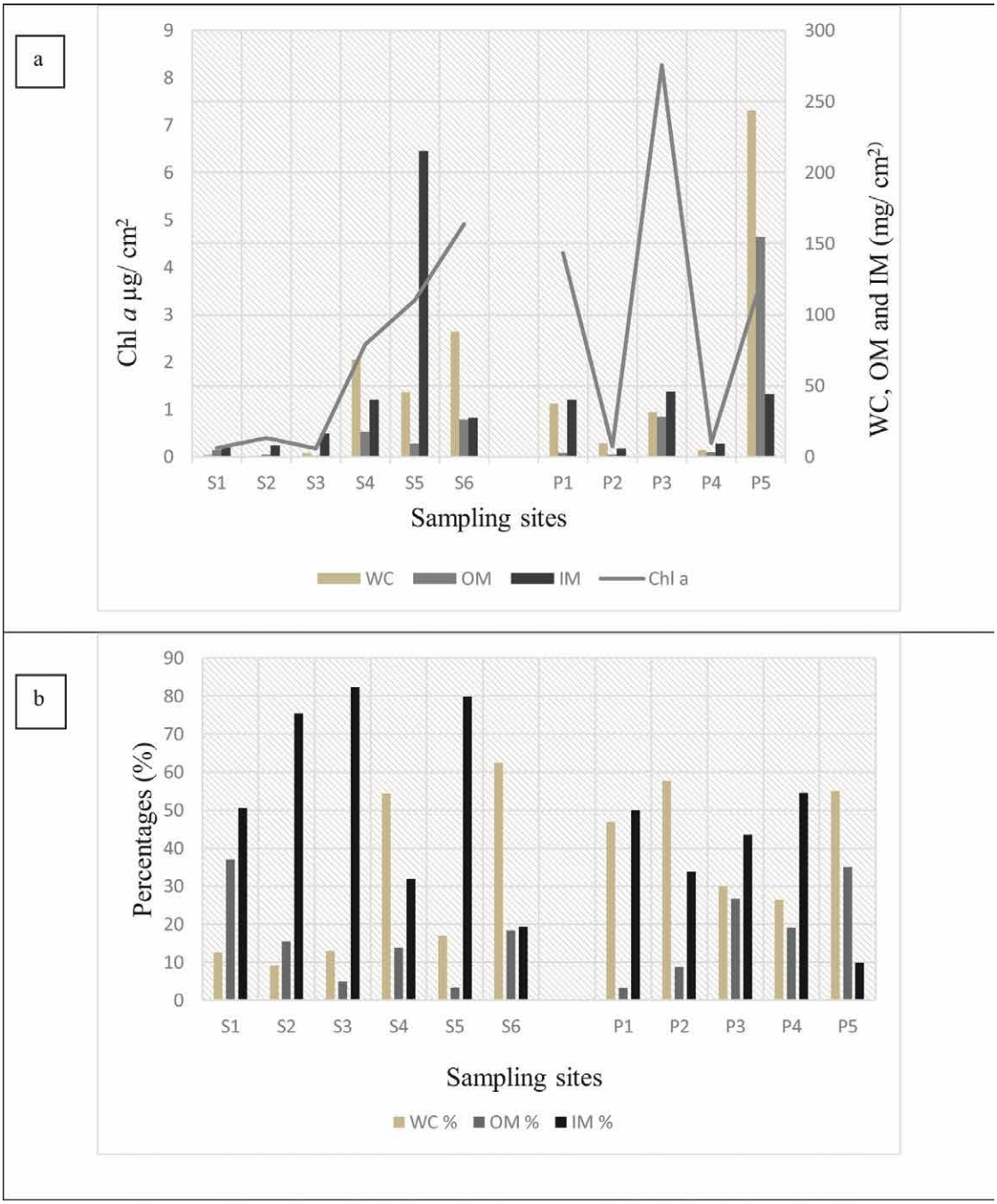

Figure 7. A – The concentration of chlorophyll *a* (Chl *a*) expressed as µg/ cm², water content (WC) and organic/ inorganic matter (OM/IM) expressed as mg/ cm² from Podpeć (P1–P5) and Stopić (S1–S6) caves. B – Water content (WC), organic and inorganic matter (OM/IM) presented as percentages from Stopić (S1–S6) and Podpeć (P1–P5) caves.

