



Smithsonian
Museum Conservation Institute

Requested Report

MCI #6304

MICROSCOPIC CHARACTERIZATION OF THE BIOCOLONIZATION ON THE
NATIONAL MUSEUM OF THE AMERICAN INDIAN BUILDING

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1. Introduction

The distribution of black staining on the NMAI building was examined during the workshop organized by the MCI in April 2010. The staining was clearly related to preferential water paths where water runs down the surfaces and it is present on the external walls facing any orientation. For these reasons the black staining was attributed to biocolonization, presumably due to cyanobacteria.

Other blackened areas not linked to water runoff were observed in some horizontal areas on the south walls. These were somewhat covered with plant debris and the blackening could be ascribed to the presence of other microorganisms, such as fungi, though these have not been examined in detail.

Three samples were collected. These were:

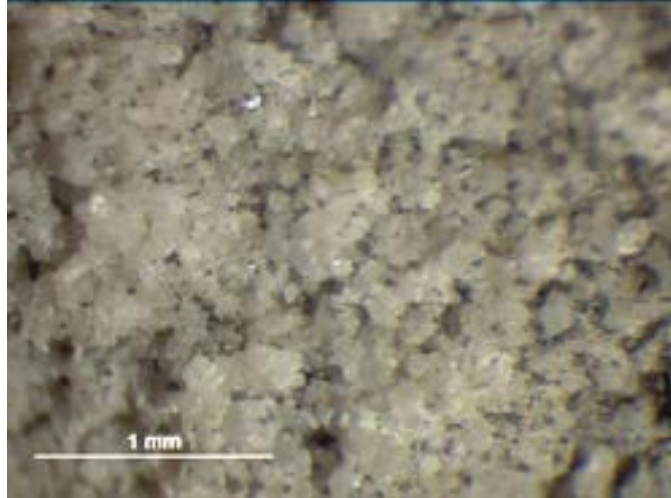
- Sample #2, corresponds to a fragment taken from a first course ashlar (prepared or dressed stonework) of the low wall by the loading dock at the north-west corner of the NMAI building;
- Sample #5, was a fragment taken from the stained area below the scupper on the east side of the wall on the roof beyond the Inouye Terrace;
- Sample #7, was a fragment taken from a rather recessed block area of a block in the ashlar course below that of sample #5.

The aim of the analyses carried out in the laboratory was to detect microorganisms causing the blackening of the wall surfaces and to investigate their relationships with stone.

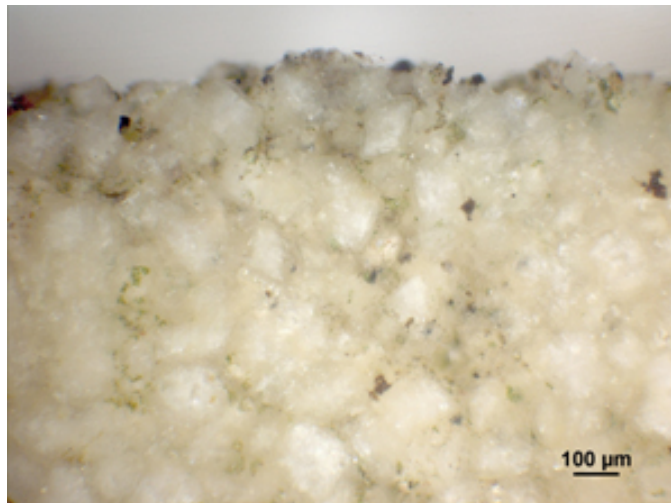
Experimental Results

The samples were first observed under a stereomicroscope. Polished cross-sections were prepared by including small pieces of them in a polyester resin. The black material on their surface was examined with optical and scanning electron microscopy (SEM). The results are presented following their numeration.

Sample #2 shows an irregular colonization of the surface, visually appearing as dark brown spots, following the boundary crystals as shown in Figure 1. The polished cross-sections show an appreciable development of photosynthetic microorganisms also within the stone, to a depth of *ca.* 1 mm (Figures 2 and 3).



**Figure 1. Photograph of sample #2 under a stereomicroscope, showing the spot-like irregular colonization. (*Subsequently published in Capitelli et al. 2012).*



**Figure. 2. Polished cross-section of sample #2 showing the penetration of photosynthetic microorganisms into the stone. (*Subsequently published in Capitelli et al. 2012).*

Capitelli F, Salvadori O, Albanese D, Villa F, Sorlini C. Cyanobacteria Cause Black Staining at the National Museum of the American Indian Building, Washington, D.C., USA. Biofouling 2012 28[3]:257-266.

