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EXPERIMENTAL ARTICLES

Microbial Communities on the Monuments of Moscow and St. Petersburg: Biodiversity and Trophic Relations

A. A. Gorbushina*, N. N. Lyalikova, D. Yu. Vlasov*, and T. V. Khizhnyak****

**Laboratory of Lower Plants, Biological Research Institute, Stary Peterhof, St. Petersburg, Russia*

***Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117811 Russia*

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Abstract—Stone monuments situated in the Alexander Nevsky Abbey, the Summer Garden, and the Smolenskoe Cemetery, St. Petersburg, and marble and limestone sculptures and tombstones situated in the Novodevichy Convent and the Donskoy Monastery, Moscow, were investigated for their microbial contamination. The architectural objects studied date back to the 12th century. The monuments in the Alexander Nevsky Abbey were found to be severely contaminated with micromycetes belonging to 24 genera (primarily of the class *Deuteromycetes*). The analysis of the samples taken from the monuments of the Donskoy Monastery by the serial dilution technique showed that they were contaminated with bacteria at a density of $(1–1.7) \times 10^5$ cells/g. This value, however, turned out to be 1 to 2 orders greater when the bacterial population was evaluated by the luciferin–luciferase method. We succeeded in identifying 12 bacterial genera; however, this number may be increased in the course of further studies. Some preventive measures to control the biodeterioration of stone heritage are discussed.

Key words: monuments, biodeterioration, micromycetes and bacteria able to grow on marble and limestone.

It is known that inorganic construction materials, including marble, limestone, and brick, are contaminated by various microorganisms, which can deteriorate these materials [1–4]. Microbial growth on the surface of stones promotes their weathering, gives rise to pigmented biofilms and incrustations, and causes marble exfoliation [5]. Biofilms are communities of microorganisms which beneficially influence each other through the secretion of extracellular substances (such as pigments, polysaccharides, and proteins), thereby providing for biofilm development on the surface of solid mineral substrates. Marble damage is very extensive in urban environments, where dust particles interact with microbial films, giving rise to firm incrustations on the surface of marble [6, 7]. The excretion of acids by autotrophic (nitrifying and thionic) and heterotrophic bacteria promotes the superficial leaching of marble.

Recent studies revealed the significant role of micromycetes in the deterioration of antique and medieval marble monuments in the Mediterranean countries [8–10], the Crimea [11], and northern Europe [12]. Microcolonial pigmented fungi can grow on and in the superficial layer of marble and other calcareous rocks and cause their deterioration [9]. Marble monuments in the open air are also often contaminated by the propagules of soil fungi, which begin growing and colonizing marble's surface under favorable environmental conditions. It is evident from this survey, that the extent of stone deterioration depends not only on the surrounding conditions but also on the composition of stone-inhabiting microflora.

Numerous open-air marble sculptures and monuments in St. Petersburg are exposed to the direct action of atmospheric factors and microorganisms. The investigation of the microbial weathering of stone historical heritage showed that marble is the most severely contaminated constructional stone and that the most abundant groups of microorganisms inhabiting damaged marble are thiobacteria, molds, and actinomycetes [1]. Wollenzien *et al.* attempted to isolate some micromycetes (*Cladosporium*, *Urocladium*, and yeastlike dark-pigmented hyphomycetes) from the samples of Carrara marble taken from the Alexander Nevsky Abbey [9]. In spite of progress in this problem, little is known on the species composition of the microflora inhabiting stone monuments and buildings.

The aim of the present work was to investigate the species composition of the microorganisms that inhabit historical marble and limestone sculptures and monuments situated in Moscow and St. Petersburg with due consideration for trophic relations between the microorganisms.

MATERIALS AND METHODS

Samples for analysis were collected from marble and limestone monuments situated in the Alexander Nevsky Abbey, the Summer Garden, and the Smolenskoe Cemetery, St. Petersburg, and the Novodevichy

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Fig. 1. Scanning electron micrograph of a black incrustation with gypsum crystals and dust particles on the marble surface.

Convent and the Donskoy Monastery, Moscow. The monuments had defects in the form of dark spots, cracks, indentations, scales, black incrustations, and so on. The samples were analyzed by the replica plating method and by the serial dilution method using agar and liquid media. In some experiments, microcolonies were directly transferred to nutrient media using a syringe needle [9]. The media used were as follows: nutrient agar, 0.005% yeast extract, potato agar, malt agar, Czapek–Dox agar with 1% glucose, peptone–glucose–yeast extract (PGY) agar, liquid media for nitrifyers and thiobacteria, sugar-containing Waksman medium, and Raymond medium for hydrocarbon-oxidizing bacteria.