Many groups of microorganisms can grow in the extreme oligotrophic conditions of cave environments (Czerwik-Marcinkowska, 2013), however, some recent studies suggest that the level of trophicity can be increased by anthropogenic factors or presence of animals (Trinh et al., 2018). The OM introduced by animals, humans, or brought by intermittent and seeping water, significantly contributes to the development of microorganisms in locations near artificial lights (Mazina and Maximov, 2009). Furthermore, throughout the cave, tourists can introduce and spread the spores and cysts of different microorganisms that remain dormant until the appearance of suitable conditions for their development, as documented by Mulec and Kosi (2008), Czerwik-Marcinkowska et al. (2015), and Meyer et al. (2017). Cyanobacteria and algae have developed mechanisms of protection from various adverse environmental conditions (Pentecost and Whitton, 2012). The development of cyanobacteria and algae depends primarily on light, but also on T and RH; all are considered the most important factors for their growth (Martinčič et al., 1981; Chang and Chang-Schneider, 1991). The microbial assemblage at the cave entrance and in lampenflora samples usually differs as a result of living in two different zones, one characterized by the presence of artificial light and nearly stable conditions, and the other influenced by the outside climate, daylight and factors that are more variable.

Differences in the diversity and assemblages of aerophytic cyanobacteria and algae in this study are obvious when samples from the cave entrance and lampenflora are compared. The diversity of phototrophic microorganisms was higher at cave entrances, where *Cyanobacteria* were dominant, and lower inside the caves. Moreover, in Figure 8, consid-

Table 1. Correlations between T, RH, LI, Chl, WC, OM and IM using Pearson coefficient.

Variables	T	RH	LI	Chl *a*	WC	OM	IM
T	**1**	0.008	0.423	0.125	**0.635**	**0.607**	0.067
RH	0.008	**1**	−0.589	0.230	**0.664**	**0.629**	0.362
LI	0.423	−0.589	**1**	−0.198	−0.112	−0.016	−0.235
Chl *a*	0.125	0.230	−0.198	**1**	0.376	0.318	0.313
WC	**0.635**	**0.664**	−0.112	0.376	**1**	**0.956**	0.153
OM	**0.607**	**0.629**	−0.016	0.318	**0.956**	**1**	0.047
IM	0.067	0.362	−0.235	0.313	0.153	0.047	**1**

Note: Values in bold are different from 0 with a significance level alpha = 0.05.

ering the number of recorded taxa, *Cyanobacteria* were dominant at the cave entrances compared to algal groups. It should be noted that besides different environmental parameters, presence of seeping water and cave morphology, many microclimatic parameters can play a role. Accordingly, we cannot be certain which factors contribute to the much higher diversity in Stopić Cave. In the lampenflora samples collected inside the caves, diversity was low, but two genera of green algae (*Chlorella* sp., *Trohiscia* sp.) were quite abundant and the green algae cf. *Chlorella* sp. was always found in biofilms. In tourist caves near artificial light, a lower diversity of cyanobacteria and algae is commonly observed near artificial lights when compared to cave entrances, green algae often being the first colonizers of stone substrata (Mulec, 2008) and dominant components (Mulec and Kosi, 2008, Czerwik-Marcinkowska et al., 2015, and Meyer et al., 2017). Frequently, these green algae assemblages include fast growing and r-selective species (Albertano, 2012; Borderie et al., 2014, Czerwik-Marcinkowska et al., 2015) such as representatives of the genus *Chlorella.* This algae is reported in many caves worldwide and is considered to be a big problem for cave conservators. The stable microclimate inside the cave and suitable conditions under artificial lighting compared to the fast changing conditions at the entrance, as well as the absence of other extreme conditions, can promote the development of green algae and diatoms, since these groups prefer a more stable environment for their growth (Mulec, 2008; Borderie et al., 2014). Dripping water with suspended nutrients can also have a positive effect on algal growth, especially on the representatives of *Bacillariophyta* (Vinogradova et al., 2009; Piano et al., 2015). Recorded *Bacillariophyta* taxa in this study are considered a typical cosmopolitan and most frequent genera in caves (Czerwik-Marcinkovska and Mrozińska, 2011; Falasco et al., 2014). Inside Podpeć Cave, only *Humidophila* sp. was registered, a genus that is distributed globally and most commonly on the wet limestone walls in caves (Lowe et al., 2014, 2017). Even though we did not record seeping water at sampling sites at the time of sampling, it does not mean that seeping water is not present during certain periods of the year. There are also cases in which cyanobacteria prevail in lampenflora communities, but according to Mulec et al. (2008), it can happen in biofilms that have been growing undisturbed for some time. On the other hand, cyanobacteria are frequently dominant in the biofilms from cave entrances, since they are capable of enduring more extreme conditions than green algae and diatoms (Pentecost and Whiton, 2012).

