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Diversity and Significance of Lithobiotic Communities at the Tomskaya Pisanitsa Rock Art Site

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Abstract—The processes of biodeterioration of the unique Tomskaya Pisanitsa monument of rock art in Western Siberia have been studied by a complex of biological and mineralogical methods. The species composition of the lithobiotic community (bacteria, fungi, and lichens) is identified using a complex of cultural, morphological, and molecular genetic methods. It is shown that the destruction of the monument is a result of interrelated physical, chemical, and biological processes, accompanied by a change in the properties of the rock and its biological colonization. The structure of microbial communities depends on the local environment and successional processes. The development of biofilms with the dominance of cyanobacteria is observed on the rock zones of increased moisture and the formation of carbonate crusts. The problems of adaptation of the lithobiotic microorganisms to existence at the Tomskaya Pisanitsa rock art monument, as well as their role in the processes of oxalate and carbonate biomineralization, are discussed. The results point to the danger of deterioration of Tomskaya Pisanitsa monument and indicate the need to find new effective ways to protect it, taking into account the accumulated scientific data.

Keywords: petroglyphs, rock, biofouling, lithobitic community, biodeterioration, biomineralization

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INTRODUCTION

There are many monuments of rock art in Siberia. Their study and preservation can be considered an extremely important task for the scientific community. The Tomskaya Pisanitsa open air museum located on the banks of the Tom River in Yashkinsky raion, Kemerovo oblast, is one such monument. Hundreds of ancient images can be found on several rock planes dating back at least 4 millennia. It was at this monument that the scientific study of rock art began 300 years ago in our country. Tomskaya Pisanitsa became the first museum–monument of this type in Russia, being the center of the Tomskaya Pisanitsa historical, cultural and natural museum reserve. Over decades of archaeological studies, a lot of scientific data has been accumulated on the features and importance of this outstanding monument and on the technique of execution, style, iconography, cultural, and chronological attribution and semantics of images (Okladnikov and Martynov, 1972; Kovtun, 2021; etc.). In recent years, interdisciplinary researches related to the study of the state of preservation and factors causing the destruction of the rock with images have intensified. Specialists in different fields recognize the complexity and undoubted importance of the task of preserving this unique archaeological monument of the Tom region. Solving this problem requires a comprehensive scientific approach, taking into account the peculiarities of the location of the object, the environmental conditions, and the main destructive factors threatening the monument.

One notable feature of the Tomskaya Pisanitsa is the rock itself (Figs. 1a–1d), where the central group of planes with petroglyphs, known as "the Sanctuary," is concentrated. The images are created on large vertical surfaces located at different heights, and the foot is

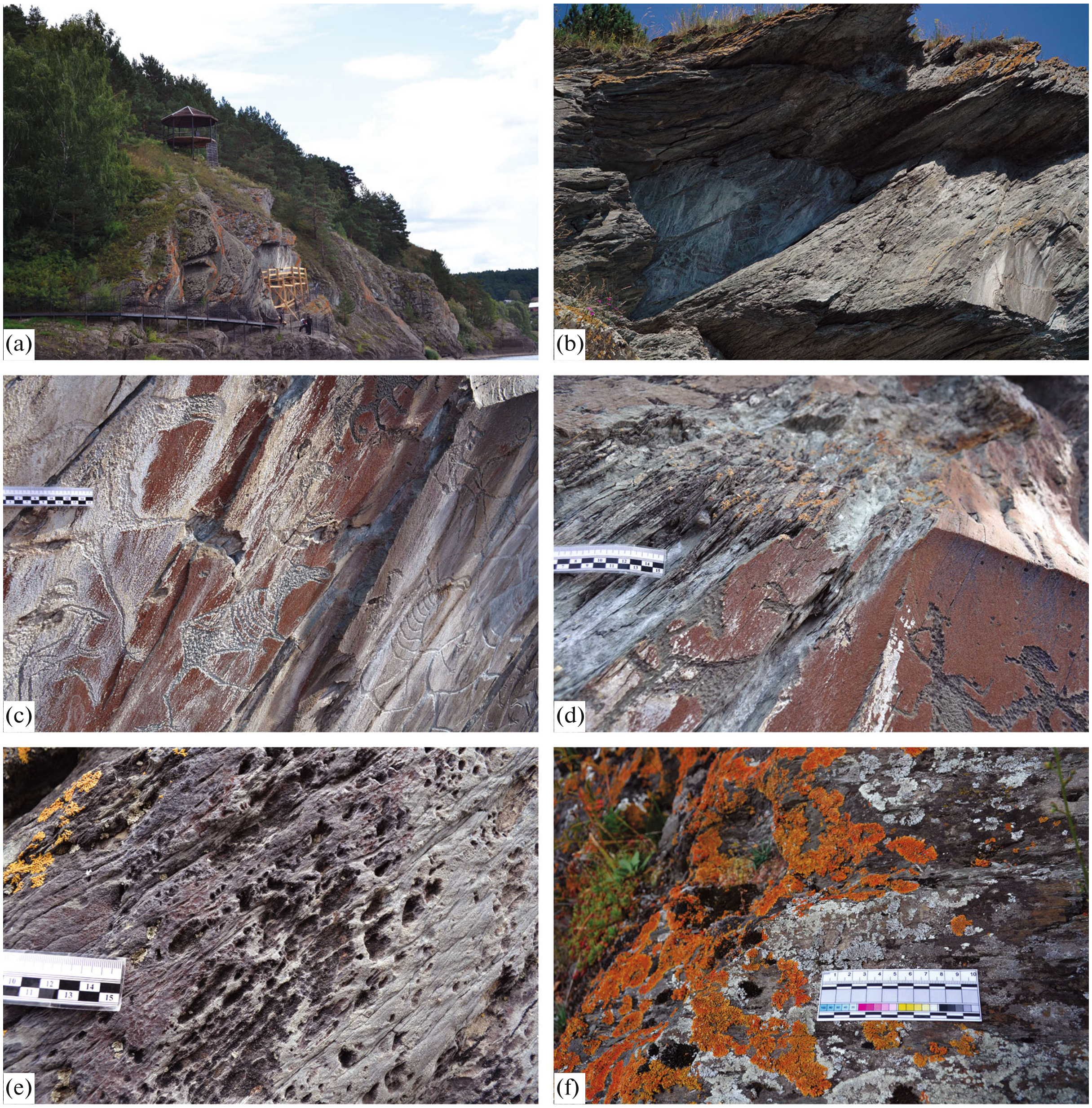

Fig. 1. Tomskaya Pisanitsa archaeological monument: (a) general view of the rock with ancient petroglyphs during the summer period of the survey in 2021, (b) structure of the rock with the planes on which the images are engraved, (c, d) rock images on planes subjected to weathering, (e) biopitting on the rock surface, and (f) biofouling of the rock surface near petroglyphs.

a massive stone step on which many spectators can stand at the same time. The rock is exposed to weathering; the destructive effect of meltwater and rainwater flowing down from the slope above the rock is particularly strong; the lower layer of the rock planes and the foot are periodically flooded with flood waters. The local destruction of the monument is associated with the peculiarities of the location and properties of the rock, the effects of moisture, temperature changes, anthropogenic impact, and the processes of biofouling of rock surfaces (Rebrikova, 2004; Miklashevich, 2011; Miklashevich and Bove, 2011; Lobzova et al., 2014; Shchigorets and Vlasov, 2021). These factors are interrelated, and the biofouling of the monument is a kind of indicator of the changes occurring.

Despite the understanding of the importance of preserving petroglyphs, the studies in the field of biofouling (biodeterioration) of rock art monuments are extremely insufficient, unlike works on the biodeterioration of historical buildings and monuments in an anthropogenic environment. In recent years, some studies have been conducted on several well-known archaeological monuments around the world (Nir et al., 2022; Rabbachin et al., 2022), including rock art monuments in Russia (Sonina and Fadeeva, 2007; Miklashevich and Mukhareva, 2011; Rusakova, 2018; Sazanova et al., 2022). The authors of these publications have shown that rocks with petroglyphs can be inhabited by microscopic fungi, bacteria, archaea, algae, lichens, and seed plants.

