Microbiota of Aquatic and Terrestrial Habitats of the Dzou Cave

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Abstract. Microbiota of the deep caves has been poorly explored. The most relevant issues of the caves biodiversity are the sources of the microorganisms’ propagules and characteristics of autochthonous microbiota. The investigation presents the assessment of microbiota biodiversity from the Dzou cave (Western Caucasus). The aim of the study was to identify the species composition of micromycetes and phototrophs of the cave and to assess the sanitary-indicative microbiota in different habitats depending on the anthropogenic load. Microscopic and cultural methods were used to identify the microbiota from the entrance zone of the cave, as well as from aquatic and terrestrial habitats of the unlit deepest parts of the cave. The analysis of the phototrophic communities composition developed in the entrance area of the cave was carried out, 22 species were identified, among which cyanobacteria prevailed. The biodiversity of micromycetes was determined: 48 species were identified in the entrance zone, 60 species – in the dark zone of the cave. Comparison of the species composition of the dark and illuminated zones of the cave showed that in addition to species \textit{Humicola grisea}, \textit{Hemicarpenteles ornatus} and \textit{Alternaria sp.}, all species of entrance area are found in the unlit part of the cave. Representatives of genus \textit{Penicillium} and \textit{Aspergillus} were dominants. Revealed increase of the micromycetes and bacteria number was driving by increase in the level of anthropogenic load, especially in places of tourist camps. It was noted that the propagules of phototrophs are present in the cave substrates even at great depth, excluding some water samples, which may indicate the periodic drift of propagules or anthropogenic factor. The largest number of micromycetes species was detected in the clay deposits and rock samples. The least number of species was isolated from the water streams of the cave. A large number of micromycetes propagules was found in substrates near tourist camps, but their biodiversity was lower than in cave soils.

1. Introduction
The popularity of extreme leisure activities leads to an increased interest in speleotourism and speleology. As a result, the anthropogenic load on both mountain ecosystems and subterranean karst ecosystems is intensifying, while there are practically no studies to assess the stability of underground ecosystems and the permissible anthropogenic load. Considering the role of karst massifs in
hydrological processes, there is a question of preserving and maintaining them in the optimal functional mode. In some cases, when subterranean cavities have been subjected to significant anthropogenic impact, there is a need for special measures for the unique cave ecosystems rehabilitation [1]. The accumulation of waste, as well as the excess of values of sanitary-indicative microbiota in hard-to-reach caves, were noted [2,3].

The entrance areas of the caves can be considered as ecotones and can serve as refugia. Characterized by more stable climatic conditions, compared to the surface, the entrance areas of the caves are illuminated and colonized by photosynthetic species. Deep entrance wells of caves with prevalence in communities of cyanobacteria and algae represent special habitats. As rule, phototrophs in the form of visual fouling in the unlit (dark) zone of caves are absent. However, lampenflora is formed in excursion caves equipped with stationary lighting. This is an indication of the species transport from the surface to the cave [4,5]. Bacteria and micromycetes represent heterotrophic component in the communities of phototrophs of both the entrance zone and the lampenflora [6-8]. The largest number of microbiota studies was conducted in caves equipped for sightseeing purposes, caves with unique paleolithic paintings, as well as caves with natural monuments status [9-12]. Hard-to-reach caves are least studied, although in recent years there have been several works devoted to the study of such objects [13,14].

One of the major cave systems is the Dzou cave, located on the Arabica massif (Caucasus). The depth of the cavity is 1090 meters at the moment. The cave was discovered in 1983 by a team of Moscow speleologists led by E. Starodubov. Since 1992 the cave has been actively explored by various cave commands. During this time, passages were completed and a topographic map of 4.7 km of cave passages was made. However, biodiversity research was not performed in the Dzou cave.

The aim of the present study was to identify the species composition of micromycetes and phototrophs and the assessment of sanitary-indicative microbiota in various habitats of the Dzou cave, depending on the anthropogenic load.