Primary production at all sampling sites was assessed by measuring the Chl *a* concentration. Chl *a* concentration is usually correlated with the degree of biofilm development (Popović et al., 2015a; 2017a). In Stopić Cave, the highest concentration of this parameter was determined at sampling site S6, which was characterized by a thick biofilm with higher WC and OM. The correlation of Chl *a* with these two parameters in general had slightly positive values, but were not significant.

Water is the most significant factor influencing the development and growth of the phototrophic community on surfaces exposed to air (Pentecost and Whitton, 2012). Moisture originates from different sources: precipitation, humidity, or groundwater seepage, and its level can be highly variable, so higher RH can contribute to better developed phototrophs reflected through higher WC and OM (Table 1). Well -hydrated biofilms contained more viable and active cells than the ones that were water deficient or temporarily dry, which is probably the reason why Chl *a* was usually higher in such samples. The rest of the sampling sites at Stopić Cave, especially the sites near artificial light, had lower Chl *a* and OM concentrations, as expected, because the lampenflora were poorly developed in this cave and existing biofilms represented the remains of the old lampenflora that had developed during the previous year and before the cave reconstruction and the installation of new and better artificial LED lighting. In Podpeć Cave the concentration of Chl *a* varied and had high values at the sampling site at the cave entrance, but also at the two sampling sites near artificial light (sites P1 and P3) where lampenflora were quite well developed (Fig. 2). Compared to Stopić Cave, lampenflora in Podpeć Cave, especially at P1 and P3, were more developed and characterized with biofilm where, among cyanobacteria, algae and many organic and inorganic particles, mosses were also present (*Amblystegium serpens* was dominant and *Tortella tortuosa* was recorded sporadically).

Biofilms from cave entrances and from the internal cave environment were also different in terms of WC, OM and IM. High WC in biofilm samples from cave entrances, which is especially evident from sampling site P5 (Fig. 8), was the

Table 2. Cyanobacterial and algal taxa from Podpeć (P1- P8) and Stopić (S1- S6) caves.

