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Cyanobacteria and Algae in the Karlamanskaya Cave (Bashkortostan Republic, Russia)

© 2024. M. Yu. Sharipova ORCID: 0000-0003-0907-5984,
I. E. Dubovik ORCID: 0000-0002-0832-6983,
Ufa University of Science and Technology,
32, Zaki Validi St., Ufa, Russia, 450076,
e-mail: Sharipovamy@mail.ru

The article presents data on the composition and structure of cyanobacterial-algal cenoses of various habitats of the Karlamanskaya Cave. The study identified 46 species and intraspecific taxa of cyanobacteria and algae belonging to 5 divisions (Cyanobacteria – 17 species and intraspecific taxa, Bacillariophyta – 12 species and intraspecific taxa, Chlorophyta – 14 species, Charophyta – 1 species, Ochrophyta – 2 species), 7 classes, 16 order, 28 families and 36 genera. *Leptolyngbya boryana* (Gom.) Anagn. et Kom., *Oscillatoria rupicola* (Hansgirg) Hansgirg ex Forti, *Luticola mutica* (Kütz.) Mann, *Mychonastes homosphaera* (Skuja) Kalina et Punc., *Chlorella vulgaris* Beijerink present the dominant complex of cyanobacteria and algae in all studied habitats. Cyanobacteria dominate in all biotopes of the cave except aquatic ones where diatoms predominated. The maximum number of species was found in the soil at the cave entrance and in the soil of the illuminated cave zone: 31 and 29 species respectively. The lowest number was found in the soil and on the walls (16 and 11 species respectively). Ecological analysis of cyanobacterium and algae revealed 12 life forms: $Ch_9B_9P_5X_5CF_5hydr_4amph_2PF_1NF_1C_1M_1H_1$. Most of the life forms were found in the illuminated cave zone: in the soil (10 forms), on the wall (10 forms) and at the cave entrance (11 forms). The ecobiomorphs composition of the dark zone is the poorest (5 forms).

Keywords: karst cave, cyanobacteria-algal cenoses.

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Цианобактерии и водоросли Карламанской пещеры (Республика Башкортостан, Россия)

© 2024. М. Ю. Шарипова, д. б. н., профессор,
И. Е. Дубовик, д. б. н., профессор,
Уфимский университет науки и технологий,
450076, Россия, г. Уфа, ул. Заки Валиди, д. 32,
e-mail: Sharipovamy@mail.ru

В статье приводятся данные о составе и структуре цианобактериально-водорослевых ценозов разнообразных местообитаний Карламанской карстовой пещеры. В результате исследования выявлено 46 видовых и внутривидовых таксонов цианобактерий и водорослей, относящихся к 5 отделам (Cyanobacteria – 17 видовых и внутривидовых таксонов, Bacillariophyta – 12 видовых и внутривидовых таксонов, Chlorophyta – 14 видов, Charophyta – 1 вид, Ochrophyta – 2 вида), 7 классам, 16 порядкам, 28 семействам и 36 родам. Доминантный комплекс цианобактерий и водорослей во всех изученных местообитаниях представлен видами: *Leptolyngbya boryana* (Gom.) Anagn. et Kom., *Oscillatoria rupicola* (Hansgirg) Hansgirg ex Forti, *Luticola mutica* (Kütz.) Mann, *Mychonastes homosphaera* (Skuja) Kalina et Punc., *Chlorella vulgaris* Beijerink. Представители цианобактерий доминируют во всех биотопах пещеры кроме водных, где преобладают диатомеи. Максимальное число цианобактерий и водорослей обнаружено в грунте при входе в пещеру и грунте освещённой зоны: 31 и 29 видов соответственно. Наименьшее – в грунте и на стенах (16 и 11 видов). Экологический анализ цианобактерий и водорослей выявил 12 жизненных форм: $Ch_9B_9P_5X_5CF_5hydr_4amph_2PF_1NF_1C_1M_1H_1$. Большинство жизненных форм были обнаружены в освещённой зоне пещеры: в грунте (10 форм), на стенах (10 форм) и при входе в пещеру (11 форм). Состав экобиоморф темновой зоны самый бедный (5 форм).

Ключевые слова: карстовая пещера, цианобактериально-водорослевые ценозы.

Caves provide special conditions for the communities of living organisms forming, including cyanobacteria and algae [1, 2]. The cyanobacteria-algal cenosis (CACs) developing in these spaces inhabits extreme biotopes. Low temperature and lack of illumination are its main distinguishing parameters and the limiting factor for the multi-species ecosystems forming

[3–5]. In addition, it was noted that the photic cave zones are mosaicity [6].

