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BIOFILM FORMING CYANOBACTERIA, ALGAE AND FUNGI ON TWO HISTORIC MONUMENTS IN BELGRADE, SERBIA

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Abstract - Biofilm on the sandstone substrata of the bridge "Brankov most" and on the granite substrata of the "Monument of the Unknown Hero" contains a complex consortia of cyanobacteria, algae, and fungi. Coccoid and filamentous cyanobacteria, green algae and diatoms make up the photosynthetic part of the biofilm while hyphal fragments, chlamydospores, fruiting bodies and spores take part as fungal components. These structures make a dense layer by intertwining and overlapping the stone surface. Five cyanobacterial, 11 algal and 23 fungal taxa were found. The interaction of the biofilm's constituents results in the bioweathering of the stone substrata through mechanical penetration, acid corrosion and the production of secondary mycogenic biominerals.

Keywords: Photosynthetic organisms, micromycetes, biodeterioration, cultural heritage

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INTRODUCTION

During the recent decades there has been a general concern about the deterioration of historic buildings. Along with chemical and physical weathering factors, microbial growth plays an important role in this process (Suihko et al., 2007). Microorganisms from all five kingdoms of the living world can colonize stone surfaces depending on stone-bioreceptivity and form a microbial sub-aerial community biofilm. Bioreceptivity is a term which indicates the potential of a material such as stone for colonization by one or more groups of living organisms without necessarily undergoing any biodeterioration (Prieto & Silva, 2005). The microbial colonization of stones depends on environmental factors such as water availability, pH, climatic exposure, nutrient sources, and on petrologic parameters, such as mineral composition porosity and permeability of the rock material (Warscheid & Braams, 2000). The stone ecosystem is subjected to harsh environmental changes, especially temperature and moisture, which exerts extreme selective pressure on any developing microbial community (May, 2003). The bioreceptivity of stone depends on its structure and chemical composition, while the intensity of the microbial contamination is caused by the climatic conditions and the anthropogenic eutrophication of the atmosphere (Prieto & Silva, 2005). Biofilm formation on clean surfaces usually starts with phototrophic organisms (algae, cyanobacteria) which use CO_2 from the atmosphere and sunlight as their carbon and energy source. Heterotrophic organisms (most bacteria and all fungi) need some organic source for their growth, and this is provided by the metabolites of phototrophic organisms or by air-borne deposition. It has been shown that the very low nutrient requirements of some rock inhabiting heterotrophic microorganisms may be fulfilled by remains of polluted air and rain or animal remains and secretion (Suihko et al., 2007).

All biofilm-forming microorganisms may cause biodeterioration and degrade stone mechanically, chemically and aesthetically through the metabolic activities and biomineralization process in these biofilms (Suihko et al., 2007). Phototrophic microorganisms can grow on the stone surface (called epilithic phototrophs) or may penetrate

some millimeters into the pore system (called endolithic phototrophs). Phototrophic microorganisms have a direct effect on the deterioration of stones due to their pigments which cause an aesthetically detrimental effect, and under certain climatic conditions they provide a protective film on the stone surface regulating humidity and temperature. The establishment of heterotrophic microbiota on rocks is possible even without the pioneering participation of phototrophic organisms. Chemoorganotrophic fungi are especially concentrated in stone crusts. They are able to penetrate into the rock material by hyphal growth and by biocorrosive activity, due to the excretion of organic acids or by the oxidation of mineral-forming cations, preferably iron and manganese. Their deterioration activities also include the discoloration of stone surfaces, due to the excretion of melanin by dematiaceous fungi (Warscheid & Braams, 2000).

MATERIALS AND METHODS

Sampling

The samples were collected from stone material with visible alteration and degradation on two localities in Belgrade: "The Monument of the Unknown Hero" on Mt. Avala and the "Brankov most" bridge (Fig.1).

Algological analyses

Samples for algological analyses (cyanobacteria and algae) were taken from the granite and sandstone surfaces of the investigated sites using a non-destructive adhesive tape sampling method (Gaylarde & Gaylarde, 1998). After rehydration in modified a Knops medium the samples were analyzed using a stereomicroscope (Zeiss Stemi DV4) and a light microscope (Zeiss Axio-ImagerM.1, with software AxioVision Release 4.6). For diatom detection the samples were treated with standard laboratory methods to prepare permanent diatom slides (Krammer & Lange-Bertalot, 1988). On the base of detailed cellular morphology the isolated cyanobacteria and algae were identified using published literature data (Elliot, 1934; Starmach, 1972; Komarek & Fott, 1983; Laundon, 1985; Krammer & Lange-Bertalot, 1988; Lange-Bertalot, 2001; Lenzenweger, 2003; Komarek & Anagnostidis, 1998; Komarek & Anagnostidis, 2005).

