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CHARACTERIZATION OF CAVE AEROPHYTIC ALGAL COMMUNITIES AND EFFECTS OF IRRADIANCE LEVELS ON PRODUCTION OF PIGMENTS

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Abstract: Aerophytic algae grow on various substrata under favourable ecological conditions. In the illuminated parts of caves, where relative humidity reaches 100%, they colonize sediments, rocky surfaces, and artificial materials. An aerophytic algal community from the cave entrance is composed almost exclusively of cyanobacteria, in contrast to lampenflora where green algae become more dominant. In the later stage of species succession in the lampenflora community, cyanobacteria are more abundant and thus community structure becomes more similar to the community from the cave entrance. Absence of correlation between photon flux density and chlorophyll *a* concentration indicates that substratum characteristics at the micro level notably influence algal growth. Chl *a* concentration per surface unit in the case of the epilithic algae from the cave entrance is lower (max. 1.71 μg cm^{-2}) compared to that for the lampenflora algae (max. 2.44 μg cm^{-2}). At cave temperatures, the light saturation point is quickly reached. At 9.0 °C and frequent low photon flux densities in a cave entrance and around lamps in show caves, biosynthesis of accessory photosynthetic pigments for two typical cave aerophytic organisms, cyanobacterium *Chroococcus minutus* and green alga *Chlorella* sp., is considerably elevated.

INTRODUCTION

Caves are one of the extreme environments generally characterized by low nutrient input (Pedersen, 2000). Low nutrient input is a limiting factor for many groups of organisms, although some species, like algae, find this environment still suitable for colonization and growth. In caves, algae can be found in bodies of water (Kuehn et al., 1992; Sanchez et al., 2002) and aerophytic habitats (Golubić, 1967; Dobat, 1970). In caves, many surfaces serve for algal colonization including: sediments, rocky surfaces and artificial material. We recently published the composition of algal communities from another two interesting cave aerophytic habitats, from stromatolitic stalagmites and from stalactites, where growth is enhanced by carbonate deposition promoted by cyanobacteria towards sun light (Mulec et al., 2007). Development of aerophytic vegetation is influenced by light, temperature, high relative humidity (reaching 100%) and/or seeping water, and substratum characteristics (Golubić, 1967; Martinčič et al., 1981; Chang and Chang-Schneider, 1991). Aerophytic algae are easily observed in the cave entrance illuminated by direct or indirect sunlight and, in show caves equipped with artificial illumination, as a part of a lampenflora community around lamps (Mulec, 2005). Several approaches have been tested to control growth of this alien lampenflora vegetation (Olson, 2006). Illuminated spots in a generally nutrient-poor cave environment are quickly colonized by aerophytic algae. The large amount of energy and consequent biomass introduced into the cave ecosystem indirectly influence cave fauna, as well as affecting the survival and transport of organisms entering the cave, either actively or passively. Higher nutrient input enables new comers to be more competitive in the cave environment than specialized troglomorphic organisms. Consequently, obligate cave-dwelling organisms are threatened and may become extirpated or extinct without restoration of previous natural conditions (Pipan, 2005).

Caves are generally not considered to be isolated habitats; however, there is an example of the spatially isolated Movile cave where the existence of complex animal communities is based only on bacterial chemolithotrophy (Sarbu et al., 1996; Kinkle and Kane, 2000). Three key modes of transport of viable algal propagules into the karst underground can be distinguished: air currents, water flow, and introduction by animals and humans (Dobat, 1970). The existence of lampenflora deep in show caves proves the efficient transport of propagules from sources above caves, which is more or less constant. Species composition of aerophytic algal communities from cave entrances differs compared to lampenflora. In illuminated cave entrances cyanobacteria prevail (Palik, 1964a; Golubić, 1967; Buczkó and Rajczy, 1989; Vinogradova et al., 1995, 1998; Asencio and Aboal, 2000). Vinogradova et al. (1998) established that light is a key factor that influences zonation of cyanobacteria in the cave entrance.

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Table 1. Location and characterization of the caves and mines in this study.