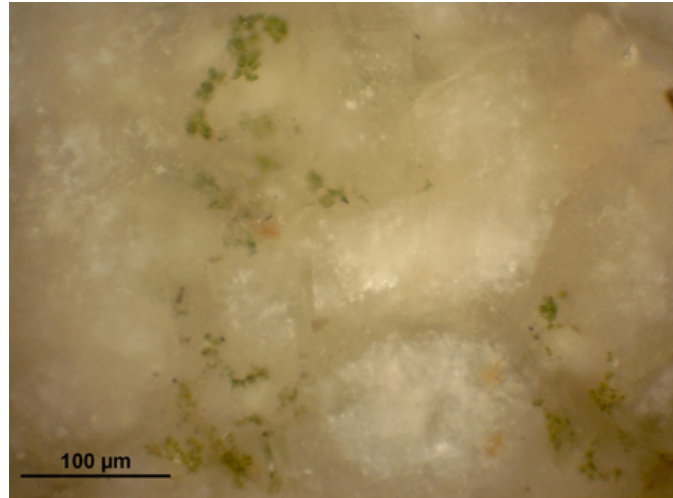


Figure 3. Higher magnification view of the inner part of the polished cross-section of sample #2 showing the green cyanobacteria colonies growing along grain boundaries.

The examination of dark material scraped off the surface and suspended in water on a slide showed colonies of cyanobacteria closely joined side by side that are very difficult to separate as shown in Figure 4. The predominant colonies are *Gloeocapsa* sp. with coloured sheaths (Figure 5); few other cyanobacteria species were recognized: *Aphanocapsa* sp. and *Chroococcus lithophilous*.

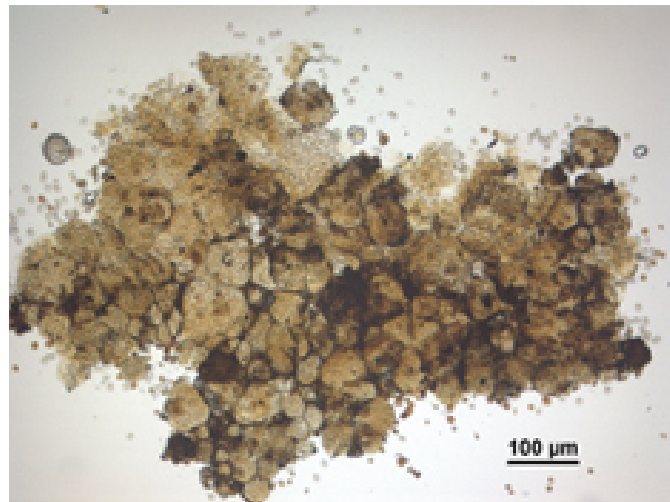
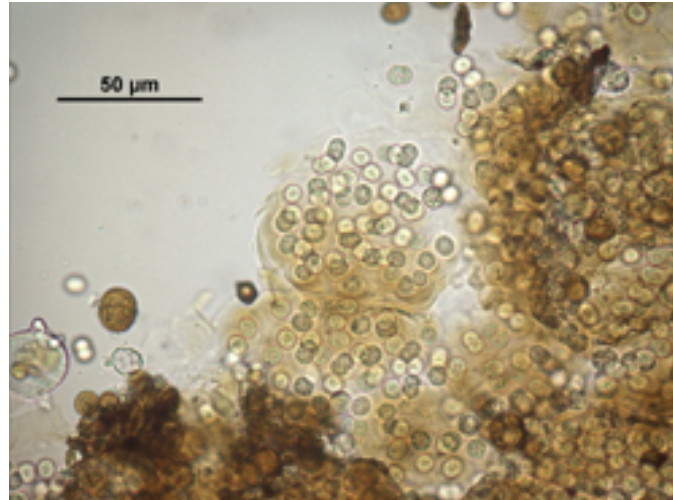
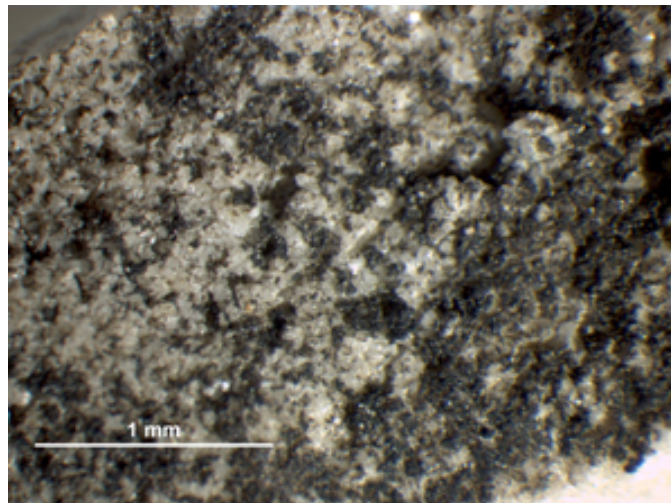


Figure 4. Fresh slide of the black material growing on the surface of sample #2.