Mycological analyses

All samples from the granite and sandstone were taken for mycological analyses by swabbing the surfaces with sterile cotton swabs. The swab samples were diluted in 10 ml sterile distilled water and shaken for 10 min. A malt-streptomycin-agar medium (MA) prepared according to Boot (1971),

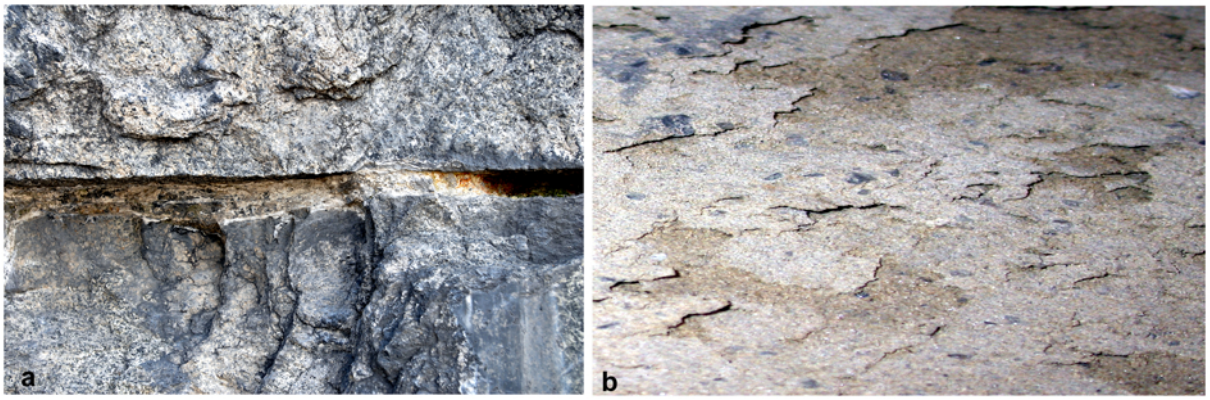

Fig. 1. Some sampling sites with visible stone alterations: a. *Haematococcus pluvialis* in red resting stage, covers the incavation of granite monument "The Monument of the Unknown Hero" on Avala; b. Exfoliation of sandstone substrata on the Brankov most bridge.

with 500 mg streptomycin per liter, was inoculated with 1 ml of the resulting suspensions. Every sample was done in triplicate. The plates were incubated on 24°C in a thermostat. Isolation of the colonies that formed was done successively, using a standard mycological medium (Malt extract agar, Potato-dextrose agar and Czapek's solution agar). All the cultures were grown for 7 days in a thermostat at 24 °C and the macroscopic and microscopic characteristics of the obtained isolates were examined. Identification of fungi was based on the macroscopic features of the colonies growing on the agar plates and the micromorphology of the reproductive structures using identification keys (Raper & Fennel, 1965; Ainsworth et al., 1973; Arx, 1974; Pitt, 1979; Ellis & Ellis, 1997).

RESULTS

The organisms forming the biofilm were identified to the species or genus level, based on their macro- and micromorphological characteristics.

The detected photosynthetic organisms

The photosynthetic organisms of the biofilm were comprised of 5 taxa of cyanobacteria, 5 taxa of green algae and 6 diatom taxa (Table 1). The most abundant biofilm-forming cyanobacteria on the sandstone substrata was coccoid *Gloeocapsa novacekii* while the

