biology and life sciences forum

MDPI

Proceeding Paper

Exploring Lampenflora of Resavska Cave, Serbia †

Slađana Popović *, Marija Pećić and Gordana Subakov Simić

Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia

* Correspondence: sladjana.popovic@bio.bg.ac.rs or spopovic.bio@gmail.com

† Presented at the 2nd International Electronic Conference on Diversity (IECD 2022)—New Insights into the Biodiversity of Plants, Animals and Microbes, 15–31 March 2022; Available online: https://sciforum.net/event/IECD2022.

Abstract: Lampenflora, a complex phototrophic community that develops near artificial light in show caves, is recognized as a major concern for cave management and its regular monitoring has become necessity. For the purpose of this work, the monitoring was performed in one of the most visited show caves in Serbia—Resavska Cave, directly before and after the main season in 2021 in March and November. Lampenflora was localized and developed mainly near the artificial light, but further parts of the cave were not affected by it. Different sampling sites (twelve in total) regarding the type of artificial light and the type of biofilm were chosen for the lampenflora sampling. Two biofilm types were recognized in situ: the epilithic/endolithic ones which were dominated by algae and/or Cyanobacteria and the moss-dominated biofilms. An analysis of the phototrophic microorganisms revealed the presence of Cyanobacteria, Chlorophyta and Bacillariophyta. The highest diversity was found among the Cyanobacteria where the genera *Aphanocapsa*, *Eucapsis*, *Gloeocapsa* and *Leptolyngbya* were recorded during both of the samplings, *Nostoc* and *Synechocystis* were recorded in March, while *Hassalia*, *Oscillatoria* and *Pseudocapsa* were only recorded in November. Chlorophyta were represented by *Chlorella*, *Desmococcus*, *Klebsormidium*, *Mesotaenium* and *Stichococcus* in March, and they were represented by same taxa except for *Klebsormidium* in November. *Humidophila* was the most widespread diatom in the fresh biofilm samples. Aside from there being higher a level of diversity of it, Cyanobacteria were sporadically found in the samples except on two sampling sites where *Aphanocapsa* cf. *muscicola* and *Hassalia* sp. were abundant. Chlorophyta dominated the samples, which is in accordance with many other studies. The ecological parameters—temperature, relative air humidity, light intensity, substratum moisture and substratum pH were also determined and related to a degree of colonization and the community composition.

Keywords: show cave; lampenflora; cyanobacteria and algae

check for updates

Citation: Popović, S.; Pećić, M.; Subakov Simić, G. Exploring Lampenflora of Resavska Cave, Serbia. *Biol. Life Sci. Forum* **2022**, *15*, 33. https://doi.org/10.3390/IECD2022-12425

Academic Editor: Ipek Kurtboke

Published: 15 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

The natural cave environment is characterized with a stable temperature during the year, usually a high relative humidity, a lower amount of nutrients and specific microclimatic conditions. Since caves have remarkable geological features, various structures and many are as well recognized as archaeological sites, they have always been natural attractions for humans [1]. For that reason many of them are transformed into show caves that are easily accessible to tourists. The transformation of a natural cave habitat implies that a lot of changes must occur; first of all, the introduction of artificial light [1,2]. All of these changes cause modifications of the natural environmental conditions, but the installation of light produces one more possible threat to the cave environment: the appearance of phototrophs. Phototrophic microorganisms naturally can find their place only in the parts of the cave which are illuminated by daylight (entrances, openings) and they inhabit cell walls, sediment or water. However, those that develop and proliferate around artificial light form one specific microorganism community called lampenflora [1,2].