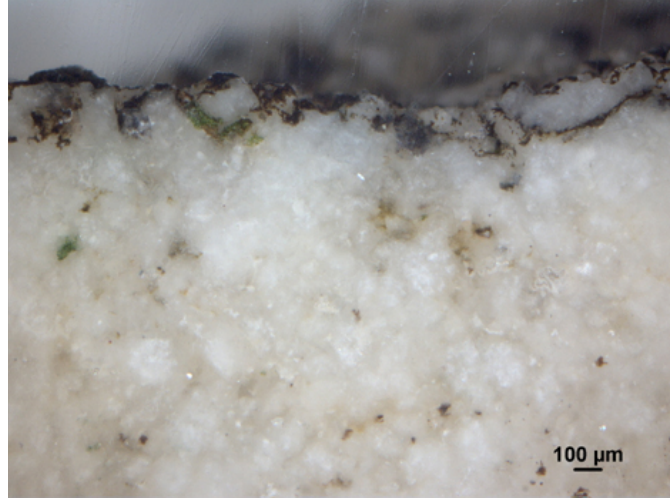
*Figure 5. Higher magnification of the previous sample, showing *Gloeocapsa sp.* colonies on the surface of sample #2. (*Subsequently published in Capitelli et al. 2012).

Sample #5 has a more abundant biocolonization coating than sample #2 with clusters of microorganisms closely joining together as shown in Figure 6.



*Figure 6. View of the surface of sample #5 under a stereomicroscope, showing clusters of microorganisms that start to join each other. (*Subsequently published in Capitelli et al. 2012).

The polished cross-sections show an irregularly distributed penetration of microorganisms into the stone to a maximum depth of *ca.* 1 mm (Figure 7). The presence of endolithic growth is higher in correspondence with the more colonized areas on the stone surface (Figure 8).



*Figure 7. Polished cross-section of sample #5 showing the penetration of biocolonization down to 1mm. (*Subsequently published in Capitelli et al. 2012).

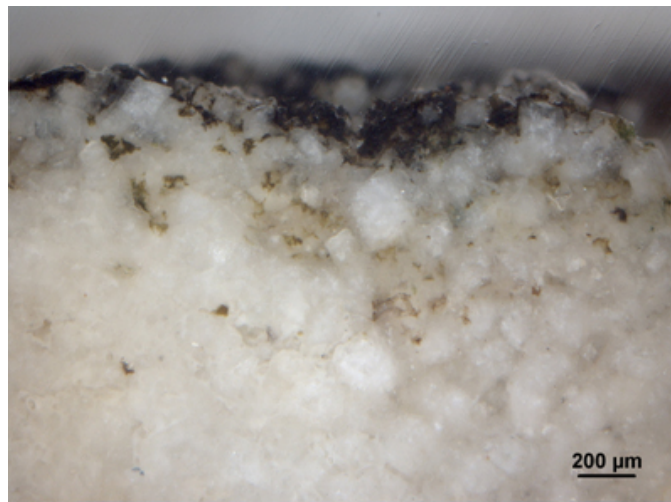


Figure 8. The same sample, at higher magnification, showing colonization developing along grain boundaries.

Examination of the dark material on the surface proved to be very similar to that of sample #2 as shown in Figure 9. Again, colonies of *Gloeocapsa* sp., with dark sheath, are closely joined and very difficult to separate. Few other cyanobacteria species were recognized: *Aphanocapsa* sp. and *Chroococcus* sp. (Figure 10).

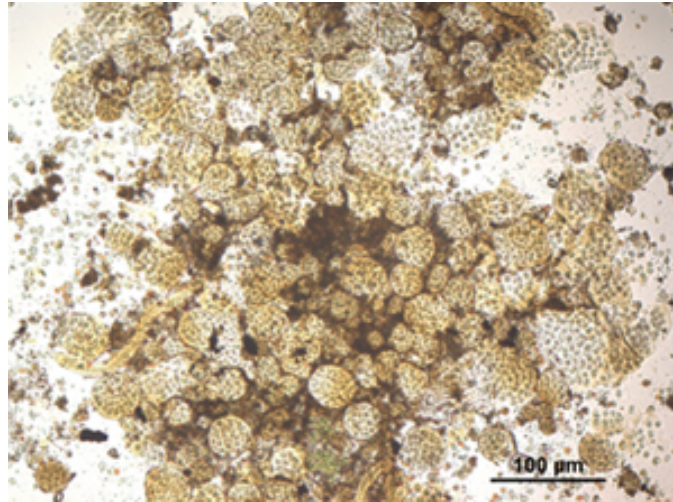


Figure 9. Fresh slide of the black material scraped from the surface of sample #5.

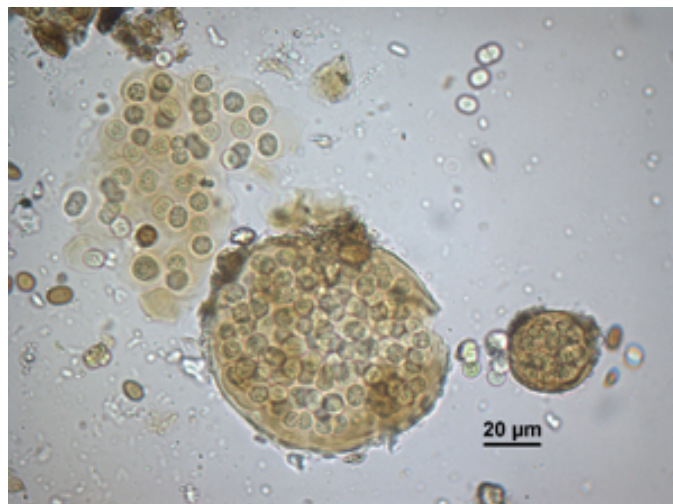


Figure 10. Higher magnification of the previous figure showing *Gloeocapsa* sp. colonies recognizable by their dark sheath.