Samples were obtained from more than 50 monuments: 25 monuments in the Alexander Nevsky Abbey, from each of which 1 to 3 samples were taken; 3 monuments in the Summer Garden; 13 monuments in the Novodevichy Convent, from each of which 2 to 5 samples were taken; and 9 monuments in the cemetery at the Donskoy Monastery (6 of these monuments were sampled for 3 years at certain intervals).

Some samples of biofilms were fixed with osmium and glutaraldehyde for examination in a JEM-100C scanning electron microscope.

Some samples were analyzed by the luciferin–luciferase method for the content of intracellular ATP, which was calculated per unit surface of stone [13]. ATP was extracted with dimethylsulfoxide. The accuracy of measurements was within 5%. When microalgae in a sample were absent, the number of bacterial cells in the sample was estimated by assuming that the content of ATP in the cells is 1 to 10 μg/mg dry biomass [14].

Fig. 2. Black biofilms on the horizontal parts of the Khitrovo tombstone, 18th century necropolis, the Alexander Nevsky Abbey.

RESULTS AND DISCUSSION

The deterioration of marble monuments in St. Petersburg could be characterized by the following features: (1) the presence of a black gypsum incrustation on marble's surface; (2) the presence of a crumble sugary surface layer 1 to 4 cm in thickness; and (3) the presence of a black film formed primarily by colonies of dark-pigmented micromycetes.

X-ray analysis and scanning electron microscopy of marble samples showed that the black incrustation on marble's surface resulted from the decalcination and sulfatization of the superficial layer of the marble and the incorporation of dust particles into this layer (Fig. 1). When the black incrustation was tightly bound to the marble's surface, the latter did not contain dust particles and was almost undamaged. However, the marble surface below the black incrustation became crumbly and sugary with time, which resulted in the incrustation tending to exfoliate from the marble together with deteriorating marble particles. The formation of the black incrustation and the subsequent marble crumbling were obviously related to bacterial activity, whereas the active growth of microscopic fungi in the black incrustation and on the underlying marble surface was not observed.

Thin black films were found on almost all of the marble monuments examined. Initially, a marble's surface appears to be covered with small black spots, which then grow to confluence in high-moisture places. As a result, the horizontal parts of the monuments were often covered with a confluent black film (Fig. 2), composed of fungal hyphae (Fig. 3) and numerous microcolonies of micromycetes. These microcolonies considerably differed from those found earlier on marble monuments in Cherson (the Crimea), which had a size

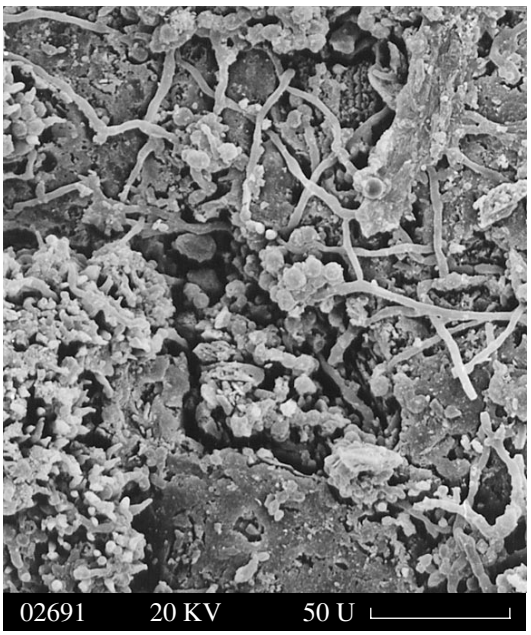

Fig. 3. Scanning electron micrograph of a black biofilm with extensively developed fungal hyphae.