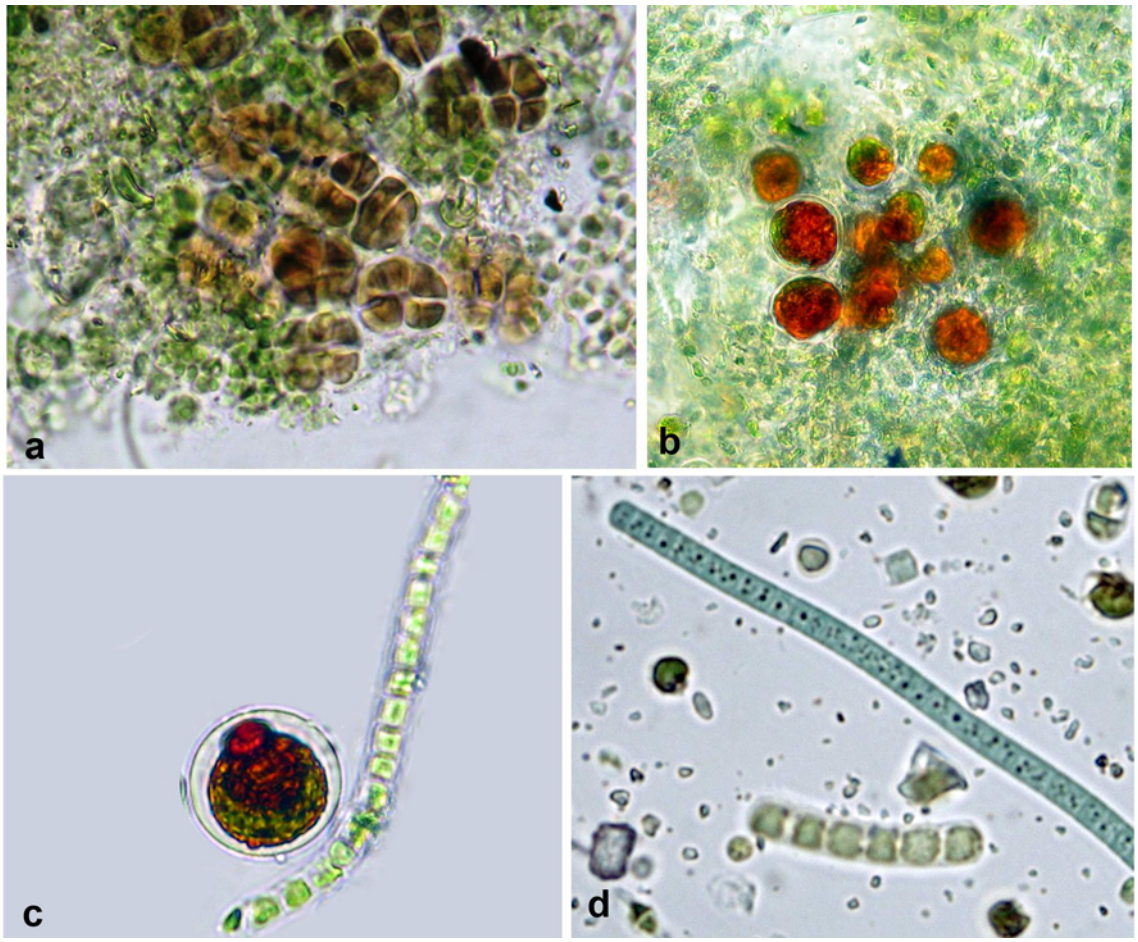

Fig. 2. Photosynthetic biofilm layer: a. dense mucoid biofilm formed by coccoid and filamentous cyanobacteria and green algae; b. *Haematococcus pluvialis*; c. *Microspora* sp.; d. *Phormidium* sp.

only cyanobacteria detected on the granite substrata was a filamentous species belong to the *Leptolyngbia* genus which included subaerophytic species, inhabiting different types of wet stone surfaces. On both investigated sites, the dominant green alga was coccoid *Desmococcus olivaceus* while this species formed an association with filamentous *Microspora* sp. on the granite substrata. Coccoid and filamentous cyanobacteria and green algae formed a dense mucoid biofilm (Fig. 2).

The detected fungi

From all the samples analyzed 23 fungal taxa were identified (Table 1., Fig. 3). The fungal species for both the granite and sandstone substrata were *Alternaria* spp., C*ladosporium cladosporoides, C. sphaeospermum, Epicoccum purpurascens, Fusarium* sp., melanized and non-melanized *Mycelia sterilia*, and one undetermined yeast isolate. Species found on the granite but not on the sandstone substrata were *Alternaria* sp., *Aspergillus flavus, Aspergillus versicolor, Cunninghamela echinulata, Drechlera dematoidea,* fungi from the *Moniliales* order and *Mucor* sp. On the other hand, the sandstone surface's biofilm included other species not present on the granite surface: *Aureobasidium pullulans* var. *melanigerum, Fusarium oxysporum, Mucor racemosus, Paecilomyces varioti, Penicillium verrucosum var. cyclopium, Penicillium* sp. and *Phoma* sp.