Covering the surface of a stone, biological objects damage or destroy it in most cases. This is due to the release of aggressive metabolites that lead to dissolution, the leaching of the rock and secondary crystallization on its surface (biomineralization), and the introduction of organisms into structural spaces and the mechanical destruction of the surface layer of the substrate (Gaylarde and Little, 2022). Special attention is paid to lithobiont microbial communities, which have been studied in recent years using molecular genetic methods (Esposito et al., 2019; Nir et al., 2022; Rabbachin et al., 2022). These studies are aimed at identifying the full spectrum of microorganisms inhabiting the surface of monuments, analyzing their physiological and biochemical properties and features of interaction with a rocky substrate.

Archaeological open-air monuments are primarily colonized by lichens, microscopic algae, and cyanobacteria (Tratebas, 2004). The primary biological colonization of rock with petroglyphs mainly depends on the location of the monument and its moisture and illumination. However, fungi and organotrophic bacteria also play a significant role in the formation of lithobiont communities. Microorganisms are often part of crustlike layers and even desert varnish (Sazonova et al., 2022; Rabukhina et al., 2022). Attempts to remove biological layers using mechanical and chemical methods give only a temporary effect. The recovery of lithobiont communities occurs quickly enough and largely depends on environmental conditions, which has been shown for stone monuments in different environmental conditions (Rusakova, 2018; Bobir et al., 2019).

It is important to note that petroglyphs are a kind of rock carvings created by changing the texture of the stone surface or deepening into it. For this purpose, techniques such as incising, engraving, and grinding, as well as their modifications and combinations, are used. The creation of the image on the stone in such ways already presupposes local damage of the rock surface and creates a special microrelief, which largely determines the subsequent processes of biological colonization of rock carvings. It is noted that the primary colonization of the rock with microbial biofilms is often determined precisely by the microrelief of the stone surface (*The Effect*..., 2019).

The present study is aimed at identifying the biodiversity and functional activity of lithobiont communities, as well as their possible role in the processes of transformation of the rock with petroglyphs at the Tomskaya Pisanitsa rock art site. This goal is related to solving the problem of preserving a unique archaeological monument through a comprehensive study involving methods used primarily by biologists (mycologists, microbiologists, lichenologists, algologists, and biochemists), as well as mineralogists, archaeologists, and restorers.

MATERIALS AND METHODS

Sampling

Studies of the biofouling processes of Tomskaya Pisanitsa and sampling were carried out from 2014 to 2021. The material for research was collected from rock surfaces (planes) with ancient images, as well as on rock sections (model ones) adjacent to the locations of petroglyphs (Fig. 1). The model sites were chosen taking into account their similarity with the planes with images by the biological colonization. Sampling sites were determined taking into account violations of the rock integrity (fragmentation, flaking, and shedding of stone material) and characteristic signs of biological colonization of the rocky substrate (Figs. 1e, 1f). Fragments of damaged rock with biological fouling were selected for a comprehensive study. Samples of biofilms and carbonate crustlike layers for the detection of cyanobacteria were collected in sterile containers (up to 120 mL in volume). In addition, lichen thalloms and carbonate deposits (light-colored crusts without signs of biofouling) were scraped from the rock surface. Part of the samples from the surface of the rocky substrate was taken by nondamaging methods (an imprint on the nutrient medium or a smear with subsequent sowing on the nutrient medium).

In total, over the period from 2014 to 2022, more than 100 biological samples from the Tomskaya Pisanitsa were studied.

Study Methods of the Rock

The polished sections of the underlying rock samples were studied under POLAM-113 and LEICA DM 4500 petrographic microscopes. The qualitative phase composition of the samples was identified using a Bruker D2 Phaser powder diffractometer with a copper anode. X-ray diagrams were obtained at room temperature in the range $2\theta = 5–70°C$. The phase identification was carried out using the ICDD PDF-2 database (release 2016).

Study Methods of Diodiversity and Metabolism of Lithobiont Organisms

Fungi identification

Traditional mycological methods have been used to detect and identify microscopic fungi in biofilms and in a destructing rock. For the primary isolation, maintenance in culture and, identification of micromycetes, a Czapek–Dox culture medium was used, on the surface of which small fragments of crushed rocky substrate with signs of biodeterioration were placed, as well as washings from the rock surface. The grown colonies were counted and identified by a number of morphological characters using common methods.

Bacteria identification

Cyanobacteria were identified by morphological characters using light microscopy (a Leica DM 1000 microscope) after their cultivation in different media: in distilled water for a month, in Z8 and BG-11 nutrient media. Species were verified in accordance with modern nomenclature using the L. (https://isling.org) and AlgaeBase (http://www.algaebase.org/) electronic databases. Some cyanobacteria isolated in pure culture were identified based on the 16S rRNA gene nucleotide sequence. DNA isolation was performed using DNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's manual. Amplification and sequencing of the 16S rRNA gene region was performed using the primers 1 (5'-CTC TGT GTG CCT AGG TAT CC-3') (Wilmotte et al., 1993) and 2 (5'-GGG GGA TTT TCC GCA ATG GG-3') (Nübel et al., 1997).

To identify organotrophic bacteria, DNA isolation from the obtained pure cultures (on the EMH medium, enzymatic meat hydrolysate) was carried out according to the classical CTAB protocol (Doyle, Doyle, 1987). Primers to the V4 region of the 16S rRNA gene were used for identification (Caporaso et al., 2011):

16S-F GTGCCAGCMGCCGCGGTAA

16S-R GGACTACHVGGGTWTCTAAT

Lichen identification was carried out using common anatomical and morphological methods; standard color reactions (spot tests) were carried out to identify taxonomically significant lichen substances (*Flora*..., 2014).

Metagenomic analysis

The weighed portion of each sample was carefully mechanically crushed. The DNA extraction was carried out according to the classical CTAB method (Doyle, J.J. and Doyle, J.L., 1987). Primers from the manual were used in the analysis of the bacterial microbiome by the 16S rRNA gene sequence:

https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

The composition of cyanobacteria was identified using primers that were recommended in the publication (Nübel et al., 1997).

The fungal diversity was identified using the ITS primers from the manual https://www.illumina.com/content/dam/illumina-marketing/documents/products/appnotes/its-metagenomics-app-note-1270-2018-001-web.pdf.

Sequencing was performed on a MiSeq device with a MiSeq Reagent Kit v3 (600-cycle) in a 2*300 paired-end mode of cycles. The data were analyzed using the QIIME2 software package.

Scanning electron microscopy (SEM-analysis)

Scanning electron microscopy was used to identify the features of the distribution and interaction of microorganisms in the surface layer of the stone material to find out the ways they penetrate deep into the rock and detect secondary mineral formations in biofilms. Samples of the damaged rock (0.5–1.0 × 0.5–1.0 cm) were previously examined under a binocular magnifier. The selection criterion for SEM analysis was the presence of microbial structures on the surface, as well as the localization of microorganisms in microzones (heterogeneous areas, cracks, and cavities) of the rock. The studies were performed using a TM 3000 desktop scanning electron microscope (Hitachi, Japan) equipped with an Oxford energy dispersive microanalysis attachment.

Metabolomic analysis

A metabolomic analysis of biofouling samples, as well as of the damaged rock substrate, was carried out to identify the biochemical composition of lithobiont communities. The extraction was performed with cold methanol (15 mL, –25°C). Then the extracts were evaporated at 40°C, dissolved in pyridine, and TMS derivatives were obtained using N, O-Bis(trimethylsilyl)-F-acetamide. The analysis was performed by gas chromatography–mass spectrometry (GC-MS) on a Maestro device (Interlab, Russia) with an Agilent MSD5975 mass selective detector, HP-5MS column, 30 m × 0.25 mm. Chromatography was performed with linear temperature programming from 70 to 320°C (6°C/min). The processing and interpretation of mass spectrometry information was carried out using an AMDIS program (http://www.amdis.net/index.html), a NIST2005 standard library, and a compound standard library (Komarov Botanical Institute, Russian Academy of Sciences). A quantitative interpretation of chromatograms was carried out by internal standardization for tridecane using a UniChrom program (http://www.unichrom.com/unichrome.shtm).