2. Materials and Methods

The Dzou cave is located in the Northern part of the Arabica massif, composed of upper Jurassic and lower Cretaceous limestone [15]. The height of the entrance is 2240 meters above sea level (N43° 26.607' E40° 22.894').

Aquatic habitats appear in the cave at a depth of about 330 m. The constant water stream with flow rate of 20 l/sec is observed from a depth of 570 m. According to observations of speleologists, conducted during cave visits, the low-water flow rate can reach 300-500 l/sec at a depth of 1020 m [16].

The air temperature in the cave varies at different depths. According to Lavrov [16] the summer air temperature reached 3.3° C at a depth of 700 meters and 4.0° C at a depth of 1000 m (hall of Abkhazia), 3.9° C at a depth of 1020 m near the main water stream. The water temperature in the main stream during the low-water period was unstable and varied from 3.1° C in the stream at a depth of 700 m to 4.0° C in the river at a depth of 1020 m.

In August 2016, samples of cave substrates (clay sediments and rocks) and water samples were taken from different parts of the cave, as well as samples of phototrophic communities from the entrance well, which were re-selected in June 2017. Substrate samples were collected in the area of underground base camps of tourists, areas remote from visits, more or less dry vaults of the cave and along the underground river. During the study, 28 samples were taken from the entrance zone of the cave and 34 samples from the dark zone of the cave. Sampling sites are present on the cave plan (figure 1).

Samples of communities, cave substrates and water were placed in sterile vials and analyzed in a laboratory. At the sampling sites, the temperature was measured by electronic devices with an error of 1%. Acidity of water was determined using the pH meter "Expert-001", sediments were measured in aqueous extract according to the standard procedure [17].
Examined of phototrophs from fouling communities was carried out with use of methods of light microscopy (Leica DMLS (Germany) and Biolam MBS-9 (Russia) at magnification of 1200-1500). Gromov medium №6, Bristol medium and extract from substrates (similar to soil extraction) were used for algae and cyanobacteria cultivation. The liquid and agarose medium were applied. The exposure was carried out at a temperature of 11 and 25° C and illumination of 30-40 µmol×m⁻²×s⁻¹. The method of glass fouling was carried out at the primary cultivation. For a more complete identification of the species composition of phototrophs from the illuminated zone, medium samples from communities were cultured in Gromov No. 6 liquid medium. In addition, in order to identify the propagules of photosynthetic organisms brought into the caves by water streams, the specimens of substrates and water from the unlit zone were cultured in Gromov No. 6 liquid medium and Bristol medium. Preliminary the water was filtered with use of nuclear filters, and in consequence the filtrate was introduced in a culture medium [18]. The cultivation time was 11 months. Identification of algae and cyanobacteria was performed with use of the following keys [19-26]. Systematics of cyanobacteria and algae is given by [27]. Mosses were determined by Ignatov, Ignatova, [28], lichens by Andreev [29]. The abundance of phototrophs was evaluated using 5-point scale [30].

**Dzou Cave**

Western Caucasus, Arabica

until 700 m: by V. Kiselev
after 700 m to 1077 m: by D. Provalov,
O. Klimchuk

Instruments: Suunto compass, clinometer

location of sampling sites

**Figure 1.** Plan of the Dzou Cave showing locations of sampling sites.
The total microbial number in water and substrates was revealed by the method of staining with DAPI dye, a luminescent microscope Zeiss Axiostar plus was used to observe the cells [18]. Bacteria of the *Escherichia coli* group were identified by membrane filters method applying the Endo medium at 37°C. An oxidase test and Gram stain was performed for lactose-positive colonies [18].

*Clostridium perfringens* bacteria were determined on Wilson-Blair medium at 43°C [18]. The number of microorganisms was expressed in colony-forming units by the weight of dry substrate (clay deposits) [18].