The study of caves' cyanobacteria and algae has been carried out in many countries. The data was summarized in a large number of publications [3, 5, 7–10]. Cyanobacteria and algae of the Shulgan-Tash cave and some karst caves of the Bashkirskiyi Ural Biosphere Reserve have been well studied in the southern Urals to date [8, 11, 12]. By 2002 in the caves of the southern Urals, 95 species and intraspecific taxa of cyanobacteria and algae were identified. They belong to 4 divisions: Cyanoprokaryota, Chlorophyta, Bacillariophyta and Xanthophyta. *Anabaena constricta* (Szaf.) Geitl., *Nostoc punctiforme f. populorum* (Geitl.) Hollerb., *Chlorella vulgaris* Beijerink, *Bracteacoccus minor* (Chodat.) Petrova, *Stichococcus minor* Nägeli and *Hantzschia amphioxys* (Ehr.) Grun. were found most frequently in studied caves [8]. Despite this, the biota of many unique caves remains unexplored.

The aim of our study was to identify the CACs composition and structure in various habitats of the Karlamanskaya karst cave in the summer, as well as to establish possible types of CACs associated with certain biotopes. We obtained observations including the complete species composition of cyanobacteria and algae for each biotope of Karlamanskaya cave for the first time.

Objects and methods of research

Characteristic of the cave. Karlamanskaya cave is located in the Republic of Bashkortostan, Karmaskalinsky region, in the Urshak-Belsk interfluve, in the basin of the Karlaman karst River at the base of a high gypsum rock Ulu-Tau. Corridor-type cave laid in gypsum of the Kungurian stage. Two entrances (one blocked) start at the base of a sheer cliff of Ulutau on the right slope of the Sagylelga dry valley (Karlamanka river system). Its length is 269 m, area – 1153 m^2, volume – 1970 m^3, amplitude – 6 m, and temperature – +7 °C. The extension is southeastern. The walls and ceilings have corrosive gutters and cavities. The floor is clay. It is the standard of young karst caves. Standard steppes (meadow, petrophyte, and shrub) with the growth of many rare plant species are widespread around the cave [13].

Vakhrushev first described the cave in 1935 [14]. Since 1965, the cave has had the status of a natural monument. Botanists discovered a typical steppe association of herbaceous plants of both endemic and relict forms near the cave on a high steep slope. Thus, the area around the Karlamanskaya cave is also a botanical nature monument [11]. The remaining entrance to the cave is an L-shaped bend that leads into a tunnel with sheer walls and an almost horizontal ceiling. The horizontal cave course runs parallel to the Sagylelga River valley that has gone into the karst. The cave is the standard of those cave types that result of the activity of horizontally circulating streams absorbed from the surface. It was formed at the intersection of vertical and horizontal tectonic cracks. Various dissolution forms of aesthetic value are found on the walls and ceiling of the cave.

The cave floor is covered with clay. Ephemeral reservoirs form on the clay surface. There are bats in the cave. The attendance of the cave is from 100 to 1000 people per year. It is not equipped for tourists and has no lighting [14].

Sampling and identification of cyanobacteria and algae. The algological studies of the cave were carried out in August 2019. The cave entrance was illuminated, and the passage was a dark area at a distance of 15 m from the entrance. 45 samples were taken: 30 soil samples in the illuminated and dark zones, 9 wall scrapings in the illuminated and dark zones, 6 water samples from ephemeral reservoirs in the dark zone. In addition, we studied the area in front of the cave entrance and took 12 soil samples. This site was characterized by synanthropic vegetation, represented by a community of low-growing, trampling and grazing-resistant mesophytes and hygrophytes of the *Plantaginetea majoris* R. Tx. et. Preising in R. Tx. 1950 class [15]. *Taraxacum officinale* and *Potentilla anserine* are diagnostic species. Sampling and identification of the species composition of cyanobacteria and algae were carried out by direct microscopy, on the "growth slides" [16, 17] and after cultivating samples in a liquid medium No. 6 [18]. Water from reservoirs was filtered through "Vladipor" MFAS-OS-3 membrane filters (pore diameter – 0.8 μm), which were then placed in a flask with a liquid nutrient medium No. 6. The cultivation plates in a luminostat were illuminated for 12 hours per day with a 2500–3000 lx light intensity (17.9–21.4 μmol photons · m^{-2} · s^{-1}) and temperature of +20 to +22 °C. All cyanobacteriae and algae were identified using a "Mikmed-1" light microscope (maximum magnification without immersion – ×600, with immersion – ×1350). Relative abundance of cyanobacteriae and algae was estimated against a 5-mark scale.

The Sørensen Resemblance Coefficient (SRC) was applied for the species composition similarity:

$$S = 2C / A+B, \quad (1)$$

where C – the number of species common for two communities, A – the number of species in the first cenosis, B – the number of species in the second cenosis.