SEM observations of sample #5 showed dehydrated cyanobacteria colonies (Figure 11) and a lot of extracellular polysaccharide substances firmly attached to the stone (Figure 12). In Figure 13 a cluster of cells in a small hole in the stone can be seen.

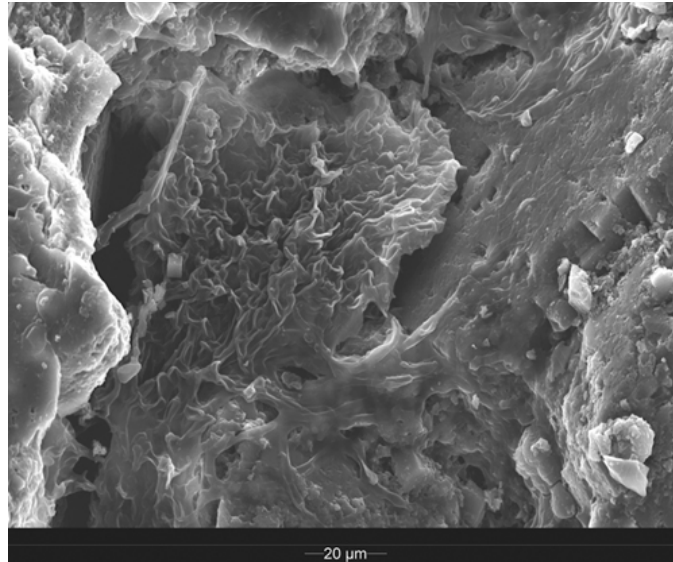


Figure 11. SEM photomicrograph of sample #5 showing dehydrated cyanobacteria colonies on the surface of a dolomite crystal.

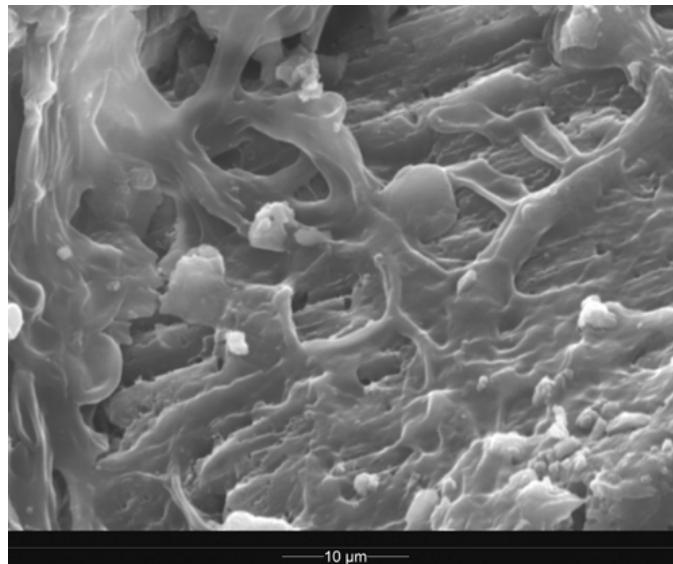
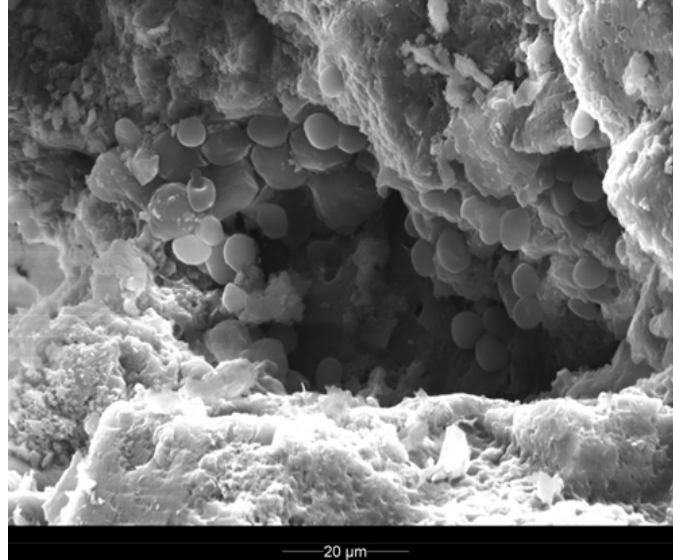


Figure 12. Higher magnification view showing significant amounts of EPS firmly adhering to the stone.