Substrates samples in the first dilution were used to identify the micromycetes. In addition, the method of fouling was used [18]. Chapek and Chapek-Dox (concentration of sucrose 0.3%) mediums and potato glucose agar, soil extract and starvation medium [18] were used. Cultivation of micromycetes was carried out at a temperature of 4, 12 and 24°C, accounting for grown colonies and isolation of pure cultures was carried out every week at low temperature. The cultivation time was at least 4 weeks (maximum 12 weeks) [5]. Samples were stored and grown in the dark. Identification of micromycetes was carried out using the following keys [31-37]. Systematics of micromycetes and lichens is given using the database [38]. Statistical processing was performed in the program Excel.

3. Results and Discussion

3.1. Entrance Area of the Cave

In the illuminated entrance zone, the flora included representatives of Bryophyta – 2 species (10% of all reported species, 1 class, 1 order, 2 families, 2 genera), Cyanobacteria – 12 species (57%, 1 class, 4 orders, 8 families, 10 genera), Bacillariophyta – 4 species (19%, 1 class, 4 orders, 3 families, 4 genera), Chlorophyta – 3 species (14%, 1 class, 2 orders, 2 families, 3 genera). Representatives of Magnoliophyta and Pteridophyta departments were not found. The analysis of the species abundance did not reveal any dominants (tables 1 and 2).

**Table 1. Photosynthetic species of the entrance area of the Dzou cave.**

| Species | The score of abundance | The species found in the samples, depth, m aquatic habitats | terrestrial habitats |
|---------|------------------------|----------------------------------------------------------|---------------------|
| **Empire Prokaryota** | | | |
| **Phylum Cyanobacteria** | | | |
| **Order Nostocales** | | | |
| *Nostoc commune* Vaucher ex Bornet & Flahault, 1888 | 3 | | |
| *Nostoc microsopicum* Carmichael ex Bornet & Flahault, 1886 | 3 | 300 | |
| *Trichormus variabilis* (Kützing ex Bornet & Flahault) Komárek & Anagnostidis, 1989 | 2 | | |
| *Scytonema sp*. | 2 | | |
| *Scytonema ocellatum* Lyngbye ex Bornet & Flahault, 1886 | 2 | | |
| *Tolypothrix tenuis* Kützing ex Bornet & Flahault, 1886 | 2 | | |
| **Order Synechococcales** | | | |
| *Leptolyngbya tenuis* (Gomont) Anagnostidis & Komárek, 1988 | 2 | | |
| **Order Chroococcales** | | | |
Aphanothece saxicola Nägeli, 1849

Gloeocapsa rupestris (Lyngbye) Bornet in Wittrock & Nordstedt, 1880

Order Oscillatoriales

Cyanothecae aeruginosa (Nägeli) Komárek, 1976

Kamptothecae chlorinum (Kützing ex Gomont) Strunecký, Komárek & J.Smarda, 2014

Oscillatoria tenuis C.Agardh ex Gomont, 1892

Table 2. Photosynthetic species of the entrance area of the Dzou cave.

| Species                                      | The score of abundance | The species found in the samples, depth |
|----------------------------------------------|------------------------|----------------------------------------|
|                                              |                        | aquatic habitats                       |
|                                              |                        | terrestrial habitats                   |

**Empire Eukaryota**

**Phylum Bacillariophyta**

**Order Naviculales**

Navicula sp. 2 - 340

Humidophila contenta (Grunow) Lowe, Kociolek, J.R.Johansen, Van de Vijver, Lange-Bertalot & Kopalová, 2014 3 900-1000 inflow «Chuma» -

Luticola nivalis (Ehrenberg) D.G.Mann in Round, R.M.Crawford & D.G.Mann, 1990 1 - -

**Order Mastogloiales**

Achnanthes sp. 1 - -

**Phylum Chlorophyta**

**Order Chlorellales**

Chlorella vulgaris Beyerinck [Beijerinck], 1890 3 1000 inflow, 1040, lake 170, 330-340, 430, 600, 1000, 1077