Cave / mine	Municipality	Altitude (m)	Length (m)	Depth (m)	Orientation of cave entrance	Lithology
Postojnska jama	Postojna	529	19555	115	NW	Cretaceous limestones
Kostanjeviška jama	Krško	170	1726	47	S	Cretaceous limestones and dolomites
Pekel pri Zalogu	Žalec	314	1159	40	NE	Triassic limestones and dolomites
Pivka jama cave	Postojna	540	794	77	E	Cretaceous limestones
Lead and zinc mine Mežica	Ravne na Koroškem	500	3500	300	S	Carnian limestones, Triassic dolomites, shales
Mercury mine Idrija	Idrija	330	1000	22	S	Permocarbonian shales and dolomites
Škocjanske jame	Sežana	425	5800	250	NW	Cretaceous and Paleogene limestones
Županova jama	Grosuplje	468	682	70	NW	Jurassic limestones

In caves, new algal species were identified (Jones, 1964; Palik, 1964b; Van Landingham, 1966a,b; Sant'Anna et al., 1991; Hernandéz-Mariné and Canals, 1994). Beyond taxonomy, ecophysiological studies on cave algae are rare, although caves represent an almost ideal natural laboratory for algological studies with practically constant ecological parameters. The purpose of this study was to ascertain how different irradiance levels influence quantity and quality change in the algal community, how low irradiances affects the ratio of photosynthetic pigments in algae, and to compare floristic analysis from the same caves conducted 22 years ago by Martinčič et al. (1981).

Study Area

Six caves (Postojnska jama, Kostanjeviška jama, Pekel pri Zalogu, Pivka jama, Škocjanske jame, Županova jama) and two mines (Idrija, Mežica) were studied in the karst region of Slovenia (Table 1). Škocjanske jame is, due to its importance from the speleological and ecological point of view, listed in the UNESCO World heritage list and as important underground wetlands (Ramsar convention). Sampling was performed in the summer of 2003. Where lampenflora were sampled in show caves, lamps are periodically turned on due to tourist visits or maintenance of the tourist infrastructure. Only in the Mežica lead and zinc mine are lamps on 24 hours due to the constant monitoring of underground water flow. Sampling sites for lampenflora were selected randomly. Sampling of aerophytic algae in the cave entrance of Škocjanske jame was performed in one of the entrances named Schmidlova dvorana, which is a cave space of huge dimensions, 22 m wide, 25 m high and 100 m deep. This large shady area in the cave mouth is directly illuminated by sunlight in the morning. Due to the orientation and position of the cave entrance, in the early spring and late autumn, an even wider area is directly illuminated. Growth experiments were carried out in the part of Postojnska jama that is not open to the public.

Materials and Methods

Samples for floristic analysis were taken from eight caves and mines. Prior to taking specimens photon flux density at the sites was measured using a LICOR LI−1000 DataLogger (USA). In the cave entrance several measurements of photon irradiance were made; the most representative ones were used for statistical analysis. We inoculated Jaworski medium (Warren et al., 1997) at the sampling sites using sterile scrapes of the algal mat. In the case of lampenflora, if confluent growth was observed around a lamp, up to seven samples were taken around the same lamp at different distances to observe differences in the community composition. Mixed and pure algal cultures were isolated in Jaworski liquid and on solid 1% Jaworski agar media. Jaworski medium is frequently used in algology as it supports growth a variety of groups of algae. Cyanobacteria were selectively isolated when the medium was supplemented with 100 $\mu g\ ml^{-1}$ of DCMU (diuron, N-3, 4-dichlorophenyl-N′-dimetil urea). Cultivation conditions were: 20 °C, 8:16 light/dark period with a photon flux density of 100 $\mu mol\ m^{-2}\ s^{-1}$ for several weeks. Cultures were regularly screened using a magnifying glass and light microscope (Nikon Eclipse TE300). Floristic data obtained from culture material were supplemented with microscopic data of the same field material fixed with 4% formalin solution. Diatom samples were processed and identified as described by Clesceri et al. (1998). Sputtered gold specimens were screened using a SEM microscope (JSM-840, JEOL, USA). Several keys and articles were used to identify algal species: Abdelahad (1985, 1989), Asencio and Aboal (2000), Couté (1982), Ettl and Gärtner (1995), Garbacki et al. (1999), Geitler (1932), Golubić (1967), Hindak (1996), Hoffmann (1986), Komárek and

Anagnostidis (2000, 2005), Krammer and Lange-Bertalot (1986, 1988, 1991), Lemmermann et al. (1915), Sulek (1969).