Species diversity estimated with Shannon index. It calculates by the formula 2:

$$H = -\sum_{i=1}^{n} p_i \ln p_i \quad (2)$$

where p_i – the i-th species proportion in the general population, which is determined by the species proportion in a sample of n_i / N [19].

Sampling and identification keys were consulted as in our previous paper [20]. Cyanobacteria and algae life forms (Ch-, C-, B- et al.) are given in accordance with the generally accepted in soil algology [21–23]. Taxonomy is presented according to AlgaeBase (www.algaebase.org: searched on 20.11.2021) [24].

Results and discussion

As a result 46 species and intraspecific taxa of cyanobacteria and algae, belonging to 5 divisions (Cyanobacteria – 17 species and intraspecific taxa, Bacillariophyta – 12 species and intraspecific taxa, Chlorophyta – 14 species, Charophyta – 1 species, Ochrophyta – 2 species), 7 classes, 16 order, 28 families and 36 genera have been identified in the material collected in the Karlamanskaya Cave (Table 1). The predominance of cyanobacteria in caves was noted earlier [1, 25].

The dominant species of cyanobacterial-algal flora for each of the 6 habitats are shown in Table 2. Diversity in different habitats ranged from 11 to 31 species, averaging 21 taxa. This is a high degree for such extreme habitats and can be explained by the wide cave passages. In spring, meltwater enters the cave via the old channel, and in summer, the cave soil remains filled with water.

Leptolyngbya boryana (Gom.) Anagn. et Kom., *Oscillatoria rupicola* (Hansgirg) Hansgirg ex Forti, *Luticola mutica* (Kütz.) Mann, *Mychonastes homosphaera* (Skuja) Kalina et Punc., and *Chlorella vulgaris* Beijerink represents cyanobacteria and algae dominant complex in all studied habitats. These species have already been repeatedly noted as predominant in abundance and frequency of occurrence for caves, in particular, in our studies of the Shulgan-Tash Cave and the caves of the Bashkirskiy Ural Biosphere Reserve [20].

The highest abundance of species in families was observed in Nostocaceae family – 6 species. The medium abundance of species in genera was not too high – 1–2 species per genus. Two genera of cyanobacteria were differed: *Nostoc* – 4 species, *Leptolyngbya* – 3 species, and one genus of green algae *Chlorococcum* – 3 species. It is the evidence of extreme habitat conditions as about half of all genera are represented by only one species [8].

Various biotopes of the illuminated and dark zones were studied to identify the difference in cenosis composition. The highest number of species was in the soil of the cave entrance and in the soil of the cave illuminated zone: 31 and 29 species, respectively. The highest value of the Shannon biodiversity index was also noted here. This indicates a significant species diversity of the CACs in these biotopes (Table 3). Cyanobacteria predominated in all cave biotopes, diatoms predominated in the water. The highest species diversity was noted in the cave entrance: 13 species of Chlorophyta. However, as we moved deeper into the cave, their diversity decreased to 4–5 species. Algae species of Ochrophyta and Charophyta divisions were only in the cave illuminated zone.

The comparison of the taxonomic composition of algae and cyanobacteria showed the differences in CACs in the studied cave habitats (Table 4). CACs of wall scrapings in the dark and illuminated zones demonstrates the maximum

Table 1

CACs taxonomic spectrum of the Karlamanskaya Cave

Taxon	Class	Order	Family	Genus	Species
Cyanobacteria	1	3	7	11	17
Bacillariophyta	1	6	9	10	12
Chlorophyta	3	5	10	12	14
Ochrophyta	1	1	2	2	2
Charophyta	1	1	1	1	1
Total species	7	16	29	36	46