**Order Prasiolales**

Desmococcus olivaceus (Persoon ex Acharius) J.R.Laundon, 1985 2 600 -

Stichococcus bacillaris Nägeli, 1849 2 - -

**Bryophyta**

**Order Hypnales**

Stereodon pallescens (Hedw.) Mitt, 1859 2 - -

Sciurohypnum starkei (Brid.) Ignatov et Huttunen, 2003 2 - -

Mosses’ Protonema 2 - 170, 330-340, 430, 600, 1000, 1077

**Phylum Lichinomycetes**
In the phototrophic biofouling zone, 57 species of microscopic fungi were isolated from the substrates, including one lichen, *Lichinella* sp. Zygomycota included 9 species from 4 genera, which accounted for 16% of the total species diversity of micromycetes in the illuminated zone. Ascomycota included 48 species from 10 genera, which accounted for 84% of the total number of species. The richest genera were *Penicillium* with 19 species accounted, including associated teleomorphs (33% of the total composition of micromycetes input zone), followed by *Aspergillus* with 13 species (23%), *Cladosporium* and *Fusarium* with 4 species for each (7%), *Mucor, Mortierella* and *Trichoderma* with 3 species for each (5%) (table 3).

The predominance of Cyanobacteria revealed in the composition of phototrophic communities in the entrance zone in summer is typical for the conditions of lower air and substrates humidity (Popović et al., 2017). Differences between species composition in the various years were not found.

**Table 3.** Micromycetes of the Dzou cave.

| Species | Cave entrance well | Camps of tourists | aquatic habitats | terrestrial habitats |
|---------|--------------------|-------------------|------------------|---------------------|
| Mucoromycota |                     |                   |                  |                     |
| Absidia coerulea Bainier, 1889 | 1 | 1 | - | 1 |
| Mortierella elongata Linnem., 1941 | 1 | - | - | 1 |
| Mortierella hyalina (Harz) W. Gams, 1970 | 1 | 1 | - | 1 |
| Mortierella minutissima Tiegh., 1878 | 1 | - | - | 1 |
| Mucor circinelloides Tiegh., 1875 | 1 | 1 | - | 1 |
| Mucor circinelloides f. griseocyamus (Hagem) Schipper, 1970 | 1 | 1 | - | 1 |
| Mucor hiemalis Wehmer, 1903 | 1 | 1 | 1 | 1 |
| Rhizopus stolonifer (Ehrenb.) Vuill., 1902 | 1 | 1 | - | 1 |
| Umbelopsis isabellina (Oudem.) W. Gams, 2003 | 1 | 1 | - | 1 |
| Ascomycota |                     |                   |                  |                     |
| Alternaria sp. | - | - | - | 1 |
| Alternaria alternata (Fr.) Keissl., 1912 | 1 | 1 | 1 | 1 |
| Aspergillus candidus Link, 1809 | 1 | 1 | - | 1 |
| Aspergillus clavatus Desm., 1834 | 1 | 1 | - | 1 |
| Aspergillus flavipes (Bainier & Sartory) Thom & Church, 1926 | 1 | 1 | - | 1 |
| Aspergillus fumigatus Fresen., 1863 | 1 | 1 | 1 | 1 |
| Aspergillus nidulans (Eidam) G. Winter, 1884 | 1 | 1 | 1 | 1 |
| Aspergillus niger Tiegh., 1867 | 1 | 1 | 1 | 1 |
| Species                                      | Author                  | Year |
|----------------------------------------------|-------------------------|------|
| Aspergillus flavus var. oryzae (Ahlb.)       | Kurtzman, 1986          |      |
| Aspergillus reptans                         | Samson & W. Gams, 1985  |      |
| Aspergillus sydowii (Bainier & Sartory)      | Thom & Church, 1926     |      |
| Aspergillus sulphureus (Fresen.)             | Wehmer, 1901            |      |
| Aspergillus terreus                         | Thom, 1918              |      |
| Aspergillus versicolor (Vuill.)              | Tirab., 1908            |      |
| Aspergillus wentii                          | Wehmer, 1896            |      |
| Aureobasidium pullulans (de Bary) G. Arnaud | 1918                    |      |
| Botrytis cinerea                            | Pers., 1794             |      |
| Chaetomium globosum                         | Kunze ex Fr., 1829      |      |
| Cladosporium cladosporioides (Fresen.)       | G.A. de Vries, 1952     |      |
| Cladosporium gossypicola                    | Pidopl. & Deniak, 1953  |      |
| Cladosporium herbarum (Pers.)                | Link, 1816              |      |
| Cladosporium sphaerospermum Penz., 1882      |                         |      |
| Fusarium culmorum (W.G. Sm.) Sacc., 1895    |                         |      |
| Fusarium equiseti (Corda) Sacc., 1886        |                         |      |
| Fusarium lateritium                         | Nees, 1817              |      |
| Fusarium oxysporum Schltl., 1824            |                         |      |
| Hemicarpenteles ornatus (Raper, Fennell & Tresner) Arx, 1974 | -                       |      |
| Humicola grisea Traena, 1914                 |                         |      |
| Paecilomyces variotii                       | Bainier, 1907           |      |
| Penicillium adamentzii K.M. Zalessky, 1927   |                         |      |
| Penicillium canescens Sopp, 1912             |                         |      |
| Penicillium citrinum                         | Thom, 1910              |      |
| Penicillium chrysogenum                      | Thom, 1910              |      |
| Penicillium vulpinum (Cooke & Massee)        | Seifert & Samson, 1985  |      |
| Penicillium cyanescum (Bainier & Sartory)    | Biourge, 1923           |      |
| Penicillium cyclopium Westling, 1911         |                         |      |
| Penicillium glandicola                      | Seifert & Samson, 1985  |      |
| Penicillium funiculosum                      | Thom, 1910              |      |
| Penicillium corylophilum Dierckx, 1901       |                         |      |
| Penicillium simplicissimum                   | Thom, 1930              |      |
3.2 Main Part of the Cave