Cyanobacterium *Chroococcus minutus* and green alga *Chlorella* sp., isolated in pure culture from the lampenflora community, which frequently inhabit aerophytic habitats including the cave entrance, were used in growth experiments in that part of Postojnska jama that is not open to the public (9.0 °C, RH 95%). Cultures in liquid Jaworski medium were cultivated in triplicate using a photon flux gradient of 100, 50, 20, 10, 5, 2.5 and 0 μmol m^{-2} s^{-1} with the same lighting period (8:16 light/dark period) and regular mixed. The inoculum was 10^4 cells per ml. After 25 days of incubation, cells were counted, harvested, and the concentration of photosynthetic pigments established: Chl *a*, Chl *b* and carotenoids for *Chlorella* sp. after Wetzel and Likens (1995) and phycocyanin after Lee et al., (1994) and Chl *a* after Vollenweider et al. (1974) for *C. minutus*.

Sites for ascertainment of Chl *a* levels of epilithic cave algae were carefully selected. Known flat rocky surfaces with minor substratum irregularities with confluent overgrowth of algae were scraped off with an alcohol flame sterilized pocket knife and collected in a test tube. The concentration of Chl *a* was established using the procedure described by Vollenweider et al. (1974). At each site photon flux density was measured.

Results

In the aerophytic algal community from the cave entrance of Škocjanske jame cyanobacteria prevailed (69% of identified taxa) while Chlorophyta (19%) and Chrysophyta (12%) represented the minor part of the community (Table 2). Samples were also taken at the same sites to determine Chl *a* levels of epilithic algae, which ranged from 0.14 to 1.71 μg cm^{-2} (Table 2). There is no correlation ($r = 0.04$, $p > 0.05$) between irradiance and Chl *a* as well as between the number of algal taxa and Chl *a* concentration ($r = 0.19$, $p > 0.05$). Taking into account only the cyanobacterial component, we established that with increasing irradiance the number of coccoid cyanobacteria lowers ($r = -0.42$, $p < 0.05$).

Lampenflora can be observed in the immediate vicinity of artificial lighting. In Slovenian caves zonation of vegetation is not observed. We identified 60 algal taxa in the lampenflora community from eight show caves (Table 3). Cyanobacteria were the most abundant (47%) followed by Chlorophyta (30%) and Chrysophyta (23%).

In Pekel pri Zalogu cave, samples were taken around one selected lamp with an illumination gradient and confluent phototrophic growth to determine Chl *a* levels of epilithic algae. Concentrations ranged from 0.57 to 2.45 μg cm^{-2} (data not shown). As in the case of aerophtytic algae from Schmidlova dvorana, lampenflora showed no correlation between irradiance and Chl *a* levels ($r = -0.19$ $p > 0.05$).

Irradiances used in our growth experiment were similar to the measurements of irradiance experienced in caves around lamps and in the shady parts of cave entrances. From Figure 1, it is evident that in cyanobacterium *C. minutus* the concentration of Chl *a* increased up to a photon flux density of 50 μmol m^{-2} s^{-1} and at 100 μmol m^{-2} s^{-1} a slight decline is observed. Concentration of phycocyanin at low photon flux densities (e.g., 2.5, 5 and 10 μmol m^{-2} s^{-1}) were higher compared to 20 and 50 μmol m^{-2} s^{-1}. Kirk (1983) experienced a similar effect that at low irradiance the ratio of biliproteins to Chl *a* increases. At the highest photon flux density of 100 μmol m^{-2} s^{-1} phycocyanin concentration elevated a little more. With green alga *Chlorella* sp. at low photon flux densities (e.g., 2.5, 5 and 10 μmol m^{-2} s^{-1}) the concentration of accessory photosynthetic pigments is also elevated (Fig. 2). By gradually increasing from 20 to 100 μmol m^{-2} s^{-1}, the molar ratio of accessory pigments (i.e., Chl *b* and carotenoids) was lowered in favour of Chl *a*. At 100 μmol m^{-2} s^{-1} the ratio of Chl *a* vs. Chl *b* was 3:1, which is typical for green algae (Kirk, 1983).

Discussion

Algae frequently grow in the illuminated parts of caves. In caves, two distinct aerophytic microhabitats colonized by epilithic algae can be distinguished: (1) cave entrances illuminated by sunlight and (2) areas around lamps in show caves and mines. Cyanobacteria prevail in the algal community from cave entrances. They can colonize into the deepest parts of the cave entrance where biodiversity of phototrophic organisms is the lowest (Vinogradova et al., 1998). Many of the identified algae from poorly illuminated cave environments cannot be considered typical cave species (Hoffmann, 2002), although the presence of some of them (e.g., *Pediastrum boryanum*, Table 2) indicates not only efficient transport but also the existence of a suitable niche in water droplets for non-aerophytic algae.