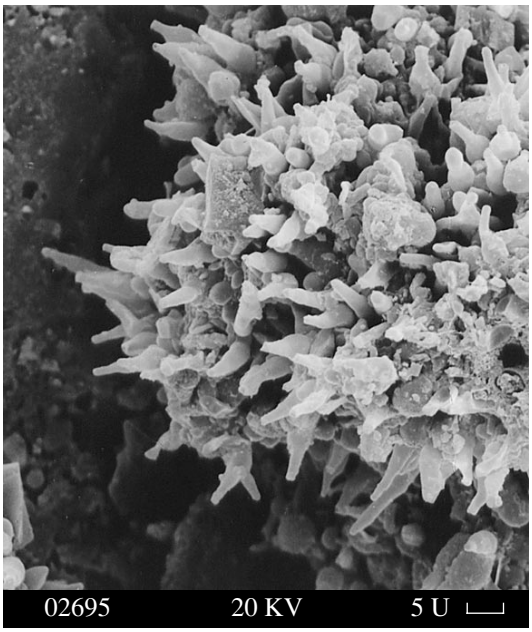

Fig. 4. Scanning electron micrograph of microcolonies on the marble surface.

of 70–80 μm and were located in small cracks and pits on the marble surface [11]. At the same time, the colonies found on marble monuments in the Alexander Nevsky Abbey had a size of 100 to 150 μm and protruded from cracks and pits. These microcolonies contained elongated cells situated radially on the colony edges and had some other signs of active fungal growth (Fig. 4). The most active fungal growth was observed on the waxed surfaces of the monuments. It should, however, be noted that these microcolonies could also be formed by lichen soredia that began colonizing the marble.

Mycological analysis allowed 26 micromycete species of 24 genera belonging to 3 classes (*Zygomycetes, Deuteromycetes,* and *Ascomycetes*) to be identified (Table 1). The identified micromycetes were dominated by *Deuteromycetes* (22 species of 20 genera), the most abundant being representatives of the soil-inhabiting genera *Alternaria, Cladosporium, Penicillium, Phoma,* and *Trichoderma.* These micromycetes were isolated from the marble surface by the replica plating method and were characterized by active growth in liquid media. The micromycetes that showed poor growth in liquid media (*Aureobasidium pullulans, Exophiala jeanselmei, Coniosporium*-like micromycetes, and *Rhinocladiella* sp.) were isolated by picking up their microcolonies from the marble surface with a syringe needle and plating them on dilute Czapek–Dox or Tiller agar media. Some micromycetes, such as *Bromella* sp., *Candida* sp., *Chaetomium globosum, Phialophora fastigiata, Rhinocladiella* sp., *Sporothrix* sp., and *Verticillium nigrescens* were isolated from marble sources for the first time.

The high abundance and diversity of hyphal micromycetous fungi on the marble monuments of St. Petersburg can be explained by favorable environmental conditions (the high air humidity and moderate summer temperatures) in this city and by the contamination of the monuments with organic matter and fungal propagules from the surrounding vegetation. For instance, many species isolated from the monuments (*Acremonium kilienze, Aurebasidium pullulans, Candida* sp., *Cladosporium cladosporiuides Rhizopus stolonifer, Sporothrix* sp., *Trichoderma viride* were also found on nearby plants. These data are in agreement with the observation of de Leo *et al.* [15], who showed that the microflora of the surrounding vegetation and soil influence the species composition of micromycetes inhabiting marble statues.

Sixty strains isolated from the five stone samples collected at the Donskoy Monastery cemetery, the Novodevichy Convent, and the Alexander Nevsky Abbey were classified into 14 genera. About one-half of the isolates were black-pigmented micromycete belonging to the genera *Phoma, Cladosporium, Ulocladium,* and *Rhizopus.*

To show the ability of micromycetes to grow on stones, two cubic samples of Myachkovskii limestone, the main construction stone in the 12th through 17th centuries, were sterilized and placed in sterile petri dishes with tap water. The upper face of one stone was inoculated with the micromycete *Phialophora melinii,* and the upper face of the other stone, with the micromycete *Exophiala moniliae.* These micromycetes, which belong to the family *Dematiaceae,* were isolated from, respectively, a marble monument (1880) situated