Usually these new inhabitants compete successfully to occupy this new ecological niche [2]. The most common photosynthetic organisms in the lampenflora communities are algae and Cyanobacteria, but very often, mosses, lichens, and sometimes even ferns and higher plants can also be present as well [3]. Lampenflora can cause undesirable changes to occur in caves that range from aesthetic ones to serious ones. The serious impact of lampenflora mostly refers to the physical and/or chemical biodeterioration of the stone substrate [1] or consequences regarding the fauna in the caves [4]. If the caves are neglected and if lampenflora proliferation occurs, they should be treated by different methods [2]. However, if possible, preventive measures should be taken to hinder the excessive development of lampenflora and one of them is certainly the lampenflora monitoring, which should be performed regularly so the situation can be intervened in time if it is necessary. The aim of this work was the screening of lampenflora in Resavska Cave in Serbia on two occasions over one year as a basis for further continuous monitoring.

2. Material and Methods

Resavska Cave is located in eastern Serbia, 15 km of Despotovac (44°04′22.4″ N; 21°37′47.4″ E), with an entrance that is at an altitude of 485 m [5]. It consists of three levels, the upper, lower and third galleries. The cave is rich in various structures among which many stalactites and stalagmites that are made of white and red calcite are present. It is believed that it is about 80 million years old. The cave is 4.5 km long: 2830 m of it has been explored, of which 800 m has been adapted for tourists to use (the upper and lower galleries only).

The cave was visited directly before and after the main touristic season in 2021 in March and November. Prior to the sampling, a screening of the cave was performed to record places with lampenflora. We tried to cover all of the parts of the cave, the different types of lamps and the different biofilms (Figure 1), after which twelve sites were chosen for sampling.

Figure 1. Types of biofilm: (**left**)—biofilm rich in mosses (down) and endolithic biofilm rich in Chlorophyta (up); (**middle**)— epilithic biofilm rich in Cyanobacteria; (**right**)—epilithic biofilm rich in Chlorophyta.

The ecological parameters, temperature (T), relative air humidity (RH), light intensity (LI), substrate moisture (SM) and pH of the substrate were determined.

For the lampenflora sampling, the adhesive tape method [6,7] was used and a flame-sterilized scalpel was utilized occasionally. Adhesive tape strips and/or microscopic slides with a small amount of biofilm that was mixed with a drop of glycerine were analyzed using a light microscope Zeiss Axio-ImagerM.1, software AxioVision 4.8, Zeiss (Oberkochen, Germany). The standard literature [8–12] was used for the identification of the phototrophic microorganisms.

A principal component analysis (PCA) was performed to represent the potential relationship of the phototrophic taxa that were organized into divisions using option "trait average" and the type of lamps that were present in the cave.

3. Results and Discussion

The lampenflora was present in the immediate vicinity of the artificial lighting but did not cover the larger areas of the rock substrate in the cave. Two types of biofilms were distinguished, biofilms that were dominated by phototrophic microorganisms and biofilms that were dominated by mosses. The biofilms that were dominated by phototrophs were less developed and presented in a thin layer on the rock substrate (epilithic), or they developed inside the rock substrate (endolithic). The biofilms that were dominated by mosses were found both around the lamps that emit the warm and cold light, and the most frequently in the passages that connect the different parts of the cave, closer to the cave floor.

When we were considering the ecological parameters (Table 1), the temperature was higher in all of the sampling sites in November, as well as for the RH. Additionally, the T was always higher in the deeper parts of the cave, and the RH was higher in the parts of the cave that are closer to the entrance. The LI varied from site to site as a consequence of the different types of lighting (reflectors), and the different distances of sampling sites from the light source, but also their exposure to them. Considering the SM, in most sites, the values did not exceed 40%; higher values only occurred in two sites in November. The pH was higher in sites that were rich in Cyanobacteria.

Table 1. Range of ecological parameter values.

Parameter	March	November
T (°C)	9.7–10.6	10.3–12.2
RH (%)	63–78	68–86
LI (Lux)	62–1379	72–1755
SM (%)	11.5–37	10.1–98
pH	5.8–6.4	6.9–7.22

An analysis of the biofilm samples revealed the presence of three divisions of phototrophs: Cyanobacteria, Chlorophyta and Bacillariophyta. A similar number of taxa was detected during both of the visits. A list of the recorded photosynthetic microorganisms and the number of sites in which they were found in March and November is given in Table 2.