Table 2

Cyanobacteria and algae dominant complex in the Karlamanskaya Cave

Taxon	Life forms	Abundance of species in different habitats					
		Gi	Gd	Wi	Wd	Wa	E
Phylum Cyanobacteria Class Cyanophyceae							
Order Synechococcales							
Family Leptolyngbyaceae							
Leptolyngbya boryana (Gom.) Anagn. et Kom.	P	5	5	5	5	5	5
Order Nostocales							
Family Nostocaceae							
Nostoc punctiforme f.populororum (Geitl) Hollerb	CF	2	5	3	3	0	2
Nostoc paludosum Kützing ex Bornet & Flahault	CF	3	0	5	0	1	0
Nostoc paludosum f. longius Kossinsk.	CF	5	0	0	0	3	0
Trichormus variabilis (Kütz. ex Born. et Flah.) Kom. et Anagn.	CF	4	5	0	0	0	5
Family Oscillatoriaceae							
Oscillatoria rupicola (Hansgirg) Hansgirg ex Forti	P	5	5	4	3	1	2
Phylum Bacillariophyta							
Class Bacillariophyceae							
Order Naviculales							
Family Diadesmidaceae							
Luticola mutica (Kütz.) Mann	B	5	4	5	4	0	5
Family Naviculaceae	B						
Mayamaea atomus (Kützing) Lange-Bertalot	B	5	2	0	0	0	0
Order Bacillariales							
Family Bacillariaceae							
Hantzschia amphioxys (Ehr.) Grun.	B	5	3	0	0	0	1
Nitzschia palea (Kütz.) W. Sm.	B	2	5	3	3	3	1
Phylum Chlorophyta							
Class Chlorophyceae							
Order Chaetophorales							
Family Chaetophoraceae							
Gongrosira debaryana Rabenhorst	amph	4	0	0	0	0	5
Order Sphaeropleales							
Family Mychonastaceae							
Mychonastes homosphaera (Skuja) Kalina et Punc.	Ch	5	5	5	5	5	5
Order Chlamydomonadales							
Family Chlorococcaceae *Chlorococcum infusionum* (Schrank) Meneghini	Ch	5	0	0	0	5	4
Chlorococcum minutum R.C.Starr	Ch	0	0	0	0	0	4
Class Trebouxiophyceae							
Order Chlorellales							
Family Chlorellaceae							
Chlorella vulgaris Beijerink	Ch	5	5	5	4	5	4
Muriella terrestris J.B.Petersen	Ch	5	2	1	1	4	3

Note (here and further): Gi – soil samples of the illuminated zone, Gd – soil samples of the dark zone, Wi – wall scrapings of the illuminated zone, Wd – wall scrapings of the dark zone, Wa – water, E – soil samples of the cave entrance.

Table 3

Number of species and species diversity of CACs in different cave biotopes

Taxon	Biotopes					
	Gi	Gd	Wi	Wd	Wa	E
Cyanobacteria	15	7	7	4	4	10
Bacillariophyta	6	5	3	3	6	6
Chlorophyta	8	4	5	4	6	13
Ochrophyta	–	–	1	–	–	2
Charophyta	–	–	1	–	–	–
Total species	29	16	17	11	16	31
Shannon Index	1,31	0,68	1,0	0,91	0,78	1,36

Note: "–" – undetected.

Table 4

The comparison of the taxonomic composition (%) of various habitats in the Karlamanskaya Cave

Soil samples from the illuminated zone	66				
Wall scrapings from the illuminated zone	54	43			
Wall scrapings from the dark zone	60	40	70		
Water	37	40	60	60	
Soil samples from the cave entrance	56	70	46	48	34
	Soil samples from the dark zone	Soil samples from the illuminated zone	Wall scrapings from the illuminated zone	Wall scrapings from the dark zone	Water

Table 5

Distribution of algae life forms in different cave biotopes

Life forms	Gi	Gd	Wi	Wd	B	E	Total
Ch	6	5	4	3	4	8	9
P	5	2	2	2	2	2	5
PF	1	–	–	–	–	1	1
C	–	–	1	1	1	1	1
NF	1	–	–	–	–	1	1
B	5	5	2	2	2	6	9
amph	1	–	1	–	1	1	2
hydr	1	–	1	1	4	–	4
X	2	–	2	–	–	5	5
CF	6	3	2	1	2	3	5
M	1	1	1	1	–	2	2
H	–	–	1	–	–	1	2
Total	29	16	17	11	16	31	46

Note: "–" – undetected.

similarity, and those in the cave entrance soil and in water – the minimum similarity.

Ecological analysis of cyanobacterium and algae revealed 12 life forms: $Ch_9B_9P_5X_5CF_5$ $hydr_4amph_2PF_1NF_1C_1M_1H_1$ (Table 5).

The maximum number (12) of life forms were in soil samples from the cave entrance, minimum (5) – in soil samples from the dark zone. Life forms spectra from zones with various illuminations differed slightly, but more species of CF-, Ch- and P-forms identified in the illuminated zone (Table 5). P-forms are drought-tolerant and "prefer bare areas of mineral soil", CF-forms are nitrogen fixing, Ch-forms are unicellular and colonial green and partly Ochrophyta algae that live in the soil, but under favorable humidity they grow on the soil surface. These species are characterized by exceptional resistance to various extreme conditions and are usually classified as ubiquists.

Summary

Examining of 45 samples from the Karlamanskaya Cave revealed quite high species diversity of algae and cyanobacteria with 46 species and intraspecific taxa. *Leptolyngbya boryana, Oscillatoria rupicola, Mychonastes homosphaera, Chlorella vulgaris, Muriella terrestris, Nitzschia palea* were constant in the studied cave. Cyanobacteria prevailed in all cave biotopes cave except aquatic, where diatoms predominated. A total of 12 life forms have been identified. The most common were the Ch-forms – unicellular immobile Chlorophyta and Ochrophyta, resistant to various adverse conditions and widespread in nature, as well as B-forms – unicellular Bacillariophyta of aquatic and wet substrates.

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