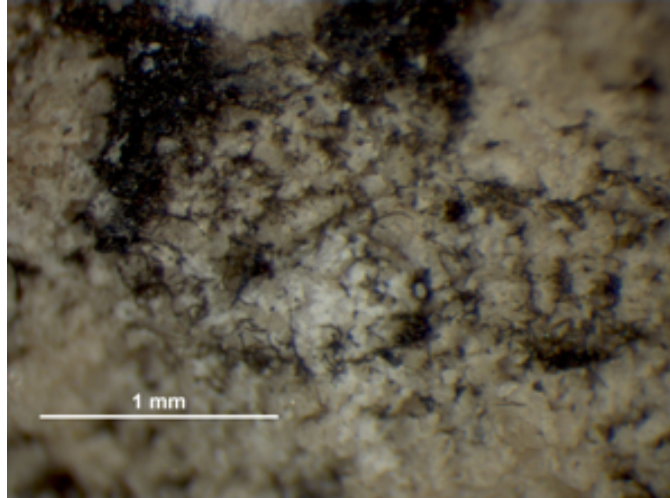


**Figure 13. SEM photomicrograph showing a cluster of cells growing within a small hole in the stone surface. (*Subsequently published in Capitelli et al. 2012).*

Sample #7 observed under a stereomicroscope shows the presence of black material irregularly distributed on the surface, with localized concentrations as shown in Figure 14. At higher magnification dark filamentous structures are noticeable in the less colonized areas (Figure 15).



Figure 14. Stereomicroscope view of the sample surface (#7) with localized biocolonization.



**Figure 15. A detailed view of the localized colonization from Figure 14. (*Subsequently published in Capitelli et al. 2012).*

Polished cross-sections show the presence of microorganisms on the surface forming a layer reaching some 0.2 mm thickness in the clusters as shown in Figure 16. In addition they penetrate into the stone to an average depth of *ca.* 0.6 mm, although some cyanobacteria can reach a maximum depth of 1 mm (Figures 17 and 18). Greater penetration into the stone generally corresponds to the thicker surface colonization, a result of the amount and quality of insolation. The penetration seems to follow porosity and pre-existing microcracks of the stone.

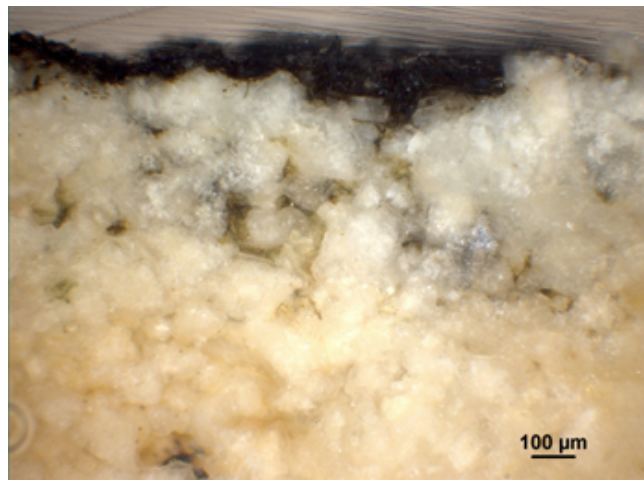


Figure 16. Polished cross-section of sample #7 showing the thick layer of biocolonization on the surface. (Reprinted with permission from [4] Capitelli et al. 2012.)

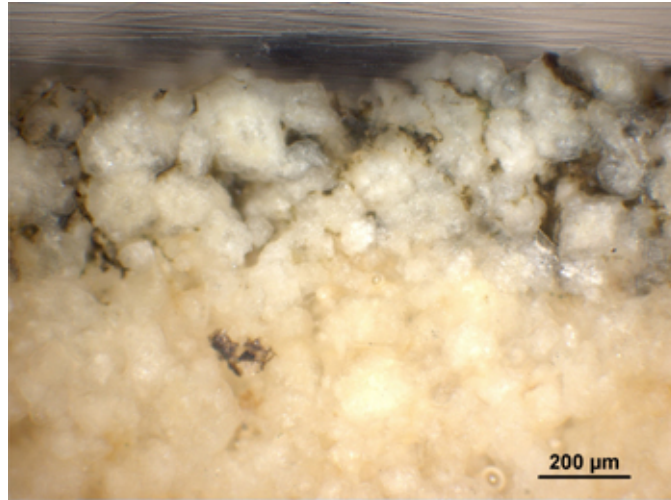


Figure 17. Detail of Figure 16 polished cross-section showing the penetration of the colonization into the stone.

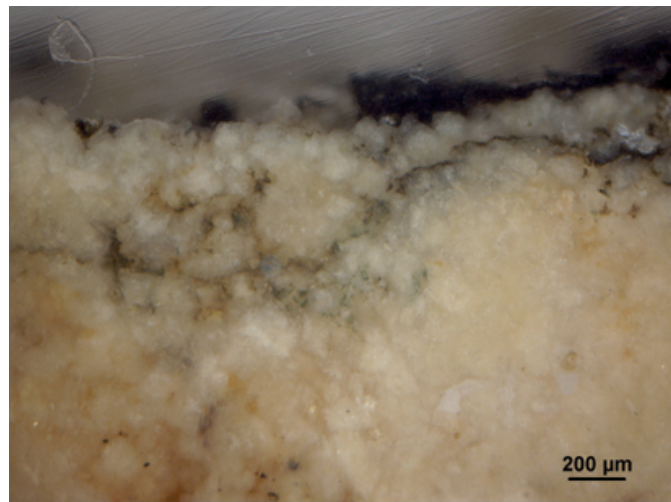


Figure 18. Another area of Figure 16 showing how the colonization grows into fissures of the stone.