During the analysis, the air temperature in the cave gradually increased from 3.3 °C at a depth of 300 m to 4.9 °C at a depth of 1000 m. The water temperature varied between 3.4-3.8 °C. The acidity of water was in the range from 7.7 to 8.3, the pH of clay sediments was 7.0–8.4 (table 4).

A total of 60 species of microscopic fungi were revealed in the dark zone of the cave. Zygomycota included 9 species from 4 genera, which accounted for 15% of the total species diversity in the dark zone of the cave. Ascomycota included 51 species from 12 genera, which accounted for 85% of the total number of species. The largest species diversity was found to be genera *Penicillium* with 19 species, including associated teleomorphs (32% of the total number of species of micromycetes dark zone of the cave), and *Aspergillus* - 13 species (22%), genus *Cladosporium*, *Mortierella* and *Fusarium* included 4 species for each (7%), *Mucor* and *Trichoderma* 3 species (5%) (table 3).

Comparison of the species composition of the dark and illuminated zones of the cave showed that in addition to species *Humicola grisea*, *Hemicarpentes ornatus* and *Alternaria sp.*, all of the species found in illuminated entrance zone were detected in deep dark part of the cave. This may be a result of introducing of fungal propagules into the cave by water and air streams [39] and also this fact may testify to the similarity of conditions in both the entrance area and the main part of the cave. It can be assumed that fungal biota of the main part of Dzou cave consists of species isolated from the surface above the cave, including the area of potential catchment, as revealed in other caves [40,41].

Representatives of genus *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium genera* are often isolated from plant substrates, whereas genus *Humicola*, *Mucor*, *Paecilomyces*, *Penicillium*, *Rhizopus* are typical soil species [42]. It is possible that several paths of propagation of propagules are realized in the cave, both with air currents, and with particles of soil and plant debris. Powerful water streams entering the cave in the period of flood are able to transfer large masses of organic matter. This organic matter can be distributed over long distances and its stocks provides the nutrition for fungi [39].