As reported in other papers, the lampenflora community shows lower biodiversity compared to the algae from cave entrances. In 1981 Martinčič et al. published a floristic analysis of lampenflora from six Slovenian show caves: Črna jama, jama Pekel pri Zalogu, Pivka jama, Postojnska jama, Škocjanske jame and Taborska jama (now called Županova jama) where they identified a total of 44 algal species, with the highest portion of cyanobacteria. Comparing these results and the results of the present study 20 years later, we did not observe any major difference in the composition of the lampenflora community. The green alga *Trentenpohlia aurea* appears with the highest frequency in Slovenian show caves.

The key question here would be: What is species succession like in the case of lampenflora? Although cyanobacteria are the most adaptable phototrophs under stressed conditions, in microhabitats with less environmental stress, like illuminated spots around lamps, they are

Table 2. Composition of algal community with regard to concentrations of Chl *a* and photosynthetic active radiation at sampling sites in the Schmidlova dvorana cave entrance from Škocjanske jame.

	Sampling site																					
Chl *a* (µg cm^{-2})	0.14	0.20	0.49	0.56	0.94	0.61	0.37	1.71	1.34	0.54	0.51	0.93	0.37	0.32	0.57	0.14	0.95	0.81	1.87	0.93	0.21	0.50
Species\ Photosynthetic active radiation (µmol m^{-2} s^{-1})	0.30	0.27	0.28	0.24	0.21	0.31	0.11	0.90	0.10	0.80	0.40	0.70	0.20	0.06	0.57	0.45	0.51	0.48	0.11	0.75	0.41	1.96
PROKARYOTA																						
CYANOPHYTA																						
Aphanocapsa sp. Nägeli	·	·	·	·	·	·	·	+	+	+	·	·	+	·	·	·	·	+	·	·	·	·
Aphanocapsa muscicola (Meneghini) Wille	·	·	+	+	+	+	+	+	+	+	·	·	+	+	+	+	+	+	+	+	+	+
Aphanothece castagnei (Brébisson) Rabenhorst	·	·	·	+	·	·	·	·	·	·	·	·	+	·	·	·	·	·	·	·	·	·
Chondrocystis dermochroa (Nägeli) Komárek et Anagnostidis	·	·	·	·	·	+	·	·	·	·	·	·	·	·	+	+	+	·	·	+	·	·
Chroococcus sp. Nägeli	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	+	·
Chroococcus helveticus Nägeli	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	+	·	·	·	·
Chroococcus lithophilus Ercegović	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	+	·	·	·
Chroococcus minutus (Kützing) Nägeli	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·
Chroococcus montanus Hansgirg	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·
Chroococcus varius A. Braun in Rabenhorst	·	·	·	+	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Chroococcus turgidus (Kützing) Nägeli	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·
Cyanothece aeruginosa (Nägeli) Komárek	·	·	·	+	·	·	·	+	·	+	·	+	·	·	·	·	·	·	·	·	·	·
Geitleria calcarea Friedmann	·	·	+	+	+	·	·	+	·	+	+	·	+	+	·	·	·	+	+	·	·	·
Gloeocapsa sp. Kützing	·	·	·	·	·	+	·	·	·	·	·	·	·	·	+	·	·	·	·	+	·	·
Gloeocapsa aeruginosa Kützing	·	·	·	·	·	+	·	·	·	·	·	·	·	·	+	·	·	·	+	+	·	+
Gloeocapsa atrata Kützing	·	·	·	·	·	+	·	·	·	·	·	·	+	·	·	·	+	·	+	·	+	·
Gloeocapsa kuetzigiana Nägeli	·	·	+	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·
Gloeocapsa rupestris Kützing	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	+	·
Gloeocapsopsis sp. Geitler ex Komárek	·	+	+	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·
Gloeocapsopsis pleurocapsoides (Nováček) Komárek et Anagnostidis	+	·	·	·	·	·	+	·	·	·	·	+	·	·	·	·	·	·	·	·	·	·
Gloeothece palea (Kützing) Rabenhorst	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	+	+	·	·
Gloeothece rupestris (Lyngbye) Bornet, in Wittrock et Nordstedt	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	+	·	·	·	·
Leptolyngbya foveolarum (Rabenhorst ex Gomont) Anagnostidis et Komárek	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	+	·
Leptolyngbya fragilis (Gomont) Anagnostidis et Komárek	·	·	·	·	+	+	·	+	·	+	+	+	·	+	+	·	+	+	+	·	·	·
Leptolyngbya gracillima (Zopf ex Hansgirg) Anagnostidis et Komárek	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·	·	·	·
Leptolyngbya perelegans (Lemmermann) Anagnostidis et Komárek	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	+	·
Leptolyngbya schmidlei (Limanowska) Anagnostidis et Komárek	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·	·	·	·
Leptolyngbya tenuis (Gomont) Anagnostidis et Komárek	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·
Lyngbya sp. C. Agardh ex Gomont	+	·	+	+	·	·	+	+	+	+	+	+	+	+	·	+	+	+	+	+	+	+
Lyngbya attenuata Fritsch	·	·	·	+	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Nostoc minutum Desmaz ex Born	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·
Oscillatoria sp. Vaucher ex Gomont	·	·	·	·	·	·	·	·	·	·	+	·	+	+	·	·	·	+	·	·	·	+
Oscillatoria subbrevis Schmidle	·	·	·	+	·	·	·	·	+	·	·	·	·	·	·	·	·	·	·	·	·	·
Phormidium inundatum Kützing ex Gomont	·	·	·	+	·	·	·	·	·	+	·	·	·	·	·	·	·	·	·	·	·	·