RESULTS

Characteristics of the Rock with Petroglyphs

The results of the study of the underlying rock (optical microscopy and X-ray phase analysis) at the sampling sites of biofouling showed that it is represented by sandstones with thin (3–5 mm) interlayers of argillites (Fig. 2a) and aleurolites (Fig. 2b), as well as their transitional varieties (aleurolite sandstones, etc.), often discontinuous along the strike. The size of clastic grains varies from 0.05 to 0.4 mm, sometimes

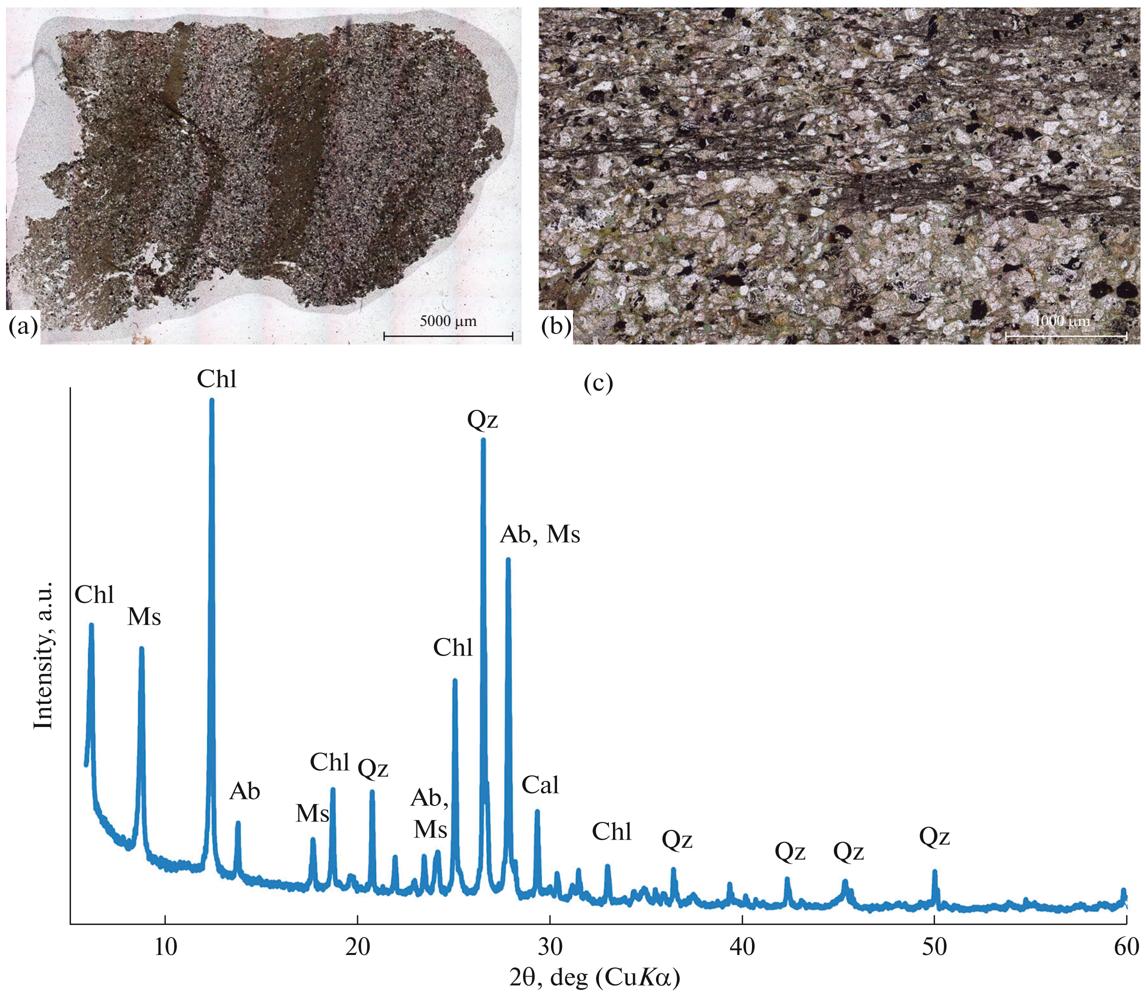

Fig. 2. General characteristics of the rock of the Tomskaya Pisanitsa rock art monument: (a, b) interlayering of sandstones with argillites and aleurolites, respectively; (c) X-ray of a rock fragment. Chl, chlorite-serpentine; Qz, quartz; Ms, muscovite; Ab, albite; and Cal, calcite.

up to 0.6 mm. The main minerals of the rock are quartz, feldspar, calcite, chlorite, micas, hydromicas, ore minerals, etc. (Fig. 2c). Quartz is present in the form of angular, sometimes wedge-shaped and sickle-shaped grains; feldspar, in the form of tabular well-faceted crystals; carbonates and ore mineral, in the form of individual grains and crystal clusters.

The rock also contains small (with a thickness of several millimeters) lens-shaped inclusions of carbonates (Figs. 3a, 3b). A system of numerous branched cracks divides these (mainly calcite) inclusions into blocks of no more than 5 mm in size (Fig. 3c), forming a breccia-like texture. Secondary minerals (ferruginous micas, chlorite, and dolomite) develop along the grains of the main minerals, replacing them partially or entirely, which indicates the occurrence of low-temperature metamorphic processes. The mica-chlorite fine-scaled aggregate often develops along cracks and acts as a cementing mass. Significant fracturing and discontinuity of the rock (Fig. 3d) confirm that it was formed under conditions of tectonic movements, possibly upon the contact of friable carbonate and harder silicate rocks.

Thus, sufficiently strong layers of rock, on which the main petroglyphs are created, are interlayered with soft layers that are subject to the greatest destruction. Such heterogeneity of the rock contributes to its biological colonization and the development of the lithobiont community.

Characteristics of the Lithobiont Community and Biofilm Mineralization

The studies have shown that the biofouling of rocks with petroglyphs is observed mainly in the cracks, depressions, places of moisture movement, and microrelief on the surface of the stone. Microbial biofilms of different compositions are formed here, as well as fouling formed by lichens and spore and seed plants.

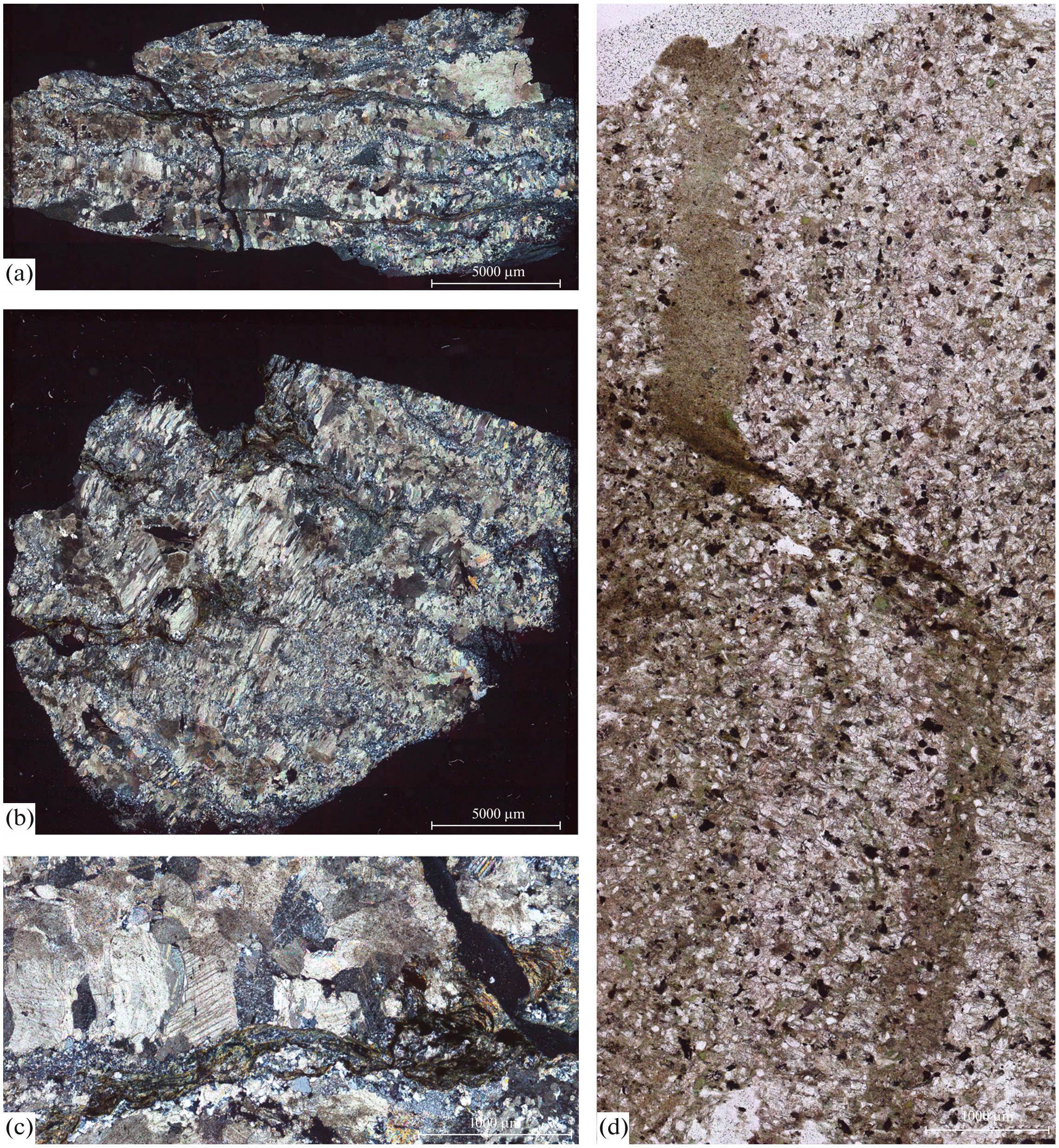

Fig. 3. Calcite breccia: (a, b) general view in two sections, (c) blocks formed by cracks, and (d) rupture and displacement along the crack of argillite interlayer (an example of a violation of the continuity of the rock).