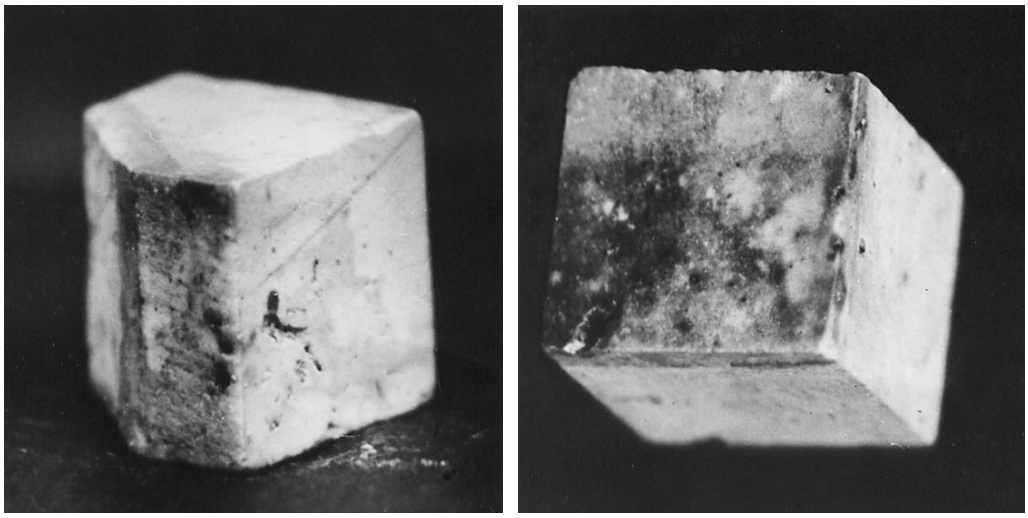

Fig. 5. Growth of *Phialophora melinii* and *Exophiala moniliae* on white stones.

in the Donskoy Monastery and a marble monument (1812) situated in the Alexander Nevsky Abbey. After 5 days of incubation, fungal growth on both limestone samples could be observed in the absence of any nutrient added (Fig. 5). The growth was obviously supported by organic matter present in the limestone.

The results of the microbiological analysis of monuments by standard methods and by the luciferin–luciferase method are presented in Tables 1 and 2, respectively. One of the studied monuments of the Alexander Nevsky Abbey, the marble Khitrovo tombstone (1728), exhibited the superficial growth of dark-pigmented micromycetes (Fig. 2). Two samples that were taken from this tombstone in 1996 were found to contain ATP in amounts of 0.85×10^{-7} and 0.275×10^{-8} g/cm^2, which corresponded to, respectively, 2.1×10^6 and 0.69×10^5 arbitrary microbial cells/cm^2. After one year, the microbial contamination of the first sampling site decreased to 1.4×10^6 cells/cm^2. At the same time, a new dark-pigmented mycelium, containing 2.06×10^6 arbitrary microbial cells/cm^2, was revealed on one of the shoulders of the Khitrovo statue.

The mean number of microbial cells detected on the monuments of the Donskoy Monastery by the serial dilution technique was $(1–1.7) \times 10^5$ cells/g sample. The evaluation of the degree of the microbial contamination of the monuments by the luciferin–luciferase method gave values greater by 1 or 2 orders. The population of microbial cells was particularly dense (up to 1.6×10^6 cells/g) at the sites with intense growth of algae and lichens, where photosynthetic organisms serve as a source of organic matter for heterotrophs.

Some samples were found to be contaminated with both microscopic algae and actinomycetes, which is in agreement with the observations of Zenova *et al.* [16], who studied the associations of algae and actinomycetes in soil. The transmission electron microscopy of biofilms stripped from a stone's surface revealed the presence of bacterial cells grown in a dead fungal hypha (Fig. 6). The microscopic analysis of thin sections also showed the presence of azotobacters, which are able to fix molecular nitrogen and, hence, are very important to other members of the communities that grow in nutritionally poor habitats. In general, the relative number of pigmented bacteria of the genera *Rhodococcus* and *Micrococcus* on the monuments studied was smaller than on southerly stone substrates. In some samples, however, their relative content was as high as 50–70%.

The number of nitrifying bacteria, which were found on the Knyaginya (Princess) Saltykova monument (1863) and the Knyaz (Prince) Salagov monument (1820) situated in the Alexander Nevsky Abbey and on three monuments situated in the Novodevichy Convent, was as low as 1 to 100 cells/g sample. The greatest number (1×10^4 cells/g) of these bacteria, which produce nitrous and nitric acids and therefore are very dangerous to stone heritage, was found in the sample taken from the southern portal of the Uspensky Sobor (Cathedral of the Assumption) in the Kremlin under the layer of an exfoliated paint. In this case, the luciferin–luciferase method gave a close value, $(2–2.75) \times 10^5$ cells/g, which can be explained by the small content of heterotrophic bacteria in this sample.