Table 2. Continued.

Species\ Photosynthetic active radiation (µmol m^{-2} s^{-1})	Sampling site																					
Chl *a* (µg cm^{-2})	0.14	0.20	0.49	0.56	0.94	0.61	0.37	1.71	1.34	0.54	0.51	0.93	0.37	0.32	0.57	0.14	0.95	0.81	1.87	0.93	0.21	0.50
	0.30	0.27	0.28	0.24	0.21	0.31	0.11	0.90	0.10	0.80	0.40	0.70	0.20	0.06	0.57	0.45	0.51	0.48	0.11	0.75	0.41	1.96
Planktolyngbya limnetica (Lemmermann) Komárková-Legnerová et Cronberg	·	+	+	·	·	·	·	·	·	·	·	·	+	·	+	·	·	·	·	·	·	+
Plectonema sp. Thuret ex Gomont	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	+	·	·	·	+	·
Pseudophormidium tenue (Thuret ex Gomont) Anagnostidis et Komárek	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+
Scytonema sp. Agardh	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	+	·	·	·
Scytonema hofmanni Agardh	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·	·
Synechococcus elongatus Nägeli	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·	·
Synechocystis sp. Sauvageau	+	+	·	+	+	+	+	+	+	·	+	·	+	·	·	·	·	·	+	·	·	·
EUKARYOTA																						
CHRYSOPHYTA																						
Eunotia argus (Ehrenberg) Kützing	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·
Cymbella ehrenbergii Kützing	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·	·	·
Navicula sp. Bory	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·	+	·
Navicula contenta var. *biceps* (Arnott, Grunow in Van Heurck) Cleve	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	+	·	·	+	+	·	+
Navicula gallica var. *perpusilla* (Grun) Lange-Bertalot	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·	·	·	·
Navicula mutica Kützing	+	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·	·	·
Navicula stroemii Hustedt	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·	·
CHLOROPHYTA																						
Chlorella sp. Beijerinck	·	+	·	·	·	+	+	·	·	·	·	·	+	·	·	+	·	+	+	+	·	+
Chlorosarcina sp. Gerneck	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·	·	·	·
Gloeocystis polydermatica (Kützing) Hindák	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·
Klebsormidium flaccidum Silva, Mattox et Blackwell	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	+
Muriella sp. J.B. Petersen	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·	·
Pediastrum boryanum (Turpin) Meneghini	·	·	+	·	·	·	·	·	·	+	+	·	·	·	·	+	·	+	+	·	+	·
Pleurococcus sp. Meneghini	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+
Scenedesmus sp. Meyen	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+
Scenedesmus bijugatus (Turpin) Meneghini	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	·	·	·	·	·	·	·
Stichococcus bacillaris Nägeli	·	·	·	·	·	+	+	·	·	·	+	·	+	·	+	+	·	·	·	+	+	·
Trentepohlia aurea Martius	·	+	·	·	·	·	·	·	·	·	·	·	·	·	·	·	+	+	+	·	·	·

Table 3. Species composition of the lampenflora algae from 8 show caves and mines.