Lichens

Special attention should be paid to lichens, which are able to colonize the planes with petroglyphs, often covering a significant area of the rock. Among them, representatives of the genera *Rusavskia* and *Caloplaca,* which have an orange color and are found on different parts of the rock, are distinguished. Crustose lichens with small thallomas (including those from the genera *Acarospora, Candelariella, Circinaria,* etc.) were found in the areas of the rock paintings themselves. They pose a certain danger to the preservation of petroglyphs, since they have the ability to penetrate deep into the rock and contribute to smoothing the boundaries and the loss of the relief of rock images.

A comparison of the results of surveys in 2014, 2018, and 2021 shows that the maintenance work on the surfaces with petroglyphs leads to a gradual decrease in the abundance of lichens. At the same time, species diversity has changed little over the past period. At the experimental sites (at a short distance from the main rock with petroglyphs), lichens form a continuous cover, which characterizes their ability to intensively colonize stone surfaces in the climatic conditions of Yashkinsky raion, Kemerovo oblast. Below we provide a list of frequently occurring lichens on

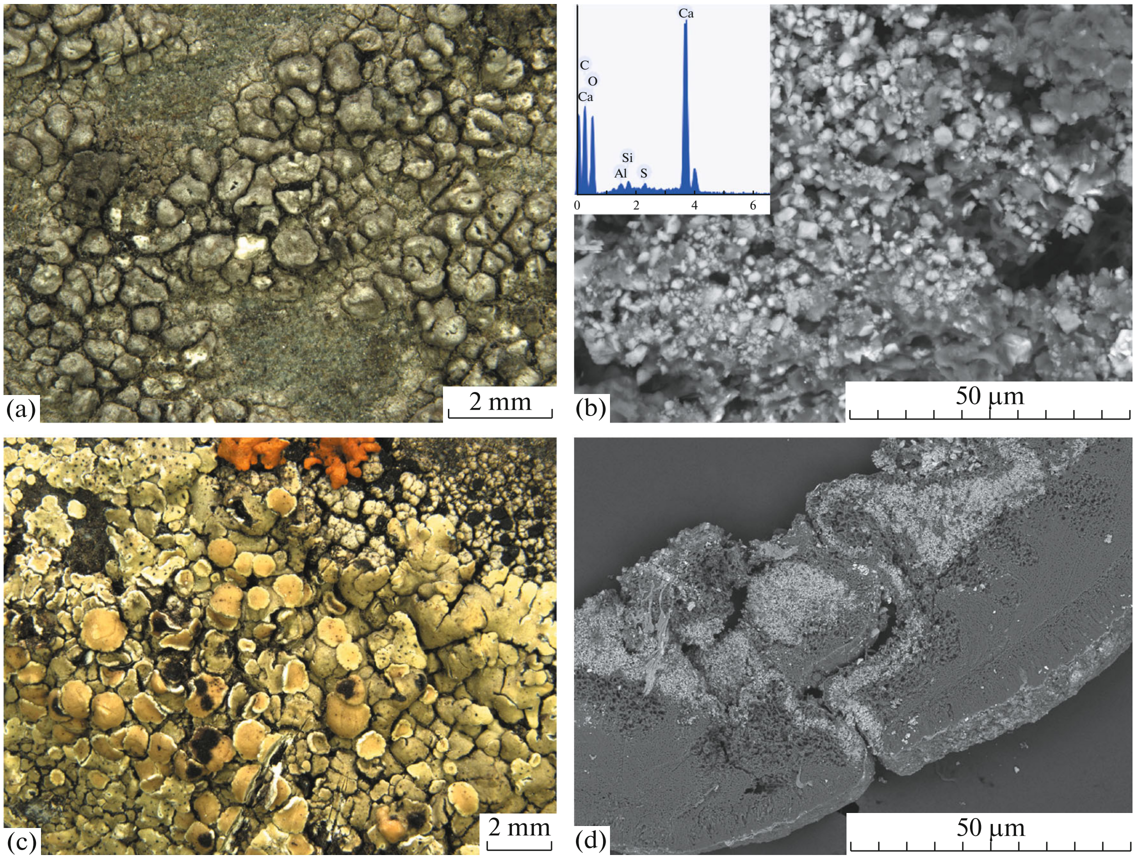

Fig. 4. Lichens containing calcium oxalates on the Tomskaya Pisanitsa rock art monument: (a) lichen *Pertusaria* sp., (b) mass formation of weddellite crystals in lichen *Pertusaria* sp. (SEM image)—the EDX spectrum is shown, (c) lichen *Protoparmeliospis muralis,* and (d) mass formation of calcium oxalates (lighter zones) in the lichen *Protoparmeliospis muralis* (SEM image).

rock outcrops directly adjacent to the planes with petroglyphs, as well as on the planes themselves with images.

(1) *Acarospora moenium* (Vain.) Räsänen

(2) *Caloplaca* sp.

(3) *Candelariella aurella* (Hoffm.) Zahlbr.

(4) *Candelariella vitellina* (Hoffm.) Müll. Arg.

(5) *Circinaria contorta* (Hoffm.) A. Nordin et al.

(6) *Lecidella carpathica* Körb.

(7) *Pertusaria* sp.

(8) *Protoparmeliopsis muralis* (Schreb.) M. Choisy

(9) *Rusavskia elegans* (Link) S.Y. Kondr. & Kärnefelt

(10) *Staurothele frustulenta* Vain.

Calcium oxalates (whewellite, calcium oxalate monohydrate, weddellite, and calcium oxalate dehydrate), as well as substrate minerals, quartz (mainly), feldspar, hydromica (glauconite), serpentine, etc., were identified in lichens *Pertusaria* sp. (Fig. 4a), *Protoparmeliospis muralis* (Fig. 4c), *Circinaria contorta* by the X-ray fluorescence. Weddellite is characterized by dipyramidal-prismatic and dipyramidal crystals ranging from 3 to 10–15 µm in size (Fig. 4b). The crystals have traces of dissolution and splitting. Whewellite is represented by lamellar crystals and their intergrowths ranging from 2 to 5 µm in size. The formation of oxalates is of mass character (Fig. 4d) and characterizes the chemical interactions between lichens and rocky substrate. The production of organic acids, especially oxalic acid, contributes to the effective dissolution of minerals and chelation of metal cations, which leads to the transformation of the surface layer of the rocky substrate (Chen et al., 2000). At the same time, lichens also mechanically damage the rock, which is due to the penetration of mycobiont hyphae into the rocky substrate and the resulting wedging effect.

Micromycetes

Microscopic fungi (micromycetes) also have a significant effect on the processes of biological destruction of the rock, which have also been identified in the samples of damaged rock and biofouling. Dark microcolonies of fungi are visible on the surface of the rock, as well as light surface mycelium (Fig. 5a–5d).