Thionic bacteria, which produce sulfuric acid, were detected only on the severely damaged Countess Kochubei monument (1810) in the Alexander Nevsky Abbey. Hydrocarbon-oxidizing bacteria were found on this monument and on the monument to the Unknown (1800), which is also severely damaged.

In total, the marble and limestone historical monuments investigated were polluted with bacteria of the genera *Pseudomonas, Rhodococcus, Dietzia, Micrococcus, Arthrobacter, Bacillus, Azotobacter, Nitrosospira,*

Table 1. The species composition of micromycetes isolated from marble monuments situated in St. Petersburg

Fungus	Alexander Nevsky Abbey	Smolenskoe Cemetery	Summer Garden
Zygomycetes			
Mucor hiemalis Wehmer	–	+	+
Rhizopus stolonifer (Ehrenb.: Fr.) Vuil.	+	–	+
Ascomycetes			
Chaetomium globosum Kunze	+	–	–
Candida sp.	+	–	+
Deuteromycetes			
Acremonium kiliense Gruetz	–	+	+
Alternaria alternata (Fr.: Fr.) Keissler	+	+	+
Aspergillus fumigatus Fres.	+	+	–
A. niger van Tieghem	–	+	+
A. terreus Thom	+	+	–
Aureobasidium pullulans (de Bary) Arnaud	+	+	+
Botrytis cinerea Pers.: Fr.	+	+	–
Broomella sp.	–	+	–
Cladosporium cladosporioides (Fres.) de Vries	+	+	+
C. herbarum (Pers.: Fr.) Link	+	+	–
Exophiala jeanselmei (Langer.) McGinnis & Padhye	+	–	–
Fusarium sporotrichioides Sherb.	+	–	+
Harzia acremonioides (Harz) Cost.	–	–	+
Coniosporium-like	+	+	+
Paecillomyces variotii Bainier	+	+	+
Penicillium citrinum Thom	–	+	–
P. funiculosum Thom	–	+	–
P. purpurogenum Stoll.	–	–	+
Phialophora melinii	+	–	–
Phialophora fastigiata (Lagerb. & Melin) Conant	+	–	–
Phialophora sp.	+	+	–
Phoma glomerata (Corda) Wollenw. & Hochapfel	+	+	+
Ph. leveillei Borema & Bollen	+	–	–
Rhinocladiella sp.	–	+	–
Sporothrix sp.	–	–	+
Trichoderma viride (Pers.) Fr.	–	+	+
Ulocladium chartarum (Preuss) Simmons	+	+	+
Verticillium nigrescens Pethybr.	+	+	–

and *Nitrobacter*, with actinomycetes of the genus *Streptomyces* and some other genera, with the green algae *Chlorella* sp. (the monument to the Unknown), *Stichococcus* sp. (the 1816 statue of Batashov), *Stichococcus bacillaris* (the 1773 statue of Bibikova), and a filamentous form of this alga (the 1806 statue of Baronesse Kolokol'tseva). All of these green algae belong to the family *Chlorococcaceae*.

To conclude, marble sculptures and buildings in St. Petersburg are subject to active biodeterioration by dark-pigmented micromycetes, whose hyphae penetrate into the pores of stones and slowly destroy them. Electron microscopic studies show that the marble surface is populated by fast-growing imperfect fungi. The black surface incrustations on the marble monuments of St. Petersburg and Moscow represent micromycete

Table 2. Determination of microbial density on the surface of monuments in the Alexander Nevsky Abbey by the luciferin–luciferase method

Monument	Sampling site	Sampling date	ATP, 10^{-8} g/cm^2	Arbitrary cells/cm^2
Kochubei statue (1852)		1996	0.200	0.50×10^5
Khitrovo statue (1728)	Cloth fold	1996	0.275	0.69×10^5
Khitrovo statue (1728)	Heel (severely damaged part)	1996	8.50	2.1×10^6
Khitrovo statue (1728)	Left hand with dark spots	May 1997	1.6	4.0×10^5
Khitrovo statue (1728)	Heel	May 1997	5.6	1.4×10^6
Khitrovo statue (1728)	Shoulder	May 1997	8.34	2.06×10^6
Litke statue	Rose marble inset	1996	0.825	2.06×10^5
Apraksin statue (1809)		1996	3.7	0.9×10^6
Kolosov statue (1819)		1996	5.57	1.39×10^6
Kosyakovskii statue (1841)		1996	0.200	0.50×10^5
Kolzakova statue (1852)		1996	0.275	0.69×10^5
Boryatinskii statue (1806)		1996	4.0	–
Marble Kolychev statue (1810) with algal growth		May 1997	0.72	1.8×10^5
Severely damaged portland-cement copy (1983) of Friedrich I statue, Summer Garden		May 1997	10.0	2.75×10^6