The examination of fresh slides showed a clear prevalence of a filamentous cyanobacterium with dark sheath belonging to the genus *Lyngbya* sp (Figure 20). Other cyanobacteria, such as *Calothrix* sp., *Pseudoanabaena* sp., *Aphanocapsa* sp. were only rarely observed.

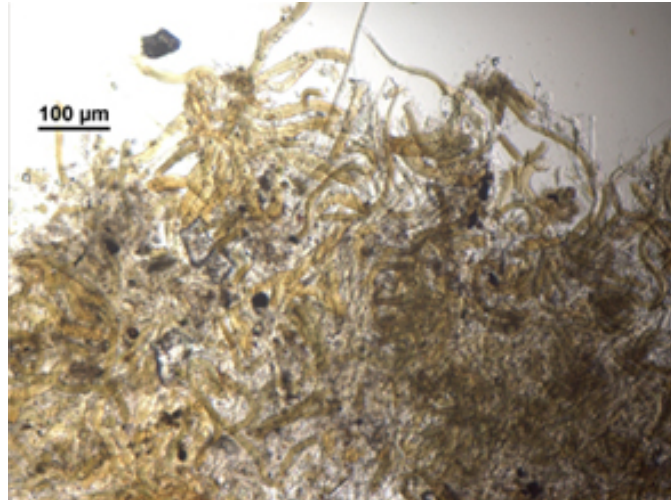
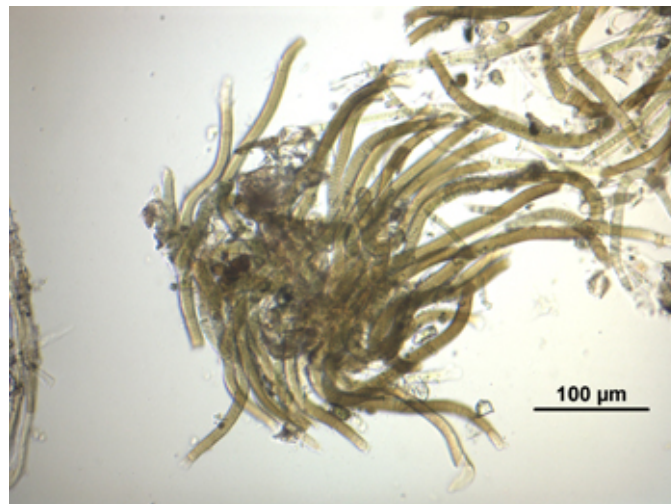


Figure 19. Fresh slide of the black material growing on the surface of sample #7 showing the prevalence of filamentous cyanobacteria.



**Figure 20. Higher magnification of the previous figure showing a cluster of the filamentous cyanobacteria. (*Subsequently published in Capitelli et al. 2012).*

SEM observations show that the network of filaments is firmly attached to the stone (Figure 21). In some areas, particulate matter (Figure 22) and pollen adhere to the sheaths of cyanobacteria.

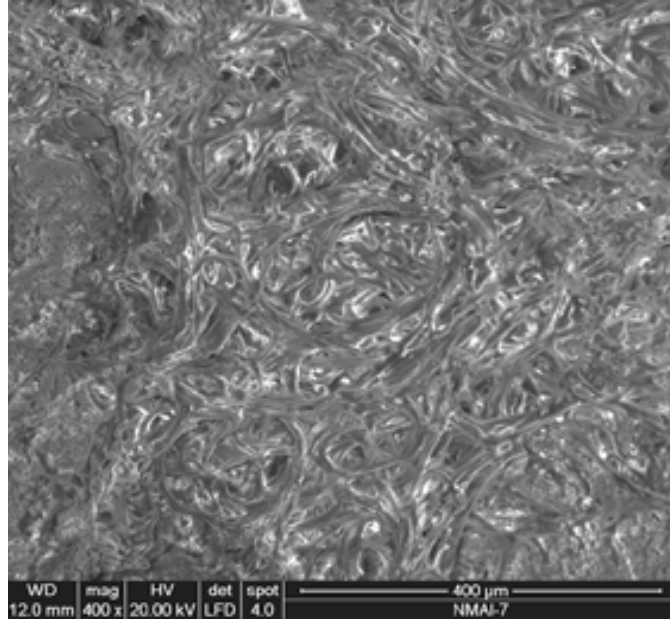


Figure 21. SEM photograph of sample #7 showing a cluster of filamentous cyanobacteria.