From samples of biofilms and fragments of crumbling sandstone, 21 species of micromycetes were iso-

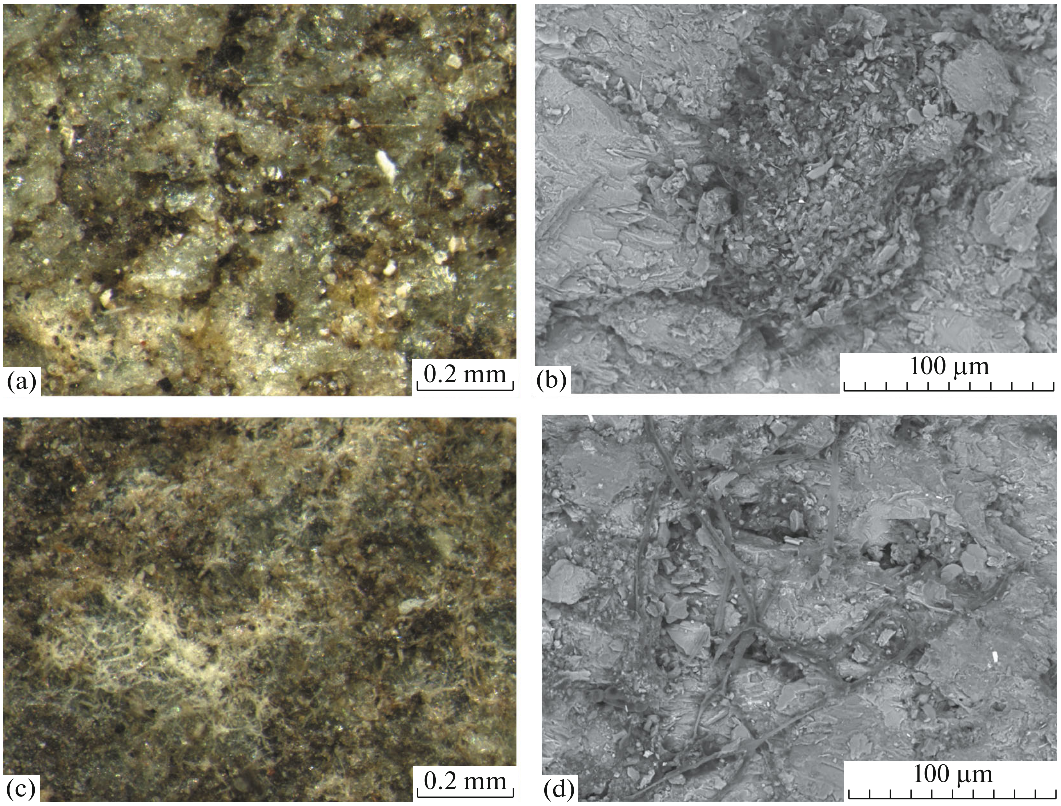

Fig. 5. Micromycetes on the Tomskaya Pisanitsa rock art monument: (a) microcolonies of dark fungi in the depressions of the surface layer, (b) fungal microcolony in the area of surface damage (SEM image), (c) light-colored hyphae of fungi forming a surface coating, and (d) hyphae growth along the microrelief of the surface (SEM image).

lated (Table 1), as well as non-spore-bearing light and dark fungi characterized by high occurrence. The abundance of micromycetes in the samples ranged from 750 to 5800 colony-forming units (CFU) per 1 g of rock. The samples are dominated by the following species of fungi: *Alternaria alternata, Marquandomyces marquandii,* and *Scytalidium lignicola,* which are known as inhabitants of soils and various organic substrates.

To specify the composition of fungi in the fouling of rock surfaces with images and in destructing sandstone, the metagenomic analysis of samples collected at the sites of development of typical surface biofilms and in the zone of deep destruction (flaking and shedding) of stone material, where fungi develop mainly in the structural spaces of damaged rock, was carried out.

The similarity of the compared habitats is in the predominance of dark fungi from the class *Dothideomycetes* and the high occurrence of fungi of the genus *Cladosporium* (dominant in the fungal community) in samples is confirmed by both the cultural method and metagenomic analysis. At the same time, the results also showed marked differences in the composition of micromycetes in biofilms and in decaying rock. Thus, according to the results of the metagenomic study, the proportion of ascomycetes in biofilms was almost 75%, whereas in the damaged rock it was 52.5%. At the same time, the proportion of basidiomycetes was significantly higher in the destructing rock (41.4% vs. 8.2% in the biofilm). More significant differences in the composition of fungi of the compared habitats are manifested already at the level of orders (Fig. 6). Thus, biofilms are dominated by representatives of the order *Pleosporales* (especially species of the family *Didymellaceae,* 44%), which are often associated with plants, whereas in the destructing rock, representatives of the order *Capnodiales* (19%) predominate which are most often referred to as extremophile fungi, characterized by high melanization of cell walls and resistance to external effects. It is among the representatives of the order *Capnodiales* that black yeastlike fungi are most often mentioned, capable of existing for a long time in unfavorable conditions, including colonizing hard-to-reach rocky substrates.

As the rank of the taxon decreases, the differences between the samples increase even more. At the same time, more known biodestructors are observed in the destructing rocks, whereas typically soil species are dominant in the biofilm. Significanjt differences may be due to the fact that moisture is better retained in biofilms on the surface of the rock and organic matter accumulates, which allows mycelial fungi usually living in the soil to actively develop. Unlike the biofilm,

Table 1. Species composition of cultivated microscopic fungi isolated from the sites of biodeterioration of the rock of the Tomskaya Pisanitsa rock art monument

Species of micromycetes	Frequency of occurrence in samples, %
1. *Alternaria alternata* (Fr.) Keissl.	71
2. *A. chartarum* Preuss	21
3. *Aspergillus flavus* Link	7
4. *A. ustus* (Bainier) Thom & Church	7
5. *Botrytis cinerea* Pers	7
6. *Chaetomium globosum* Kunze	7
7. *Cladosporium cladosporioides* (Fresen.) G.A. de Vries	7
8. *C. herbarum* (Pers.) Link	7
9. *Coniosporium* sp.	7
10. *Curvularia* sp.	7
11. *Entomortierella lignicola* (G.W. Martin) Vandepol & Bonito	14
12. *Fusarium oxysporum* Schltdl.	21
13. *Marquandomyces marquandii* (Massee) Samson, Houbraken & Luangsa-ard	64
14. *Mucor hiemalis* Wehmer	7
15. *Paecilomyces variotii* Bainier	7
16. *Phoma herbarum* Westend.	7
17. *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson	36
18. *Rhizopus stolonifer* (Ehrenb.) Vuill.	7
19. *Scytalidium lignicola* Pesante	78
20. *Trichocladium griseum* (Traaen) X. Wei Wang & Houbraken	21
21. *Trichoderma koningii* Oudem.	28
22. Non-spore-bearing light fungus	43
23. Non-spore-bearing dark fungus	57

there are many microzones in the destructing rock, which allows microcolonial yeastlike fungi to attach to them and successfully develop. The data obtained indicate that significant changes in the composition of fungal communities may occur with the biological colonization and destruction of rocks with petroglyphs.

Bacteria

In 2018, we identified the sites of biofilm proliferation with cyanobacteria dominance on the rock of the Tomskaya Pisanitsa rock art monument for the first time (Fig. 7a). Their development was confined to areas of high humidity, as well as places where carbonate deposits (layers) formed on the surface of sandstone. Biofilms showed active growth during soaking; gas formation was observed.

In total, according to the results of studies in 2018 and 2021 using cultivation and metagenomic analysis, cyanobacteria from 17 genera were identified in places of carbonate layers on the rock surface near petroglyphs (Table 2). It should be noted that six taxa were identified only in the biofilm metagenome, which may be due to their inability to develop on the used nutrient media. At the same time, two taxa were found only in conditions of accumulative cultures. Their absence in the metagenome indicates the need to isolate algologically pure cultures (genera *Chroococcus* and *Gloeocapsopsis*) and clarify the molecular identification of the strains.

The identified cyanobacteria are dominated by *Microcoleus autumnalis* (Gomont) Strunecky, Komárek & J. R. Johansen (Fig. 7b), and *Phormidium kuetzingianum* (Kirchner ex Hansgirg) Anagnostidis & Komárek. These taxa are characterized by a filamentous shape, a high growth rate, and the ability to redeposite carbonates (Figs. 7c–7e) and form mats on the rocky surface. From the given list, other species of cyanobacteria, including widespread representatives of the genera *Gloeocapsa* and *Calothrix* can participate in the processes of carbonate deposition (Kamennaya et al., 2012).