Table 3. Determination of microbial density on the surface of monuments in the Donskoy Monastery and the Novodevichy Convent by the luciferin–luciferase method

Sampling site	Sampling date and temperature	ATP, 10^{-8} g/cm^2	Arbitrary cells/cm^2
Donskoy Monastery			
Marble Yur'ev statue (the end of the 18th century)	April 1996, 10^{-12} °C	3.3	8.2×10^5
Severely damaged part of this statue	The same	4.26	1×10^6
Monument to Govorkova, damaged marble gravestone	″	5.03	1.26×10^6
Willis statue (1910) with algal growth	″	9.0	–
Novodevichy Convent			
Limestone tombstone (17th century?)	April 1996, 4°C	1.1	2.75×10^5
The same	May 1996, 20°C	7.0	1.75×10^6
The tombstone lateral face	The same	0.65	1.6×10^5
Marble D.V. Davydov statue (1840)	April 1996, 4°C	0.58	1.45×10^5
The same	May 1996, 20°C	0.65	1.6×10^5
The same	November 1997, 10°C	No data	5.94×10^7
The 17th century white stone Pokrov Church subject to powdery weathering	April 1996, 4°C	0.58	1.45×10^5
The same	May 1996, 20°C	0.4	1×10^5
One of the walls of this church	November 1997, 10°C	0.78	1.94×10^5
Nearby the same wall	November 1997, 10°C	8	2×10^6
The wall with algal growth	April 1996, 4°C	0.9	–
The same	November 1997, 10°C	40	1×10^7
Marble monument (1860) with black spots	April 1996, 4°C	0.32	0.8×10^5
The same	May 1996, 20°C	0.29	0.7×10^5
A severely damaged part of this monument	April 1996, 4°C	0.46	1.15×10^5
The same	May 1996, 20°C	1.9	4.75×10^5
Mortar	November 1997, 10°C	2.4	6×10^5
Vault wall	The same	1.8	4.5×10^5

Note: When algae were present, the amount of ATP was not converted to the number of arbitrary microbial cells.

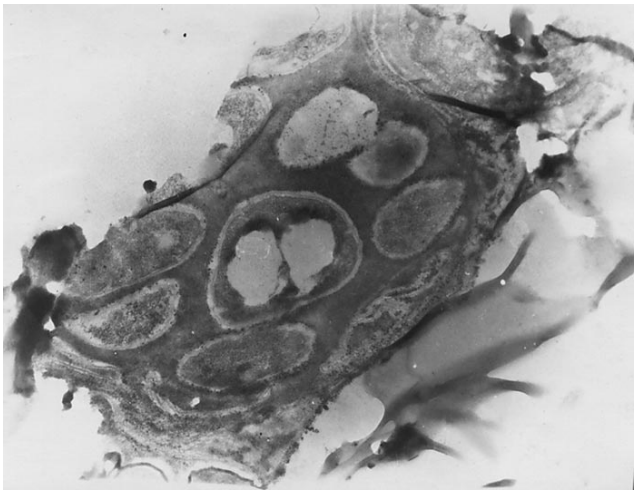

Fig. 6. Growth of bacteria in a dead fungal hypha.

hyphae associated with biofilms. The high abundance and diversity of fungi grown on the marble monuments of St. Petersburg are explained by favorable environmental conditions in this city, a high degree of air pollution, and by the contamination of the monuments with organic matter and fungal propagules transferred from the surrounding vegetation and soil.

Analysis revealed multiple trophic relations between the members of microbial biofilms. The dominant dark-pigmented micromycetes were best isolated by means of the direct transfer of their colonies from the stone surface onto nutritionally poor agars, such as Tiller agar, which support the growth of autochthonous microorganisms. To prevent the destructive processes induced by microorganisms, the following control measures can be recommended: (1) the surface of marble and limestone monuments should be mechanically cleaned without applying organic substances, (2) the monuments can be treated with biocides specific to the contaminating microflora and neutral to the stone, and (3) the disintegrating parts of the monuments should be fixed with specially chosen impregnating compounds.

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