Table 1. Algae and cyanobacteria found on different stone material.

Cyanobacteria	Stone material	
	Granite	Sandstone
Aphanocapsa muscicola		*
Gleocapsa novacekii		*
Leptolyngngbia sp.		*
Phormidium sp.	*	
Total	1 taxa	3 taxa
Algae		
Planothidium freequentissimum var. *magnum*		*
Navicula veneta	*	*
Luticola luticopsis		*
Nitzschia communis		*
Nitzschia inconspicua		*
Nitzschia frustulum		*
Haematococcus pluvialis	*	
Desmococcus olivaceus	*	*
Cylindrocystis brebissonii	*	
Microspora sp.	*	
Cylindrocapsa sp.	*	
Total taxa	6	7

DISCUSSION

Algological and mycological analyses showed that the structure of the microbiota was specific to different rock substrata, but that the total taxa for the granite and sandstone were very similar. The genera of phototrophic organisms identified are mainly of algae involved in gelatinous products, the presence of which is related to mineral fixation (Peraza Zurita et al., 2005). It was significant that *Haematococcus pluvialis* was found in a red resting stage coloring the incavation caused by bombs in Second World War. The red-stained granite surface had a dense layer consisting of cysts with astaxanthin deposits. Although according to Tomaselli et al. (2000) the taxonomic diversity of stone-dwelling photosynthetic micro-organisms appears to be rather wide, especially considering that the habitats they colonize can be classified as extreme habitats, our results emphasize the low photosynthetic biodiversity in these biofilms, with only 16 taxa found. Crispim et al. (2004) reported the presence of 11 cyanobacterial taxa on church walls in Porto Alegre (Brazil). The most dominant fungi among Deuteromycotina were dematiaceous

Table 2. Micromycetes found on different stone material.

Micromycetes	Stone material	
	Granite	Sandstone
Alternaria sp. 1	*	*
Alternaria sp. 2	*	*
Alternaria sp. 3	*	
Aspergillus flavus	*	
Aspergillus versicolor	*	
Aureobasidium pullulans var. *melanigerum*		*
Cladosporium cladosporoides	*	*
Cladosporium sphaeospermum	*	*
Cunninghamela echinulata	*	
Drechlera dematoidea	*	
Epicoccum purpurascens	*	*
Fusarium oxysporum		*
Fusarium sp.	*	*
Moniliales	*	
Mucor racemosus		*
Mucor sp.	*	
Mycelia sterilia (non-melanized)	*	*
Mycelia sterilia (melanised)	*	*
Paecilomyces varioti		*
Penicillium sp.		*
Penicillium verrucosum var. *cyclopium*		*
Phoma sp.		*
yeast	*	*
Total taxa	16	16

hyphomycetes with melanized hyphae and reproductive structures (genus *Alternaria, Aureobasidium*, *Cladosporium, Drechslera*). The production of dark conidia and pigments was recorded in culture media during the cultivation of melanized fungi. The microfungi identified, especially melanized hyphomycetes from the genus *Alternaria, Cladosporium, Drechslera, Epicoccum,* cause