In an accumulative culture (100 mL of distilled water), a calcified biofilm dominated by cyanobacteria was maintained for 2 years and was characterized by a very stable state and the preservation of calcification (Fig. 7f). Its composition, in addition to cyanobacte-

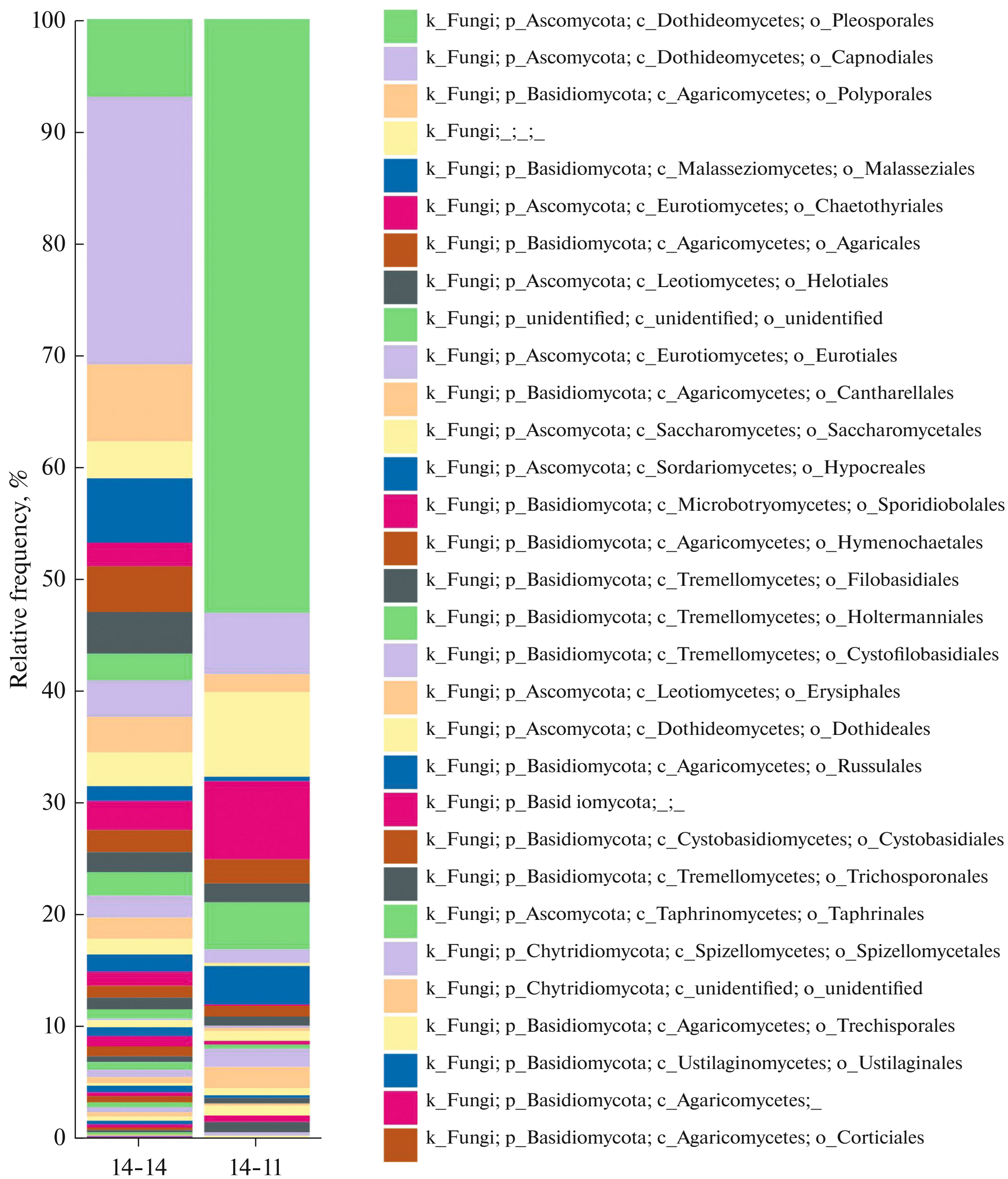

Fig. 6. Variety of fungi in the destructing rock of the Tomskaya Pisanitsa rock art monument (no. 14-14) and biofilm on its surface (no. 14-11) according to metagenomic analysis (at the level of orders).

ria, included green algae and diatoms, as well as organotrophic microorganisms. Due to the presence of rock particles and redeposited calcium carbonate in the biofilm, the composition of the necessary elements was maintained in the accumulative culture. The species *Microcoleus autumnalis* can be considered the edificator of this biofilm, forming a tangle of trichomes, outside of which there were colorless dead filaments of *M. autumnalis,* probably forming a protective layer for the entire biofilm community.

The results of a metagenomic analysis of biofilm samples and destructing rock showed that biofilm samples (1 and 2) are quite similar in the composition of bacteria (Fig. 8). Along with the dominant *Cyanobacteria* (40% of the microbiome), representatives of the group *Proteobacteria* (*Gammaproteobacteria* and *Alphaproteobacteria*, about 6 and 16%, respectively) and *Bacteroidia* (from 10 to 15%) play a significant role in biofilms.

At the same time, the proportion of alpha- and gamma-proteobacteria increases significantly in the destructing rock (no. 3), while the proportion of cyanobacteria decreases to 10%. It is interesting to note that the proportion of actinobacteria (15%)—which are mainly represented by the genus *Streptomyces,* as well as representatives of the group *Bacilli* (12%)—sig-

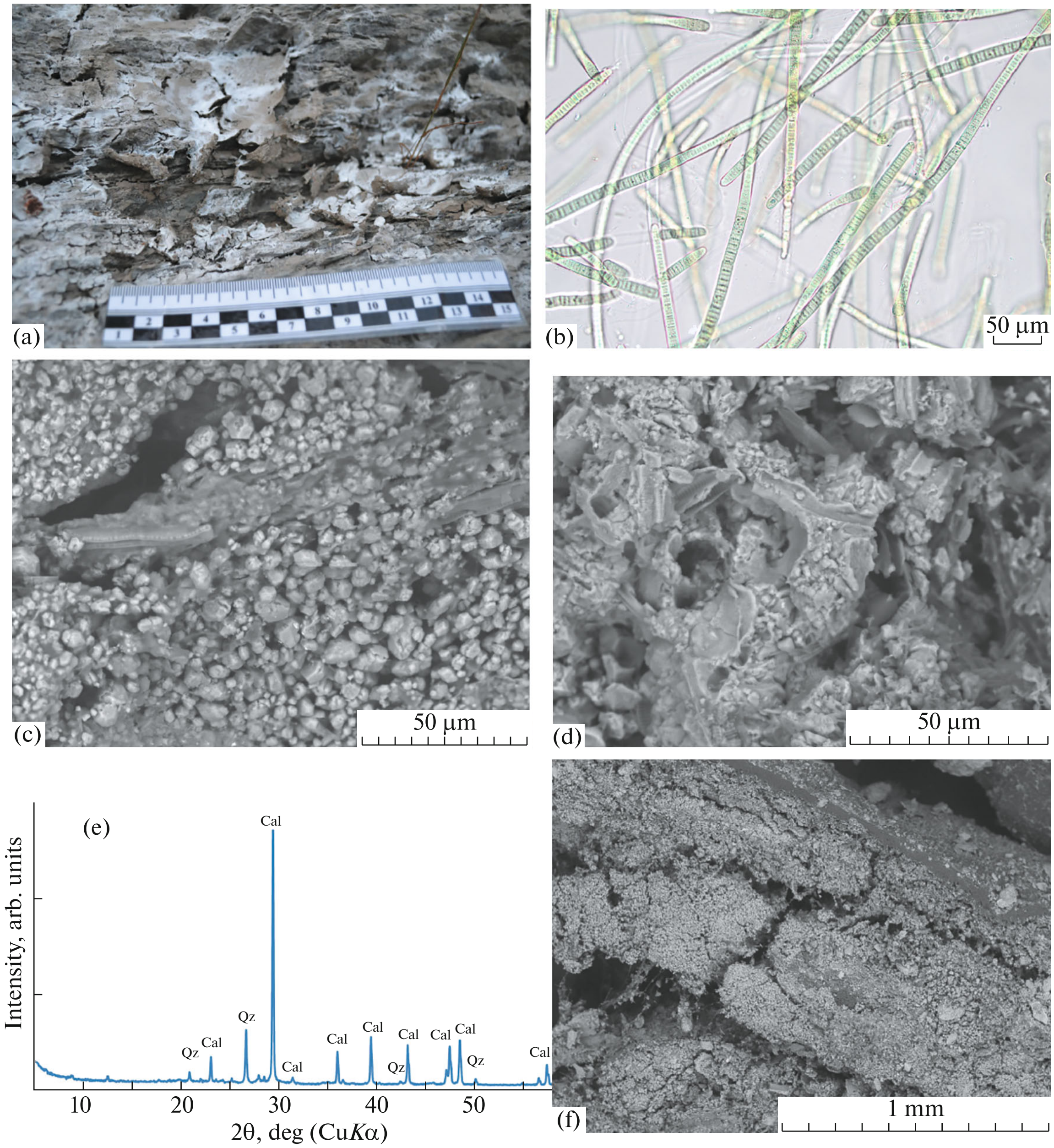

Fig. 7. Biofilms dominated by cyanobacteria on the Tomskaya Pisanitsa rock art monument: (a) external view of a typical biofilm with carbonate layers; (b) dominant species of cyanobacteria, *Microcoleus autumnalis;* (c) calcite crystals on the surface of the biofilm; (d) calcite deposits on the coats of filamentous cyanobacteria; (e) X-ray of the mineral component of the biofilm (Cal, calcite, Qz, quartz); and (f) calcified biofilm in an accumulative culture (2 years of cultivation).

nificantly increases in the damaged material. Actinomycetes and bacilli belonging to these groups are found on stone monuments in different climatic conditions. The proportion of unidentified bacteria in the studied samples did not exceed 8%.