	Stopić Cave						Podpeć Cave				
	Inside the Cave			Cave Entrance			Inside the Cave				Cave Entrance
Taxa/Samples	S1	S2	S3	S4	S5	S6	P1	P2	P3	P5	P8
Cyanobacteria											
Chroococcales											
Aphanocapsa cf. *planctonica* (G.M.Smith) Komárek & Anagnostidis						+					
Aphanocapsa muscicola (Meneghini) Wille				+	+						
Aphanocapsa rivularis (Carmichael) Rabenhorst						+					+
Aphanocapsa sp. Nägeli						+					
Aphanothece caldariorum P.G. Richter					+						+
Aphanothece saxicola Nägeli					+						
Asterocapsa spp. H.-J.Chu				+	+	+					+
Chondrocystis dermochroa (Nägeli) Komárek & Anagnostidis				+	+						+
Chroococcus cf. *spelaeus* Ercegovic						+					+
Chroococcus ercegovicii Komárek & Anagnostidis				+	+	+					+
Chroococcus pallidus Nägeli											
Chroococcus turgidus (Kützing) Nägeli					+	+					
Chroococcus varius A. Braun				+							
Chroococcus sp. Nägeli				+	+	+					+
Cyanothece aeruginosa (Nägeli) Komárek					+						+
Eucapsis sp. F.E. Clements & H.L. Shantz				+	+	+					+
Gloeocapsa alpina Nägeli				+							+
Gloeocapsa atrata Kützing					+	+		+			+
Gloeocapsa cf. *granosa* (Berkeley) Kützing				+							+
Gloeocapsa compacta Kützing				+							+
Gloeocapsa nigrescens Nägeli				+							+
Gloeocapsa punctate Nägeli				+		+					
Gloeocapsa violacea Kützing											
Gloeocapsa sp. Kützing						+					
Gloeothece cf. *incerta* Skuja				+							
Oscillatoriales											
Leptolyngbya foveolarum (Gomont) Anagnostidis & Komárek	+			+	+	+		+			+
Leptolyngbya henningsi(Lemmermann) Anagnostidis					+	+					
Leptolyngbya valderiana (Gomont) Anagnostidis & Komárek					+	+					
Leptolyngbya sp.1 Anagnostidis & Komárek				+	+	+					+
Leptolyngbya sp. Anagnostidis & Komárek				+	+	+					+
Phormidium cf. *ambiguum* Gomont											+
Phormidium corium Gomont ex Gomont						+					
Phormidium sp. Kützing ex Gomont											+
Porphyrosiphon fuscus Gomont ex Frémy					+						
Nostocales											
Nostoc commune Vaucher ex Bornet & Flahault											+
Nostoc punctiforme Hariot				+	+	+					+
Nostoc sp. Paracelsus					+	+					
Scytonema cf. *bruneum* Schmidle				+							
Scytonema drilosiphon Elenkin & V.I. Polyansky					+						
Scytonema hofmannii C. Agardh ex Bornet & Flahault						+					
Scytonema mirabile Wolle				+							
Scytonema subtile K. Möbius				+	+						
Scytonema sp. Agardh ex Bornet et Flahault				+							+
Tolypothrix tenuis Kützing ex Bornet & Flahault						+					
Chlorophyta											
Chlorella sp. Beyerinck	+	+	+				+	+	+	+	
Trochiscia sp. Kützing	+		+	+		+				+	
Bacillariophyta											
Hantzschia sp. Grunow											
Humidophila sp. (Lange-Bertalot & Werum) R.L. Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange-Bertalot & Kopalová		+									
Orthoseira spp. Thwaites	+	+	+								
Pinnularia sp. Ehrenberg						+	+				
Xanthophyta											
Xanthophyta unknown	+			+	+	+	+		+		