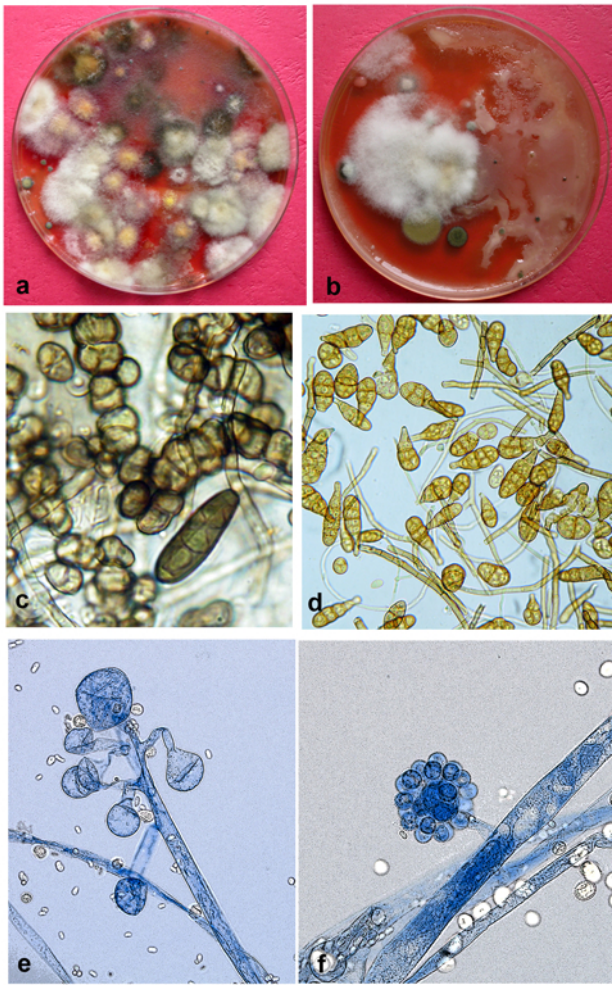

Fig. 3. Micromycetes isolates from stone substrata: a, b. Primary isolates; c. *Drechslera dematoidea,* conidia and hyphae; d. *Alternaria* sp., spores; e, f. *Cunninghamela echinulata*, sporangia and sporangiospores.

discoloration, as well as the mechanical exfoliation of building stone material analyzed through mechanical hyphae penetration and the production of different pigments and organic acids (Milanesi et al., 2005). *Aureobasidium pullulans* var. *melanigerum* is a stain fungus that usually causes staining of different stone surfaces which decreases the aesthetic qualities of historic monuments. The microscopic analyses of the biofilm samples showed the presence of an initial association of potential photobionts and mycobionts able to form lichens. The ability of microcolonial fungi to form associations with potential photobionts present on

rock surfaces was demonstrated for pure cultures *in vitro* by Gorbuschina et al. (2005). Recently it was revealed that fungi comprise a significant component of microbiota in a wide range of rocks including sandstone, granite, limestone, marble and gypsum (Burford et al., 2003), Šimonovičova et al. (2004) reported the presence of 36 different microfungi on stone in a hypogean cemetery in Bratislava. Microbial biofilms modify the capillary water uptake of the porous stone material investigated, causing alterations in the water-vapor diffusion. Surface active compounds in the biofilm provoke a decrease in the pore water tension, changing the specific moisture relationship of the stone and protecting microorganisms against water loss and desiccation and favoring subsequent microbial contamination and their bio-corrosive activity (Warscheid, 1996). Besides balancing humidity changes, the biofilm protects the stone microbiota from extreme temperatures as well as the toxic impact by salt and heavy metal accumulation. This may explain the resistance of some microorganisms to biocidal treatments (Gaylarde & Morton, 1999).

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ЦИЈАНОБАКТЕРИЈЕ, АЛГЕ И ГЉИВЕ У БИОФИЛМУ НА ДВА ИСТОРИЈСКА СПОМЕНИКА У БЕОГРАДУ, СРБИЈА

МИЛИЦА ЉАЉЕВИЋ ГРБИЋ, ЈЕЛЕНА ВУКОЈЕВИЋ, ГОРДАНА СУБАКОВ- СИМИЋ, ЈЕЛЕНА КРИЗМАНИЋ и М. СТУПАР

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Биофилм камена пешчара "Бранковог моста" и гранита "Споменика незнаном јунаку" на Авали садржи комплекс цијанобактерија, алги и гљива. Кокалне и филаментозне цијанобактерије, зелене и силикатне алге чине фотосинтетички део биофилма, док фрагменти хифа, хламидоспоре, плодоносна тела и споре чине фунгалну компоненту биофилма. Структуре гљива су испреплетане са цијанобактеријама и алгама и тако формирају густ слој биофилма на каменој површини. Са ова два локалитета укупно је изоловано 5 таксона цијанобактерија, 11 таксона алги и 23 таксона гљива. Интеракције организама биофилма резултирају пропадањем површине камена, путем механичке пенетрације, биокорозије и продукцијом секундарних биогених минерала. Биолошке анализе су неизоставни део мултидисциплинарног приступа савременог концепта сложеног система конзервације културне баштине.