When sowing on the EMH nutrient medium, it was found that the abundance of organotrophic bacteria does not exceed 10^5 CFU per 1 g of substrate. At the same time, the dominant morphotypes of bacteria were isolated which were identified by the 16S rRNA gene nucleotide sequence. The dominant cultured bacteria include the species *Bacillus cereus,* which is widespread and occurs in soil and on plant and other substrates, as well as *Kocuria turfanensis* from the family *Micrococcaceae* (*Actinobacteria*). Species of the genus *Kocuria* have been found in extreme and technogenic habitats and were isolated from the air (Zhou et al., 2008).

In general, our data indicate that, as the stone with petroglyphs is destructed, the composition of the bacterial community changes significantly, as happens in the case of fungi. At the same time, the proportion of

Table 2. Cyanobacteria on the Tomskaya Pisanitsa rock art monument

Genera of cyanobacteria	Method of cyanobacteria detection	
	metagenomic analysis	cultivation
1. *Aliterella*	+	–
2. *Calothrix*	+	+
3. *Chalicogloea*	+	+
4. *Chamaesiphon*	+	+
5. *Chroococcidiopsis*	+	–
6. *Chroococcus*	–	+
7. *Gleocapsa*	+	+
8. *Gloeocapsopsis*	–	+
9. *Leptolyngbya*	+	+
10. *Microcoleus*	+	+
11. *Nodosilinea*	+	–
12. *Nostoc*	+	+
13. *Phormidesmis*	+	+
14. *Phormidium*	+	+
15. *Pleurocapsa*	+	–
16. *Tychonema*	+	–
17. *Wilmottia*	+	+

(+) detected by this method; (–) not detected by this method.

cyanobacteria decreases markedly, and the proportion of proteobacteria with different types of metabolism increases markedly.

Metabolite Composition of the Lithobiont Community

The results of GC-MS analysis (Figs. 9a, 9b) showed significant differences in the composition of the identified metabolites in biofilms dominated by cyanobacteria and demaging rock (in the zone of foliation and deep destruction). At the same time, the presence of sugars, polyols, and fatty acids was observed in all samples. Terpenoids (phytol and neophytadiene) and phenolic compounds (benzoic acid and tocopherol), which were absent in the sample of the destructing rock, were also present in the biofilm samples. Some samples of biofilms contained sterols, camposterol, and cholesterol, as well as the alkaloid aminobenzoxazole.

Disaccharides sucrose and trehalose were dominant in all cases; trehalose prevailed. Trehalose is usually found in the cells of fungi. The highest concentrations of this compound are observed in resting structures, such as spores and sclerotia. In addition to its main reserve function (carbohydrate storage), trehalose also protects fungal cells from adverse conditions such as high and low temperatures, drying out (Ocón et al., 2007). The diversity of polyols in samples of fouling and damaged rock included manite, arabite, sorbitol, erythritol, glycerol, and myo-inosite. The most common polyols were mannitol and sorbitol. It is interesting to note that the content of polyols in the destructing rock was higher than in biofilms, and mannitol and sorbitol were dominant. Polyols play an important role in the metabolism of fungi and plants. In addition to energy functions, polyols are involved in protecting the body from salt and photooxidative stress (Williamson et al., 2002).

Diterpenes (phytol and neophytadiene) found in biofilm samples are known as metabolic products of higher plants and algae. At the same time, neophytadiene is able to have an inhibitory effect on fungi and bacteria. Its presence in the metabolomic profile of a community may influence the nature of biotic interactions of microorganisms (Gutbrod et al., 2019). The presence of fatty acids (stearic, palmitic, pelargonic, linoleic, linolenic, myristic, nonadecyl, and arachinic) in the metabolic profile is characteristic of biolayers of various compositions (Sazanova et al., 2021). Fatty acids perform many physiological functions and are components of membranes. Their content in the samples may be associated with the presence of both actively growing organisms and resting structures. It should be mentioned that, in the studied samples of biofouling and destructing rocks, a low content of monosaccharides, amino acids, organic acids, products of energy metabolism, sugar acids, and glycosides was recorded, which may be due to the predominance of resting structures of microorganisms in the studied samples and a sufficiently low metabolic activity of the lithobiont community. Thus, the metabolomic profiles of biofilms dominated by cyanobacteria and the microbial community in the zone of deep destruction of the rock are formed primarily by compounds that perform an adaptive function. In the conditions of temperature difference, variable humidity, and high insolation characteristic of the Tomskaya Pisanitsa area, these compounds contribute to the survival of microorganisms and their development on a rocky substrate.

DISCUSSION

The studies have shown that the Tomskaya Pisanitsa rock art monument is subjected to the processes of intense biological colonization. The preservation of the monument largely depends on the condition and properties of the rock and the composition and degree of development of biofouling on it, as well as external conditions. As it turned out, the rock itself (sandstones with layers of aleurolites and argillites) is extremely heterogeneous and fractured, with lenslike inclusions of carbonates. The densest areas on which petroglyphs are created alternate with loose foliated areas that are most susceptible to weathering and leaching processes. The listed properties of the rock largely determine the nature of the biological colonization of the rock with

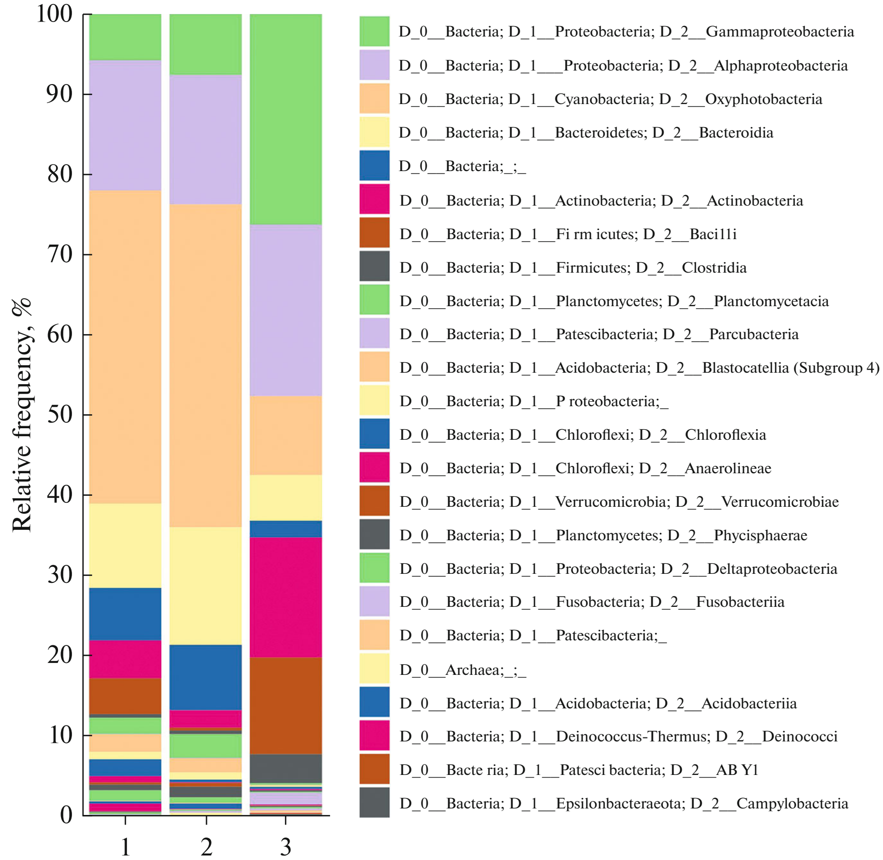

Fig. 8. Diversity of bacteria in samples of biofilms (1 and 2) and damaged stone material (3) according to metagenomic analysis.

