Structural Analysis of Historical Constructions, New Delhi 2006
P.B. Lourenço, P. Roca, C. Modena, S. Agrawal (Eds.)

Analysis of Surface Patina on the Church of Nossa Senhora do Rosario, Ouro Preto, Brazil

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ABSTRACT: The church of Nossa Senhora do Rosario, in the town of Ouro Preto, Brazil, was constructed by and for slaves in the 17th Century. It is built of siliceous stone, with most of its exterior surface being plastered and painted, but with exposed quartzite in pillars and parapets. The hot and humid conditions have led to considerable weathering of the structure. Light and fluorescence microscopy, scanning electron microscopy with EDS elemental analysis, laboratory culture on mineral medium and DNA sequencing after amplification by the Polymerase Chain Reaction with specific primers were used to determine the nature of the black patina on the quartzite parapet. It was composed solely of cyanobacteria. The filamentous organism, *Stigonema ocellatum*, was mainly responsible for the black patina; the spherical cells of *Gloeocapsa* added to the dark colouration through their deep red capsules and also caused direct degradation by penetrating into the stone, producing pits.

1 INTRODUCTION

Both modern and ancient buildings are subject to weathering, caused by biological and non-biological factors. Biological and, especially, microbiological, factors are particularly important in tropical and sub-tropical climates (Allsopp and Gaylarde 2004). In Brazil, as in other parts of the world, there has been recent increase in the attention paid to the conservation of historic and cultural properties; the colonial churches of the Brazilian states of Salvador and Minas Gerais are important examples.

The microorganisms involved in biodeterioration of buildings are bacteria, fungi (moulds) and algae, fungi generally having been considered most important for destructive process in the past (Allsopp et al. 2004, Wollenzien et al. 1995). The microbial colonisation and the subsequent degradation of the stone are influenced, however, not only by the microbial flora, but also by the porosity and permeability of the material, the pH and the chemical nature of the surface, as well as by the environmental conditions. Microorganisms can induce stone degradation by a number of mechanisms, including production of acid or alkaline metabolites, chelation of constitutive elements, production of an organic layer (biofilm), subject to water absorption and varying in mass according to temperature variations, as well as the production of surface patinas by mobilisation of ions (generally Fe and Mn) within the stone (Gaylarde et al. 2003, Warscheid and Brahms 2000).

Figure 1 : The church of Nossa Senhora do Rosario, Ouro Preto, Minas Gerais, Brazil.

The church of Nossa Senhora do Rosario, in the historic town of Ouro Preto, Minas Gerais, Brazil, is one of the churches constructed by and for the slaves in the 17th Century (Fig. 1). It is built of siliceous stone, with most of its exterior surface being plastered and painted, but with exposed quartzite in pillars and parapets. The hot and humid conditions obtaining in Minas Gerais for most of the year have led to considerable weathering of the structure. A black patina is obvious on some of the exposed quartzite surfaces and this increases the thermal effect of water on the surface, leading to stresses within the structure and increased degradation. Here, we report the analysis of the black patina present on the surface of exposed quartzite on the parapet of this church.

2 MATERIALS AND METHODS

2.1 Scanning Electron Microscopy and Electron Dispersive Spectroscopy (EDS) analyses

Loose flakes from the upper parapet of the church were broken to an appropriate size, attached to stubs and sputter-coated with carbon, without any previous treatment. They were examined in the SEM (Cambridge Stereoscan 600 operating at 20KV) and various portions of the images analysed by EDS.

2.2 Microbiological analysis

Loose flake samples from the upper parapet of the church were stored at ambient temperature until laboratory analysis was possible (approximately one month). Our previous studies have shown that this is the best strategy for preserving organisms from walls in tropical or sub-tropical environments.

Sub-samples were broken from the flakes and placed directly on nutrient poor (oligotrophic) medium, solid Modified Knop's Medium (MKM), containing (g.L^{-1}) KNO_3 1.25; KH_2PO_4 1.25; $MgSO_4.7H_2O$ 2.5; $Fe_2(SO_4)_3$ 0.004; sodium citrate, 0.3; $CaCl_2.2H_2O$ 0.036 and agar-agar 1.4, plus 1mL micronutrient mixture, containing (g.L^{-1}) H_3BO_3 2.86; $MnCl_2.4H_2O$ 1.81; $ZnSO_4.7H_2O$ 0.222; $Na_2MoO_4.2H_2O$ 0.39; $CuSO_4.5H_2O$ 0.079 and $CoCl_2.6H_2O$ 0.045 (Gaylarde and Gaylarde, 1998). Medium BG11 (Rippka et al., 1979), supplemented with 7% sodium chloride, was also used. Plates were incubated at 25 ^{o}C in an illuminated BOD incubator and were examined microscopically after 1–4 h at magnifications up to x312 to visualize rehydrated microorganisms in situ, and then after further incubation for up to 8 weeks. Microorganisms seen after the initial (4 h) rehydration period and those growing on the incubated plates were

recorded photographically at 400–1000 x magnification using a Leica DMLB microscope with digital camera attachment and IM50 software. Fluorescent light was used to verify the presence of photosynthetic pigments within the cells.

Microorganisms were identified by their morphology and cyanobacteria classified according to Boone et al. (2001) and Geitler (1932).

