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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, Belgrade, Serbia; marija.pecic@bio.bg.ac.rs(M.P.); gsubak@bio.bg.ac.rs(G.S.S.)
* Correspondence: sladjana.popovic@bio.bg.ac.rs or spopovic.bio@gmail.com
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Abstract: Lampenflora, a complex phototrophic community developing near artificial light in show caves, is recognized as a major concern for cave management and its regular monitoring became necessity. For the purpose of this work, monitoring was done 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 artificial light, further parts of the cave were not affected. Different sampling sites (twelve in total) regarding type of artificial light and type of biofilm were chosen for lampenflora sampling. Two biofilm types were recognized in situ: epilithic/endolithic ones dominated by algae and/or Cyanobacteria and moss dominated biofilms. Analysis of phototrophic microorganisms revealed the presence of Cyanobacteria, Chlorophyta and Bacillariophyta. The highest diversity was found in Cyanobacteria where genera *Aphanocapsa*, *Eucapsis*, *Gloeocapsa* and *Leptolyngbya* were recorded during both samplings, *Nostoc* and *Synechocystis* in March, *Hassalia*, *Oscillatoria* and *Pseudocapsa* only in November. Chlorophyta were represented with *Chlorella*, *Desmococcus*, *Klebsormidium*, *Mesotaenium*, *Stichococcus* in March and the same taxa except *Klebsormidium* in November. *Humidophila* was the most widespread diatom in fresh biofilm samples. Aside from higher diversity, Cyanobacteria were sporadically found in samples except on two sampling sites where *Aphanocapsa* cf. *muscicola* and *Hassalia* sp. were abundant. Chlorophyta dominated in samples, which is in accordance with many other studies. Ecological parameters—temperature, relative air humidity, light intensity, substratum moisture and substratum pH were also determined and related to degree of colonization and community composition.

Keywords: show cave; lampenflora; cyanobacteria and algae

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1. Introduction

Natural cave environment is characterized with the stable temperature during the year, usually a high relative humidity, 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 are transformed into show caves easily accessible to tourists. The transformation of a natural cave habitat implies a lot of changes, first of all, the introduction of artificial light [1,2]. All changes cause modification of the natural environmental conditions, but the installation of light means one more possible threat to the cave environment: the appearance of phototrophs. Phototrophic microorganisms naturally can find their place only in parts of the cave which are illuminated by daylight (entrances, openings) and 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 [3]. Lampenflora can cause undesirable changes in caves that range from aesthetic to serious ones. The serious impact of lampenflora mostly refers to physical and/or chemical biodeterioration of the stone substrate [1] or consequences regarding fauna in caves [4]. If caves are neglected and if lampenflora proliferation occurs, it should be treated by different methods [2]. However, if possible, preventive measures should be taken to hinder excessive development of lampenflora and one of them is certainly monitoring that should be performed regularly so it could be intervened in time if necessary. The aim of this work was screening of lampenflora in Resavska Cave in Serbia two times during 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 the entrance at an altitude of 485 m [5]. It consists of three levels, upper, lower and third gallery. The cave is rich in various structures among which many stalactites and stalagmites made of white and red calcite are present. It is believed that is about 80 million years old. The cave is 4.5 km long: 2830 m has been explored, of which 800 m has been adapted for tourists (upper and lower gallery only).

The cave was visited directly before and after the main touristic season in 2021, in March and November. Prior to sampling, a screening of a cave was performed to record places with lampenflora. We tried to cover all parts of the cave, different types of lamps and 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.

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

For lampenflora sampling, 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 mixed with a drop of glycerine were analyzed with light microscope Zeiss Axio-ImagerM.1, software AxioVision 4.8. Standard literature was used for the identification of phototrophic microorganisms.

Principal component analysis (PCA) was done to represent the potential relationship of phototrophic taxa organized in divisions using option "trait average" and type of lamps that are present in cave.

3. Results and Discussion

Lampenflora was present in the immediate vicinity of artificial lighting not covering larger areas of the rock substrate in the cave. Two types of biofilms were distinguished, biofilms dominated by phototrophic microorganisms and biofilms dominated by mosses.

Biofilms dominated by phototrophs were less developed and present in a thin layer on the rock substrate (epilithic), or develop inside the rock substrate (endolithic). Biofilms dominated by mosses are found both around lamps that emit warm and cold light, frequently in the passages that connect different parts of the cave, closer to the cave floor.

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

Analysis of biofilm samples revealed the presence of three divisions of phototrophs: Cyanobacteria, Chlorophyta and Bacillariophyta. Similar number of taxa was detected during both visits. List of recorded photosynthetic microorganisms and number of sites on which they are found in March and November is given in Table 2.

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

Cyanobacteria were the most diverse in both seasons and all three morphological groups were recorded in this division: coccoid, simple trichal and heterocytous ones. Coccoid representatives (genera *Aphanocapsa*, *Eucapsis*, *Gloeocapsa*, *Pseudocapsa*, *Synechocystis*) were the most frequently encountered. Lower diversity is observed in 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 samples. The exception were two sampling sites, one on which *Aphanocapsa* cf. *muscicola* dominated in both seasons (Figure 1—dark biofilm in the middle), and one where *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 samples and generally recorded at a higher number of sampling sites (Table 2). For example, biofilm presented in Figure 1 (right) was almost completely made of *Mesotaenium* sp.

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

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

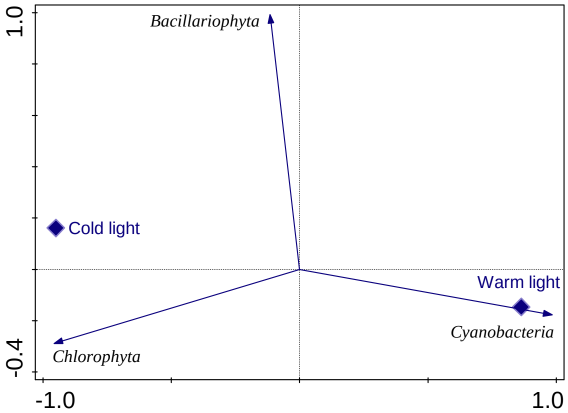

Figure 2. PCA of phototrophic taxa organized in divisions related to type of lamps that are present in cave.

Institutional Review Board Statement:

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Data Availability Statement:

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

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