Species	Cave/Mine							
	Id	Ko	Me	Pe	Pi	Po	Šk	Žu
PROKARYOTA								
Cyanophyta								
Aphanocapsa biformis A. Brown in Rabenhorst	·	·	+	+	+	·	·	·
Aphanocapsa fusco-lutea Hansgirg	·	·	·	·	·	·	+	·
Aphanocapsa muscicola (Meneghini) Wille	+	+	+	+	+	+	+	+
Aphanocapsa parietina Nägeli	·	·	·	+	·	·	·	·
Chondrocystis dermochroa (Nägeli) Komárek et Anagnostidis	+	·	+	+	·	·	·	·
Chroococcus lithophilus Ercegović	·	·	·	·	·	·	+	·
Chroococcus minutus (Kützing) Nägeli	+	·	+	·	·	·	+	·
Chroococcus schizodermaticus W.West	·	·	·	·	·	·	+	·
Chroococcus varius A. Braun in Rabehorst	·	·	+	·	·	·	·	·
Chroococcus westii (W.West) Boye-Petersen	·	·	·	+	·	·	+	·
Gloeocapsa sp. Kützing	·	+	·	·	·	·	+	·
Gloeocapsa atrata Kützing	·	·	·	·	·	+	+	·
Gloeocapsa bituminosa (Bory) Kützing	·	·	·	·	·	·	+	·
Gloeocapsa punctata Nägeli	·	·	·	·	·	+	·	·
Gloeocapsa rupestris Kützing	·	+	·	·	·	·	·	·
Leptolyngbya foveolarum (Rabenhorst ex Gomont) Anagnostidis et Komárek	·	+	·	·	·	·	·	·
Leptolyngbya fragilis (Gomont) Anagnostidis et Komárek	·	·	·	+	·	+	·	·
Leptolyngbya perelegans (Lemmermann) Anagnostidis et Komárek	·	·	·	·	·	+	·	·
Leptolyngbya scytonemicola (Gardner) Anagnostidis et Komárek	·	·	·	+	·	·	·	·
Lyngbya sp. C. Agardh ex Gomont	+	·	+	+	·	+	+	·
Oscillatoria sp. Vaucher ex Gomont	·	·	·	+	·	·	·	·
Planktolyngbya bipunctata (Lemmermann) Anagnostidis et Komárek	·	·	·	·	·	+	·	·
Planktolyngbya limnetica (Lemmermann) Komárková-Legnerová et Cronberg	·	·	·	·	+	·	·	·
Plectonema cf. *puteale* Hansgirg	·	·	·	+	·	+	+	·
Pseudoanabaena catenata Lauterborn	·	+	·	·	·	·	·	·
Pseudocapsa sp. Ercegović	·	·	·	·	·	·	+	·
Scytonema hofmanni Agardh	·	·	·	+	·	·	·	·
Synechocystis sp. Sauvageau	+	+	+	+	+	+	·	+
EUKARYOTA								
Chrysophyta								
Chlorocloster sp. Pascher	·	+	+	+	·	+	·	+
Cymbella ehrenbergii Kützing	·	·	·	·	·	+	·	·
Elipsoidon sp. Pascher	·	+	·	·	·	·	·	·
Elipsoidon oocystoides Pascher	·	·	·	·	·	·	·	+
Fragilaria pinnata Ehrenberg	·	·	+	·	·	·	+	+
Heterococcus sp. Chodat	·	·	·	·	·	·	+	·

Table 3. Continued.

Species	Cave/Mine							
	Id	Ko	Me	Pe	Pi	Po	Šk	Žu
Heterococcus furcatus Pitschmann	·	·	·	·	·	·	+	·
Monodus sp. Chodat	·	·	+	+	·	·	·	+
Navicula sp. Bory	·	+	·	·	·	·	+	·
Navicula contenta var. *biceps* (Arnott, Grunow in Van Heurck) Cleve	+	+	·	+	·	·	+	+
Navicula gallica var. *perpusilla* (Grun) Lange-Bertalot	·	+	+	·	·	+	·	+
Navicula mutica Kützing	+	+	+	+	·	+	·	+
Nitzschia sp. Hassall	·	·	+	·	·	·	·	·
Pinnularia borealis Ehrenberg	·	·	·	·	·	·	+	·
Chlorophyta								
Apatococcus cf. *lobatus* (Chodat) J.B. Petersen	+	·	·	·	·	·	·	·
Chlorella sp. Beijerinck	+	+	+	+	+	+	+	+
Chlorotetraedron sp. MacEntee	+	·	·	·	·	·	·	·
Gloeocystis polydermatica (Kütz.) Hindák	·	·	·	+	·	·	·	·
Klebsormidium flaccidum Silva, Mattox et Blackwell	+	+	+	·	·	·	·	·
Microthamnion cf. *strictissimum* Rabenhorst	·	·	+	·	·	·	·	·
Muriella sp. J.B Petersen	+	·	·	+	·	·	·	·
Myrmecia sp. Printz	+	·	·	·	·	·	·	·
Pediastrum boryanum (Turpin) Meneghini	·	·	+	·	·	+	·	·
Pseudochlorella sp. Lund	·	·	·	·	·	·	+	·
Pseudoclonium cf. *basiliense* Vischer	+	·	+	+	·	·	·	·
Scenedesmus bijugatus (Turpin) Meneghini	·	·	·	·	+	·	·	·
Scenedesmus obliquus (Turpin) Kützing	·	·	·	·	+	·	·	·
Scotiellopsis sp. Vinatzer	·	+	+	·	·	·	·	+
Stichococcus bacillaris Nägeli	+	+	+	+	+	+	·	+
Stichococcus exiguous Gerneck	·	·	+	·	·	·	·	·
Stichococcus undulatus Vinatzer	+	·	·	·	·	·	·	·
Trentepohlia aurea Martius	·	+	+	+	+	+	+	+