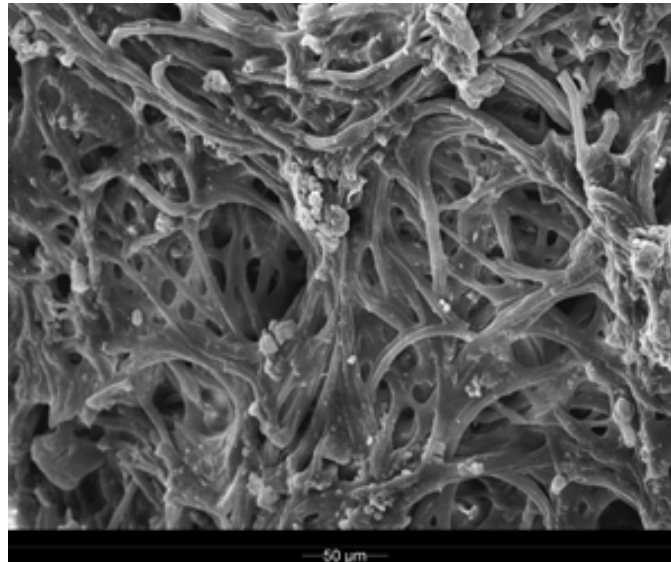


Figure 22. Higher magnification view showing filamentous cyanobacteria and particulate matter adhering on them.

Conclusions

The results of the analyses confirmed that in all three samples cyanobacteria are the predominant microorganisms, although some differences between the samples were observed. Samples #2 and #5 show a similar biological growth with the presence of a community (i.e., biocenosis) of cyanobacteria

and predominance of *Gloeocapsa* sp. colonies (Figures 4 and 9). In sample #7 a filamentous cyanobacterium, *Lyngbya* sp., is dominant (Figure 19). No fungi have been detected. The different predominant species in the samples could be ascribed to different microclimatic parameters, such as water availability—that is higher for sample #7 than for sample #5—time of wetness, substrate orientation, exposure to sunlight, in the sampled areas.

The cyanobacteria genera colonizing the NMAI building are ubiquitous and therefore their presence is not strictly related to the stone type or climate. As well known, the gelatinous sheaths act as a reservoir for water, allowing the cyanobacteria to survive even during period of dry conditions, and play an important role in the adhesion to the substrate (Crispim et al., 2004; Crispim and Gaylarde, 2005; Caneva et al., 2009). The blackening or dark color clearly visible with the naked eye can be attributed to the colored sheaths of the dominant cyanobacteria colonizing the stone surface. These sheaths contain a yellow-brown pigment, scytonemin, which acts as an ultraviolet sunscreen and may provide significant protection to other microorganisms living in the community (Singh et al., 2010).

The cross-sections show that cyanobacteria develop both on the surface as well as into the stone subsurface. They can reach a maximum depth of about 1-mm. The greater penetration into the stone is generally observed underneath the thicker surface colonization and along pre-existing microcracks and fissures. The photosynthetic microorganisms penetrating into the stone do not show, as usually found, a dark pigmentation. It is possible that the previous cleaning of the building using pressurized water contributed to push the microorganisms deeper into the stone thus favoring their development there. Being under the surface, these microorganisms can not be reached by mechanical cleaning, and if some of them survive biocide applications, they recur more rapidly than normal colonization on a clean stone would take. Therefore, a careful application of biocides that reaches the inner colonized stone is necessary to prevent a fast recolonization of the stone surface. However, it is important to remember that any dead cells remaining inside the stone can also serve as food source for other heterotrophic microorganisms. It is important that the biocide effectiveness be evaluated not only with visual observation but with the aid of at least simple techniques such as the measurement of chlorophyll a fluorescence. This can be carried out in situ by portable fluorimeters (e.g., Mini-PAM or Imaging PAM, Tretiach et al., 2010; Ryan et al., 2011) or, in the laboratory, by epifluorescence measurement on fresh slides. With epifluorescence microscopy it is possible to observe the red chlorophyll fluorescence, typical of the intact and active cells, turning white (total chlorophyll breakdown) if the biocide is effective. The fluorimeters measure the intensity of fluorescence emitted by photosynthetic organisms and are more sensitive than epifluorescence observations to reveal the effectiveness of biocides.

References

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Location of the samples taken from the NMAI building for analyses

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The samples were collected in April 2010, upon the occasion of the visit by the four invited scientists for their further analysis. Four samples were collected and subdivided into the specimens that the scientist subsequently analyzed.

Sample #2, a fairly large spalling flake with overall black surfaces on both front and back, was readily detached from the lowest course of the loading dock wall on the west side of the building. The sample area receives rainwater from sloping capstones without any overhang; some rainwater also splashes up from the black granite ledge below (Figure 1).