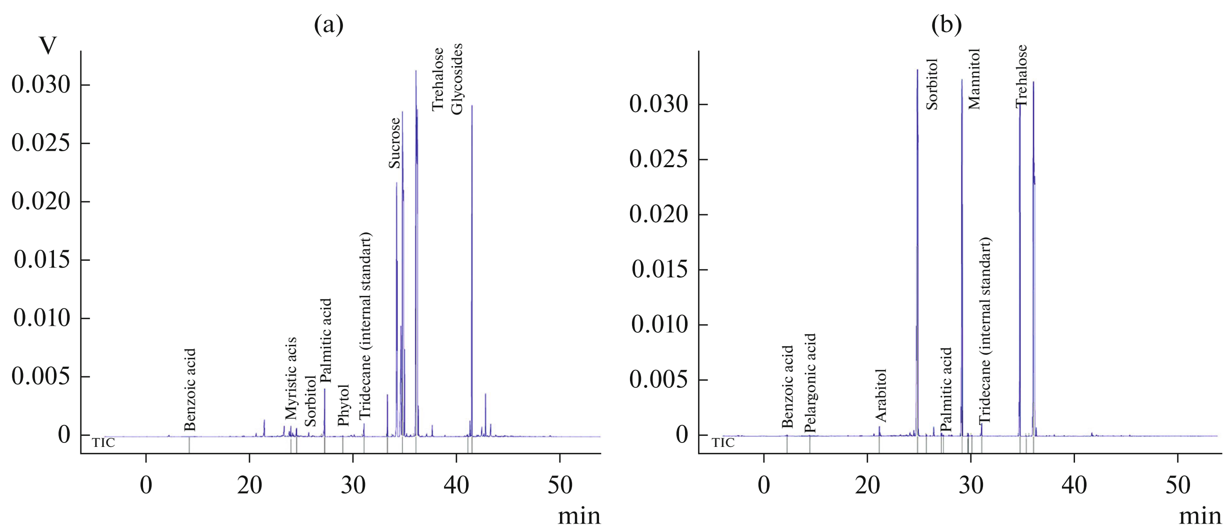

Fig. 9. Examples of chromatograms of extracts obtained from the biofilm dominated by cyanobacteria (a) and destructing rock with signs of foliation and deep destruction (b).

petroglyphs. Microorganisms use the structural spaces of the surface layer of the rock (cracks, caverns, cavities, depressions, mineral layers) for attachment and subsequent development. At the same time, microbial biofilms and lichens often develop along the microrelief of the rock carvings themselves, influencing the state of petroglyphs, as well as their aesthetic perception.

The data indicate a high destructive potential of biofouling on the Tomskaya Pisanitsa monument. Microscopic fungi, cyanobacteria, proteobacteria, actinobacteria, and lichens (crustose and foliate) can be attributed to the number of potential destructors characterized by a significant proportion in lithobiont communities. It is important to note that the identified lithobiont organisms have a sufficiently high resistance to external effects, which manifests itself at morphological and biochemical levels. For example, according to metagenomic analysis among fungi, black yeastlike fungi (representatives of the order *Capnodiales*) play a significant role in the lithobiont community, which are well known for their ability to form melanized microcolonies and exist for a long time under conditions of lack of nutrition, increased insolation, changes in temperature and humidity, and salt stress (Sterflinger, 2010). It was these fungi that dominated those areas of the rock where the rock is foliated and many microzones are formed that are favorable for colonization by these fungi. The significant adaptive potential of the lithobiont community is also evidenced by the results of the metabolomic analysis. The composition of microbial metabolites is formed primarily by compounds that perform an adaptive function. First and foremost, this refers to the high content of trehalose and polyols, which provide protective responses of organisms to various stressful effects. Under conditions of variable humidity, high insolation, and temperature differences characteristic of rock outcrops, these compounds contribute to maintaining the viability of lithobiont organisms.

The comparison of lithobiont communities in surface biofilms and in the destructing rocks on the Tomskaya Pisanitsa showed that the composition of bacteria and fungi in them differs significantly. This concerns the dominant groups of microorganisms, as well as the structure of the microbiome as a whole, revealed using the metagenomic analysis. Thus, cyanobacteria evidently dominate in developing biofilms on wet rock sites, which is quite consistent with the well-known ideas about the dominance of phototrophic organisms at the stage of primary colonization of rocks (Gorbushina, 2007). Organotrophic organisms already dominate in the destructing rock: proteobacteria, actinomycetes, and species of the genus *Bacillus*—microscopic fungi well known for their destructive activity. These data make it possible to suggest that the structure of lithobiont communities on the Tomskaya Pisanitsa rock art monument depends on local conditions that develop on particular parts of the rock with images, and may also be the result of successional processes. A high diversity of bacteria and fungi has been detected on rock surfaces with petroglyphs and adjacent sections of the rock, which form biofilms of the complex composition, often covering a significant surface of the rock with images. In most cases, the biofilms are dominated by cyanobacteria, along with which organotrophic bacteria and microscopic fungi develop. It is shown that the combination of research methods of culturing, PCR analysis, and metagenomic study provides identification of the most complete spectrum of microorganisms forming microbial biofilms on the rock with petroglyphs. Our data on the microbiome of prokaryotes at the Tomskaya Pisanitsa site are in good agreement with the recently obtained results (Nir et al., 2022; Rabbachin et al., 2022) during a metagenomic study of microbial communities in oligotrophic habitats.

The role of biochemical processes in the destruction of the Tomskaya Pisanitsa rock art monument is indicated by the fact that, among the identified microorganisms, there were many species that are known for their ability to participate in biomineralization processes. For example, among fungi, they are species of the genus *Aspergillus,* active producers of oxalic acid, the development of which leads to the active oxalate mineralization of biofilms (Vlasov et al., 2020). Among organotrophic bacteria, species of the genus *Bacillus* usually contribute to carbonate biomineralization and to oxalate biomineralization in oligotrophic conditions. Many lichens living on rocky substrates are distinguished by the formation of oxalates (Frank-Kamenetskaya et al., 2019; De los Ríos et al., 2021). In our studies, calcium oxalates were detected by the XRF method in lichens *Pertusaria* sp., *Protoparmeliospis muralis,* and *Circinaria contorta,* which indicates a chemical interaction of lichens and rock. At the same time, both whewellite—calcium oxalate monohydrate—and weddellite—calcium oxalate dihydrate—were detected in large quantities in the thallomas and fruit bodies of lichens. The development of biofilms dominated by cyanobacteria on the Tomskaya Pisanitsa occurred in places of well-marked carbonate deposits (calcite crusts). Cyanobacteria of the genera *Microcoleus*, *Phormidium*, *Gloeocapsa*, *Calothrix*, etc., capable of calcification under various environmental conditions, were identified there (Kamennaya et al., 2012). The ability to carbonatize was also confirmed when culturing biofilms with cyanobacteria dominance in an accumulative culture for two years. The interrelation of the processes of formation of carbonate crusts with the development of microbial communities also takes place at other archaeological sites in Siberia, which was recently shown during the survey of rock art monuments of the Minusinsk Basin (Sazanova et al., 2022).

CONCLUSIONS

This study has demonstrated that the destruction of the Tomskaya Pisanitsa rock art monument can be considered a consequence of interrelated physical, chemical, and biological processes accompanied by changes in the properties of the rock and its biological colonization. The existing risk of loss of unique rock carvings indicates the importance and necessity of monitoring the condition of the monument and finding new approaches to its preservation, taking into account the accumulated scientific data.

It is almost impossible to stop the processes of biofouling of the rock with petroglyphs completely, but ongoing maintenance works show the possibility of significantly reducing the rate of destructive processes and improving the preservation of the monument. Due to the blocking of the paths of moisture that fell on the planes with petroglyphs, in recent years it has been possible to significantly reduce the moisture level of rock surfaces with petroglyphs on the Tomskaya Pisanitsa and slow down the processes of biological colonization of rocks with petroglyphs. At the same time, there is an obvious need to further search for effective ways to protect this unique monument from destruction.

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