| *Penicillium lanosum* Westling, 1911 | 1 | 1 | - | 1 |
| *Penicillium multicolor* Grig.-Man. & Porad., 1915 | 1 | - | 1 | 1 |
| *Penicillium janczewskii* K.M. Zalessky, 1927 | 1 | 1 | - | 1 |
| *Penicillium simplicissimum* (Oudem.) Thom, 1930 | 1 | - | - | 1 |
| *Penicillium purpureogenum* Stoll, 1923 | 1 | 1 | 1 | 1 |
| *Penicillium roseopurpureum* Dierckx, 1901 | 1 | - | - | 1 |
| *Penicillium waksmaniai* K.M. Zalessky, 1927 | 1 | - | - | 1 |
| *Talaromyces luteus* (Zukal) C.R. Benj., 1955 | 1 | 1 | - | 1 |

3.3.1 Analysis of the Terrestrial Habitats (Table 4)

| Description | Depth, m | Place of selection | Micromycetes | Clostridium perfringens | E. coli group bacteria | Total microbial number | Air temperature | pH |
|-------------|----------|-------------------|--------------|------------------------|-----------------------|-----------------------|----------------|----|
| Entrance well | 0-18 | Wall, rock | - | 0 | 12 | 2x10^7 | - | - |
| 18 m | 18-20 | Floor, ground | 35x10^3 | 35 | 25 | 5x10^8 | - | 8.2 |
| Location                              | Depth (m) | Parameter       | Value 1 | Value 2 | Value 3 | Value 4 |
|---------------------------------------|-----------|----------------|---------|---------|---------|---------|
| Bottom of the well 14 m               | 70-75     | Floor, ground  | 12x10^3 | 4       | 16      | 3x10^8  |
| Old bottom well 19 m                  | 170       | Floor, ground  | 14x10^3 | 19      | 6       | 4x10^7  |
| Camps of tourists 300                 | 330-340   | Wall, rock     | 19x10^3 | 0       | 2       | 2x10^7  |
|                                       |           | Floor, ground  | 23x10^3 | 3       | 8       | 5x10^7  |
| Bottom of the well 48 m               | 430       | Wall, rock     | 12x10^3 | 0       | 6       | 2x10^6  |
| Wall                                  | 600       | Wall, ground   | 82x10^2 | 0       | 6       | 8x10^5  |
| Camps of tourists 700                 | 700       | Wall, ground   | 42x10^2 | 0       | 0       | 4x10^7  |
|                                       |           | Floor, ground  | 13x10^3 | 8       | 2       | 6x10^8  |
| Meander Chuma                         | 900-1000  | Wall, ground   | 12x10^3 | 0       | 0       | 5x10^7  |
|                                       |           | Floor, ground  | 24x10^3 | 0       | 4       | 9x10^8  |
| New influx                            | 1000      | Wall, ground   | 3x10^2  | 0       | 0       | 2x10^8  |
| Hall of Abkhazia                      | 1000      | Wall, rock     | 6x10^2  | 0       | 0       | 2x10^6  |
|                                       |           | Floor, ground  | 18x10^3 | 0       | 3       | 3x10^8  |
| Near the river under the ledge        | 1077      | Wall, rock     | 13x10^3 | 0       | 5       | 3x10^8  |
|                                       |           | Floor, ground  | 42x10^2 | 0       | 8       | 5x10^9  |
| Camps of tourists 1000                | 1000      | Wall, rock     | 23x10^4 | 0       | 8       | 2x10^8  |
|                                       |           | Floor, ground  | 41x10^2 | 0       | 8       | 2x10^9  |
| Kunlegis                              | 1040      | Wall, rock     | 2x10^2  | 0       | 2       | 8x10^3  |
|                                       |           | Floor, ground  | 2x10^2  | 0       | 2       | 3x10^6  |
| Far point                             | 1077      | Floor, ground  | 12x10^2 | 0       | 14      | 5x10^6  |
The greatest species diversity of micromycetes was found in the substrates of the cave. It is interesting to note that the number of species isolated from samples in different parts of the cave was the smallest for water samples, and the greatest for the cave soils, the variety of species on tourists camps was low. Thus, it can be assumed that species diversity near the tourist sites reduces due to the abundance of organic matter. It is possible that culture methods revealed stored propagules of micromycetes in the soil rather than species that actually develop in the cave environment. The isolation of micromycetes in caves is complicated by the use of a standard medium that is not adapted for the cave environment, wherefore it is difficult to assess which species in the selected sample are dominant [42].