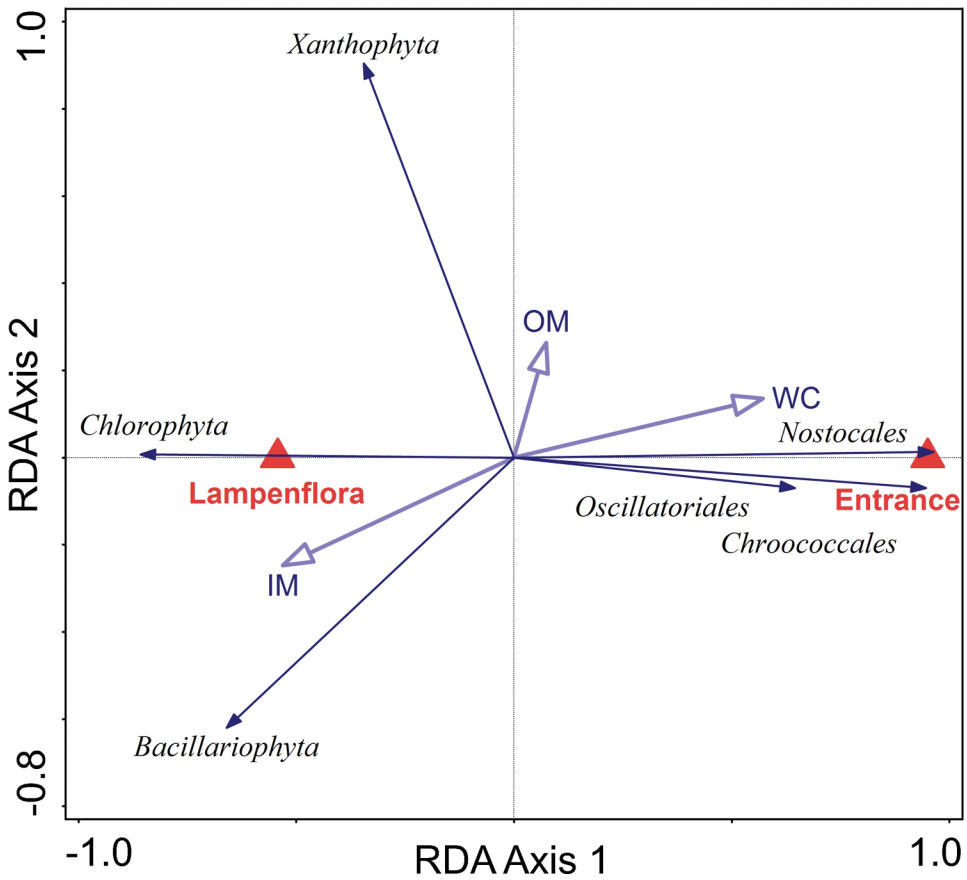

Figure 8. RDA analysis showing the relationship between explanatory variable, lampenflora and communities at cave entrances, and responce variables (*Cyanobacteria−Chroococcales*, *Oscillatoriales* and *Nostocales*), *Chlorophyta*, *Bacillariophyta* and *Xanthophyta*). Included supplemenatary variables are: content of organic (OM) and inorganic matter (IM) and water content (WC).

result of the presence and dominance of cyanobacteria. Cyanobacterial extracellular polysaccharides (EPS) play an important role in stress tolerance (Chug and Mathur, 2013) and thanks to these polysaccharides, cyanobacteria are not so vulnerable to variations in climatic conditions. One of their main roles is that they can retain water and enable cyanobacteria to better survive drought conditions, facilitating survival in an aerophytic habitat (Pentecost and Whitton, 2012; Chug and Mathur, 2013; Li et al., 2013).IM was higher in almost all lampenflora samples.

Lampenflora often cause the deterioration of stone substrata and cave formations. Lampenflora were especially very well-developed in Podpeć Cave, and at some sites, deteriorated parts of the stone base were mixed with biofilm components, which influenced IM content. The process of substrate deterioration in cave environments is of special concern, especially in caves with numerous attractive speleothems. The metabolic activities of microorganisms not only leads to undesired change in cave formations, but they can also disturb the habits of native organisms (Piano et al., 2015). The removal of lampenflora is achieved through various mechanical or chemical treatments. However, all such actions should be practicable and with minimal impact to the cave environment and organisms (Trih et al., 2018).

Conclusions

Cyanobacteria and algae were examined from biofilm samples taken from the Podpeć and Stopić caves. *Cyanobacteria*, *Chlorophyta*, *Bacillariophyta* and *Xanthophyta* were recorded, with the highest diversity found in the coccoid cyanobacteria. *Cyanobacteria* were dominant at cave entrances, while green algae were prominent elements of caves' lampenflora. Cf. *Chlorella* sp. was recorded in every lampenflora sample. Ecological parameters did not vary significantly, except for the LI that was dependent on the different aspects of cave entrances (i.e., their size) and sampling sites. Biofilm parameters (water content, content of organic and inorganic matter) varied greatly between samples collected near entrances and inside the caves. Chlorophyll *a* did not show clear correlations with any of the other measured parameters. The metabolic activity of green algae, which usually compromise part of the lampenflora, causes biodeterioration of the stone substratum and can lead to the irreversible damage of cave structures. Further investigations are necessary, since the knowledge on cave biofilms on the Balkan Peninsula is limited.

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