Note: Id–mercury mine Idrija, Ko–Kostanjeviška jama, Me–lead and zinc mine Mežica, Pe–Pekel pri Zalogu, Pi–Pivka jama, Po–Postojnska jama, Šk–Škocjanske jame, Žu–Županova jama

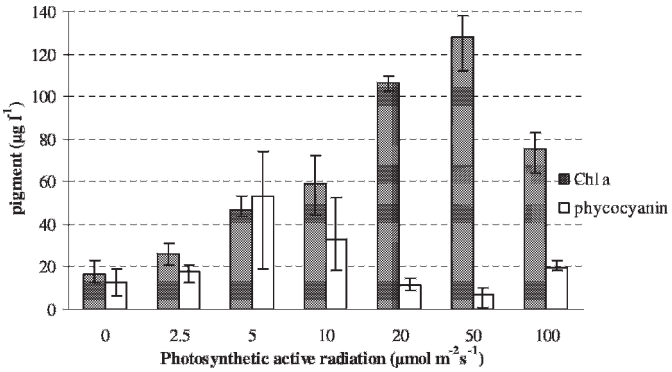

Figure 1. Chl *a* and phycocyanin concentrations at different photon flux densities after 25 days of cultivation of *Chroococcus minutus* in Postojnska jama.

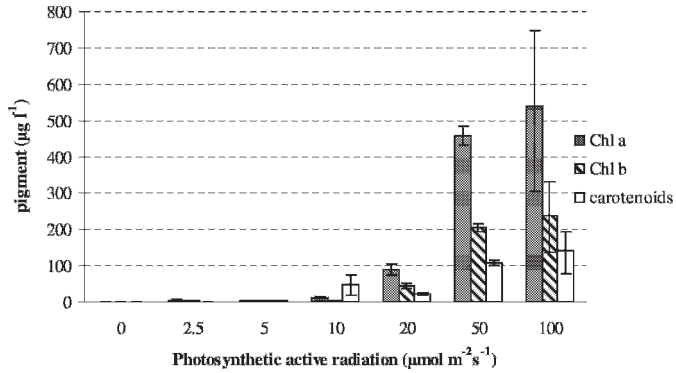

Figure 2. Concentrations of the Chl *a*, Chl *b* and carotenoids at different photon flux densities after 25 days of cultivation of *Chlorella* sp. in Postojnska jama.

overgrown by fast-growing eukaryotic algae. This observation is supported by a 10-times higher increase in cell count of *Chlorella* sp. after 25 days of cultivation under cave conditions compared to *C. minutus* (data not shown), which is similar to the findings of Gaylarde and Gaylarde (2000) who concluded that the first colonizers on the walls of buildings are eukaryotic algae, but cyanobacteria become predominant later in the species succession. This later stage does not develop if the biofilm is frequently removed from the buildings (Gaylarde and Gaylarde, 2000). The best similar example can be seen in the case of the lampenflora community from Škocjanske jame with one of the highest percentage of cyanobacteria (57%) we found, which could be due to the fact that lampenflora have not been removed since the establishment of electric illumination in 1959 (Mulec, 2005). Nevertheless lampenflora algae are usually ubiquitous, fast reproducing, and adaptable soil algae (Rajczy, 1989). Although some authors report lampenflora communities having more eukaryotic algae than cyanobacteria (Faimon et al., 2003), it could be that they sampled lampenflora in the early stage of species succession.