Indirect evidence of the implementation of the water and man-made pathways of introduction can serve as detection of phototrophs *Navicula* sp., *Humidophila contenta*, *Chlorella vulgaris*, *Desmococcus olivaceus* and protonema of mosses at considerable depths, including those near underground tourist sites (table 2). Mosses are often found in caves in the form of a protonema [10,43], which requires less light to maintain its vital activity than for algae and cyanobacteria [44].

The maximum number of microorganisms was noted in entrance zone of the cave, the total number of coliform bacteria was increased there, also the clostridia and a high number of micromycetes was noted. Deeper into the cave the total microbial number in the soil and the number of coliform bacteria decreases. Clostridia was found only in areas the tourists camps. The appearance of coliform bacteria in the water flows may be due to the proximity of the surface or a decrease in the filtration capacity of rocks (bare karst, large faults through which water passes or high water pollution at the entrance run off from pastures) (table 5).

**Table 5.** The number of microorganisms in the aquatic habitats (in 1 liter).

| Description                  | Depth, m | Place of selection | Total microbial number | Micromycetes | E. coli group bacteria | Air temperature°C | pH  |
|------------------------------|----------|--------------------|------------------------|--------------|------------------------|-------------------|-----|
| Camps of tourists 300        | 330-340  | Main watercourse   | 2x10⁶                  | 4            | 0                      | 3.3               | 8.0 |
| Stream                       | 500      | Stream             | 2x10⁶                  | 6            | 2                      | -                 | 7.9 |
| River under the ledge        | 570      | Stream             | 5x10⁵                  | 4            | 4                      | -                 | 7.8 |
| Affluent 600                 | 570 (600)| Main watercourse   | 8x10³                  | 2            | 0                      | 3.4               | 8.1 |
| Camps of tourists 700        | 700      | Main watercourse   | 3x10⁷                  | 0            | 0                      | 3.8               | 8.1 |
| Camps of tourists 700        | 700      | Watercourse        | 5x10⁶                  | 2            | 12                     | -                 | 8.3 |
| Meander Chuma                | 900-1000 | Affluent 1         | 6x10⁶                  | 2            | 5                      | 4.9               | 7.8 |
| Meander «Chuma»              | 900-1000 | Affluent 2         | 3x10⁶                  | 3            | 0                      | -                 | 8.0 |
New water after fault 1000 New affluent after fault 3x10⁵ 6 0 - 7,9

Camps of tourists 1000 1000 Main watercourse 2x10⁷ 4 0 - 7,8

River 1030 The beginning of the river 5x10⁵ 0 0 - 7,7

Camps of tourists 1000 1040 Lake (siphon) under camp of tourists 3x10⁷ 14 6 - 7,8

River under the ledge 1077 River under the ledge 7x10⁵ 2 - 7,8

The caves are characterized by an increase in the biodiversity of microorganisms in geochemically heterogeneous areas and the predominance of saprophytic microorganisms in oligotrophic environments [45]. Representatives of Enterobacteriaceae were found in aquatic communities of caves, and the presence of fecal coliforms in the waters of caves and an increase in the total microbial number are associated with anthropogenic load [10,46,47], which is confirmed in this study.

4. Conclusion
An analysis of biodiversity in the entrance zone of Dzou cave revealed the prevalence of cyanobacteria among the other phototrophs, which is typical for the caves. No obvious dominants have been found; in this case we can speak about polydominating among the phototrophs in the entrance zone of the cave. The composition of micromycetes species in illuminated and dark zones of the cave practically coincides, the largest number of species is found in the substrates. An increase in the sanitary-indicative groups of bacteria and propagules of micromycetes was found in areas of the cave with increased anthropogenic load.

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