Cyanobacteria were the most diverse in both of the seasons, and all three of the morphological groups were recorded in this division: the coccoid, simple trichal and heterocytous ones. The coccoid representatives (genera *Aphanocapsa*, *Eucapsis*, *Gloeocapsa*, *Pseudocapsa* and *Synechocystis*) were the most frequently encountered ones. A lower diversity was observed in the simple trichal (genera *Leptolyngbya* and *Oscillatoria*) and heterocytous ones (genera *Hassalia* and *Nostoc*). Even though Cyanobacteria had the highest diversity, they were found sporadically in almost all of the samples. The exception were two of the sampling sites, in one *Aphanocapsa* cf. *muscicola* dominated during both of the seasons (Figure 1—dark biofilm in the middle), and in one, *Hassalia* sp. was found in the dark parts of the biofilm.

A slightly lower diversity was observed in Chlorophyta. Unlike Cyanobacteria, Chlorophyta were more abundant in the samples, and they were generally recorded at a higher number in the sampling sites (Table 2). For example, the biofilm that is presented in Figure 1 (right) was almost completely made of *Mesotaenium* sp.

Bacillariophyta were very often recorded in the samples where the groups of different representatives of these algae were seen. Taking into account the identified genera from the fresh material, the genus *Humidophila* stands out, and it has been recorded in a large number of the sampling sites.

Table 2. List of recorded Cyanobacteria and algae and number of sites on which they are found in March and November.

Taxon	March	November
	Cyanobacteria	
Aphanocapsa muscicola	3	1
Aphanocapsa cf. *muscicola*	2	2
Aphanocapsa cf. *parietina*	1	0
Eucapsis sp.	1	2
Gloeocapsa biformis	1	1
Hassalia sp.	0	1
Leptolyngbya foveolarum	1	1
Leptolyngbya sp.	3	2
Nostoc sp.	2	0
Oscillatoria rupicola	0	1
Pseudocapsa dubia	0	1
Synechocystis pevalekii	2	0
Simple trichal Cyanobacteria	2	2
Coccoid Cyanobacteria	4	2
	Chlorophyta	
Chlorella sp.	7	6
Desmococcus olivaceus	1	1
Klebsormidium flaccidum	1	0
Mesotaenium sp.	7	6
Stichococcus bacillaris	7	6
Stichococcus chlorelloides	5	7
Coccal green algae 1	5	7
Coccal green algae various	8	5
	Bacillariophyta	
Fallacia sp.	1	0
Humidophila spp.	4	5
Orthoseira roseana	1	1
Psammothidium sp.	2	0
Various	6	7
	Others	
Mosses	6	6

Figure 2 shows phototrophic microorganisms grouped in divisions in relation to the type of the lighting. It appears that Cyanobacteria were more likely to be found in the places where the lamps that emitted the warm light are present, unlike Chlorophyta which were more diverse around the lamps that emitted the cold light.

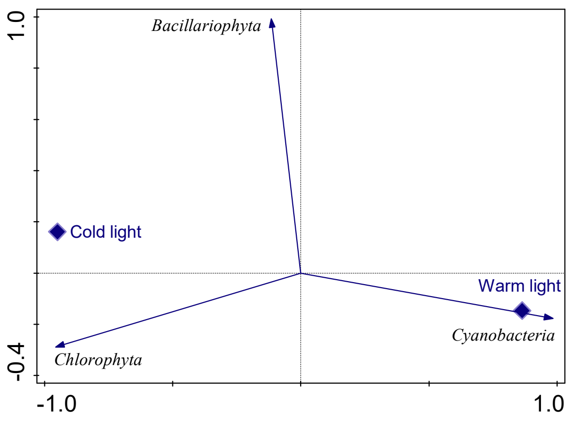

Figure 2. PCA of phototrophic taxa organized in divisions related to type of lamps that are present in the cave.