We identified 59 algal taxa from the Schmidlova dvorana cave entrance of Škocjanske jame. A higher number of identified taxa belonging to Oscillatoriales compared to Nostocales confirmed that this cave environment is exposed only to low photon flux density (Table 2). Namely cyanobacteria from the order Oscillatoriales are better adapted to constant low irradiance levels than Nostocales (Albertano et al., 2000). If we take into account that in the cave entrance relative air humidity is constant and only the photon irradiance lowers the deeper we go into the cave, a correlation between irradiance and Chl *a* concentration is expected. Our results showed there is no correlation that indicates a substratum and/or presence of seeping water with available nutrients on sites in karst caves play an important role in the colonization and growth of aerophytic algae. No correlation between irradiance and biomass (Chl *a*) was observed as in the case of lampenflora. However, there is a difference in Chl *a* concentration per surface unit between these two microhabitats. Lampenflora algae demonstrated higher values (max. 2.44 μg cm^{-2}) compared to the algae from cave entrance (max. 1.71 μg cm^{-2}). This difference can be explained due to the different light regime in both microhabitats (i.e., changing light quality and irradiance levels during the day in the cave entrance), different periods of illumination, different *in situ* moisture levels, and different species composition. Generally speaking, areas around lamps have more stable conditions since they are deeper into the constant zone of the cave. However, these values are a few magnitudes lower when compared with the concentrations from non-cave environments like the Niagara cliff where it was 7.3 μg cm^{-2} (Matthes-Sears et al., 1997).

Results presented here indicate that the light is not the only key factor for high or low algal biodiversity. Our results further show that, if we take into account only the cyanobacterial part of the community, coccoid forms tolerate low irradiance more easily; and thus, they represent the major part of the community. Aerophytic algae must develop an intimate interaction with substratum on which they are attached. Which component of the limestone substratum directs or limits algal colonization? In carbonate rocks, several different trace elements can be found in significant concentrations: Al, Ba, Cd, Co, Cu, Fe, K, Mg, Mn, Na, Si, Sr, Ti, U and Zn (Morse and MacKenzie, 1990). Some of these elements could also be lethal for algae or certain groups of algae. In caves, algae often colonize flowstone surfaces. Various minerals can be traced in flowstone in which Co, Cr, Cu, Fe, Mg, Mn, Ni and Zn are the most frequent elements deposited together with the flowstone (Hill and Forti, 1997). Tomaselli et al. (2000) determined that some algae have a preference for a specific substratum to colonize. Nevertheless, one should take into account that in caves various substances in seeping and dripping water can notably influence algal growth.

Physiological adaptations of algae in cave conditions are not well studied. When light is limiting for algal growth the Chl *a* content increases and it decreases when light is not limiting (Meeks, 1974). For cyanobacterium *C. minutus* at cave temperature (9.0 °C), the light saturation lies between 50 and 100 μmol m^{-2} s^{-1}. A similar trend is observed for green alga *Chlorella* sp. at photon flux densities slightly higher than 100 μmol m^{-2} s^{-1}. Based on our experiences from caves, the majority of algae grow generally at photon-flux density much lower than the saturation value and even lower than the compensation point. Diatoms and cyanobacteria have an average compensation point between 5 and 6 and green algae at 21 μmol m^{-2} s^{-1} (Hill, 1996). Algae in caves must include in their survival strategy other ways to obtain energy, like heterotrophy. Giordano et al. (2000) suggests that cells living in caves at low photon irradiance could have a better yield of available photons. To capture as many available photons as possible at low irradiance, cells synthesise accessory photosynthetic pigments. At 9.0 °C and below 10 μmol m^{-2} s^{-1}, the biosynthesis of accessory pigments in two typical aerophytic organisms (*C. minutus* and *Chlorella* sp.) was elevated. With *Chlorella* sp. at values higher than 10 μmol m^{-2} s^{-1}, the ratio of carotenoids to Chl *ab* approached 1:3, which is an expected ratio for green algae. At 100 μmol m^{-2} s^{-1}, a typical ratio Chl *a*: Chl *b* 3:1 for green algae was observed (Kirk, 1983). Different photon irradiance also influences the ratio of Chl *a* vs. phycocyanin in *C. minutus*. At the highest irradiance used in the experiment, the concentration of Chl *a* decreased, but phycocyanin slightly increased. Zilinskas Braun and Zilinskas Braun (1974) explained such a phenomenon with lowered efficiency of energy transfer from phycocyanin to Chl *a*. In the cave habitat, very adaptable algae can prosper if they can use low photon irradiance dosages.

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