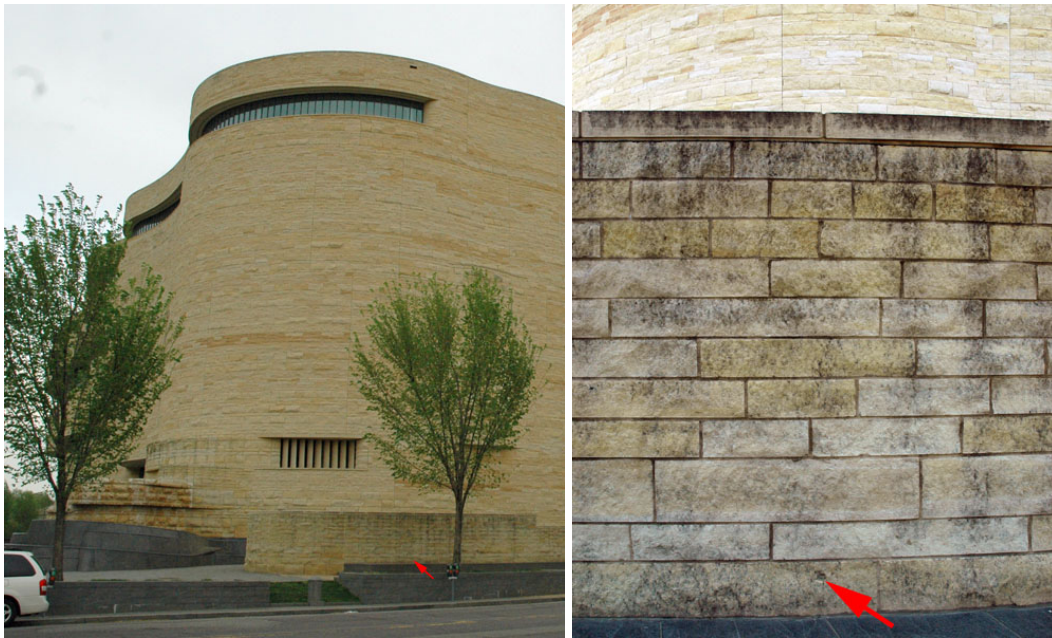


Figure 1. Sample #2 location indicated by arrows: left, general view of the exterior loading dock wall; right, detail (April 2010).

Samples #5, #7, and #9 were taken from areas of heavy biocolonization on the fifth floor terrace east wall below scuppers that drain the roofs above (Figure 2). Samples #5 and #7 were spalling flakes easily detached from below the same scupper: #5 from an area with a uniform black deposit and sample #7 from a slightly recessed area on an adjacent block with spotty surface biocolonization (Figure 3L). Sample #9 was scraped from dense black material on a membrane below a second scupper (Figure 3R).



Figure 2. Left, view of the east face of the 5th floor terrace showing locations of samples #5, #7, and #9 from darkened areas below two scuppers, which drain water from the small terrace above and, in turn, the main roof terrace with the dome (April 2010).

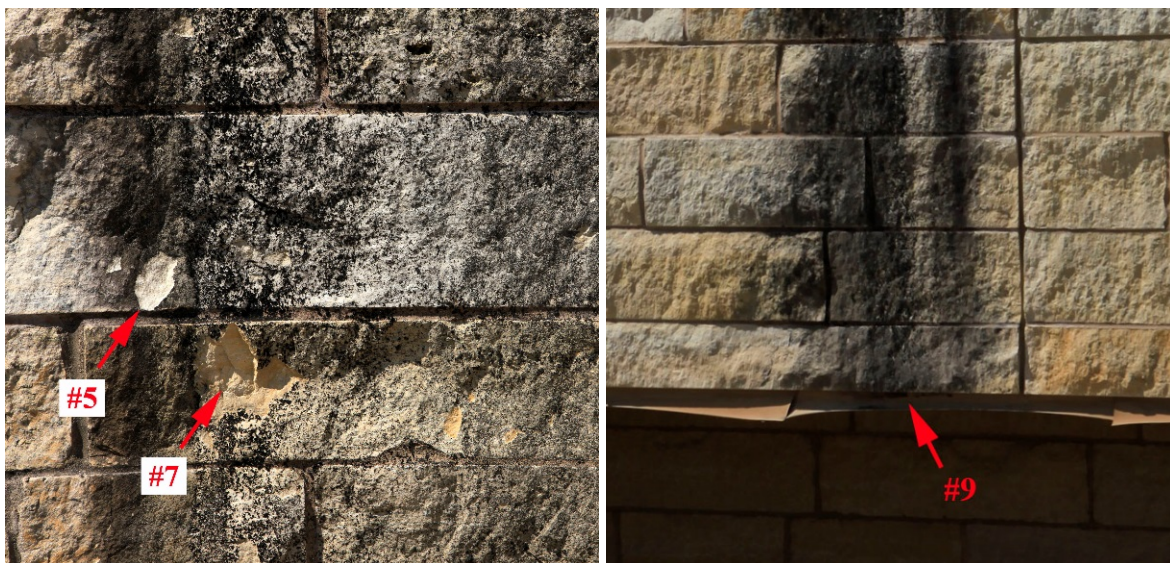


Figure 3. Details of sample locations shown in the previous figure. Left, samples #5 and #7 were flakes detached from areas indicated by the arrows. Note the uniform black colonization where sample #5 was taken, compared to the spotty area to its right where sample #7 was removed; right, sample #9 was scraped from the membrane at the bottom of the wall (April 2010).

The larger samples studied by May and Warscheid were pieces cut from extra blocks left over from the construction of the building.