Author Contributions: Investigation and sampling, S.P., M.P. and G.S.S.; conceptualization and visualization, S.P.; methodology, S.P. and M.P.; formal analysis and data curation, S.P.; resources, S.P. and G.S.S.; writing—original draft preparation, S.P. and M.P.; writing—review and editing, S.P. and G.S.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No 451-03-68/2022-14/200178.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No 451-03-9/2021-14/200178. The authors want to thank the management of the cave for support during sampling.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Albertano, P. Cyanobacterial biofilms in monuments and caves. In *Ecology of Cyanobacteria II*; Whitton, B.A., Ed.; Springer: Dordrecht, The Netherlands, 2012.
2. Baquedano Estévez, C.; Merino, L.M.; de la Losa Román, A.; Durán Valsero, J.J. The lampenflora in show caves and its treatment: An emerging ecological problem. *Int. J. Speleol.* **2019**, *48*, 249–277. [CrossRef]
3. Cigna, A. The problem of Lampenflora in Show Caves. In Proceedings of the 6th ISCA Congress, Demanovska Valley, Slovakia, 18–23 October 2010; Slovak Caves Administration: Liptovský Mikuláš, Slovakia, 2011.
4. Mulec, J.; Glažar, S. First results on use of a hydrogen peroxide solution in Postojnska jama (Slovenia) to remove lampenflora. In Proceedings of the 6th ISCA Congress, Demanovska Valley, Slovakia, 18–23 October 2010; Slovak Caves Administration: Liptovský Mikuláš, Slovakia, 2011.
5. Đurović, P. *Speleološk Atlas Srbije*; Srpska Akademija Nauka i Umetnosti: Beograd, Serbia, 1998. (In Serbian)
6. Gaylarde, P.M.; Gaylarde, C.C. A rapid method for the detection of algae and cyanobacteria on the external surfaces of buildings. In Proceedings of the Third Latin American Biodegradation and Biodeterioration Symposium, Florianopolis, Brazil, 27–30 April 1998; The British Phycological Society: London, UK, 1998.
7. Urzi, C.; de Leo, F. Sampling with adhesive tape strips: An easy and rapid method to monitor microbial colonization on monument surfaces. *J. Microbiol. Methods* **2001**, *44*, 1–11. [CrossRef]
8. Hofmann, G.; Werum, M.; Lange-Bertalot, H. *Diatomeen im Süßwasser–Benthos von Mitteleuropa: Bestimmungsflora Kieselalgen für die ökologische Praxis; Über 700 der häufigsten Arten und ihre Ökologie*; Koeltz Scientific Books: Königstein, Germany, 2013. (In German)
9. John, D.M.; Whitton, B.A.; Brook, A.J. *The Freshwater Algal Flora of the British Isles: An Identification Guide to Freshwater and Terrestrial Algae*; Cambridge University Press: Cambridge, UK, 2003.
10. Komárek, J.; Anagnostidis, K. Cyanoprokaryota 1. Teil Chroococcales. In *Süsswasserflora von Mitteleuropa; Bd. 19/1*; Büdel, B., Gärtner, G., Krienitz, L., Schagerl, M., Eds.; Gustav Fisher Verlag: Jena, Germany, 1998.
11. Komárek, J.; Anagnostidis, K. Cyanoprokaryota 2. Teil Oscillatoriales. In *Süsswasserflora von Mitteleuropa; Bd. 19/2*; Büdel, B., Gärtner, G., Krienitz, L., Schagerl, M., Eds.; Gustav Fisher Verlag: Jena, Germany, 2005.
12. Komárek, J. Cyanoprokaryota 3. Teil: Heterocytous Genera. In *Süßwasserflora von Mitteleuropa; Bd. 19/3*; Büdel, B., Gärtner, G., Krienitz, L., Schagerl, M., Eds.; Springer: Berlin, Germany, 2013.