2.3 Molecular analysis

The classification of cyanobacteria is still under revision and analysis of the DNA of the cells, in addition to cell morphology, is recommended for identification (Castenholz, 2001). The Polymerase Chain Reaction (PCR) allows the DNA of specific groups of organisms to be amplified and sequenced, using group-specific primers to initiate the reaction. After growth on solid medium, cyanobacteria were thus subjected to direct PCR with universal bacteria (27F1) and cyanobacteria-specific (809R) primers, using a modification of the methods described in Gaylarde et al. (2004).

Microcolonies or filaments were placed in 5 µl of MilliQ water in a sterile Eppendorf and freeze-thawed 5-6 times in the freezer compartment of a domestic refrigerator (71^{o}C) and at room temperature (ca. 23^{o}C). After the final thawing, all the required reagents for the PCR reaction were added directly to the Eppendorf to give a final volume of 20 µl. These were: 2.0 µl DNA polymerase reaction buffer (10x), 1.0 µl $MgCl_2$, 50 mM, 2.0 µl dNTPs, 2 mM (all from Biotech International, Perth, Australia), 7.8 µl water MilliQ, 1 µl each of forward and reverse primers (10 µM) and 0.2 µl *Taq* DNA polymerase 1 U/µl. The PCR reaction was carried out in an Applied Biosystems GeneAmp System 2400 (PE Applied Biosystems, Fullerton, CA) with the following programme: 92^{o}C for 10 min for initial denaturation, 30 cycles of 92^{o}C for 30 s, 30 s at 55^{o}C, 72^{o}C for 1 min, and a final extension at 72^{o}C for 7 min. The products were visualized by electrophoresis in 1% agarose gels using ethidium bromide staining. PCR products were sequenced using the primers 27F1 and 809R.

Automated sequencing was performed using the PRISM Big Dye cycle sequencing system and ABI 3739 Capillary Applied Biosystem (Foster City, CA) using Polymer 7. Consensus sequences were compared to those deposited in public databases using the BLAST search tool (www.ncbi.nlm.nih.gov/BLAST). Sequences giving similarities of >95%, where these agreed with the cell morphology, were accepted as the probable identity of the organism.

3 RESULTS AND DISCUSSION

3.1 Scanning Electron Microscopy and Electron Dispersive Spectroscopy (EDS) analyses

Fig. 2 shows a scanning electron micrograph (SEM) of the black patina and the EDS analysis is shown in Fig. 3. No elements were detected in sufficient quantity to account for the black colouration. The silicon, aluminium, potassium and iron identified in the EDS scan doubtless come from the quartzite itself, together with windborne clays and pollution from a nearby aluminium smelting plant.

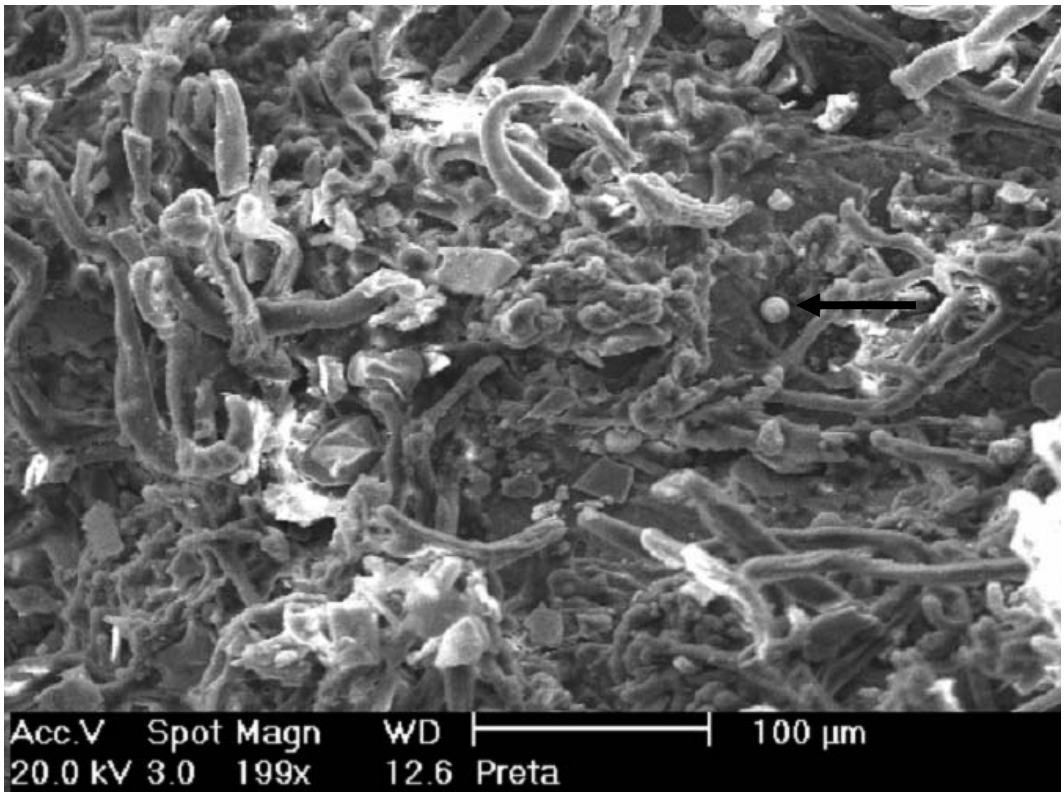

Figure 2 : Scanning electron micrograph of patina on the quartzite parapet of the church. Arrow indicates spherical cells in a similar sized pit.

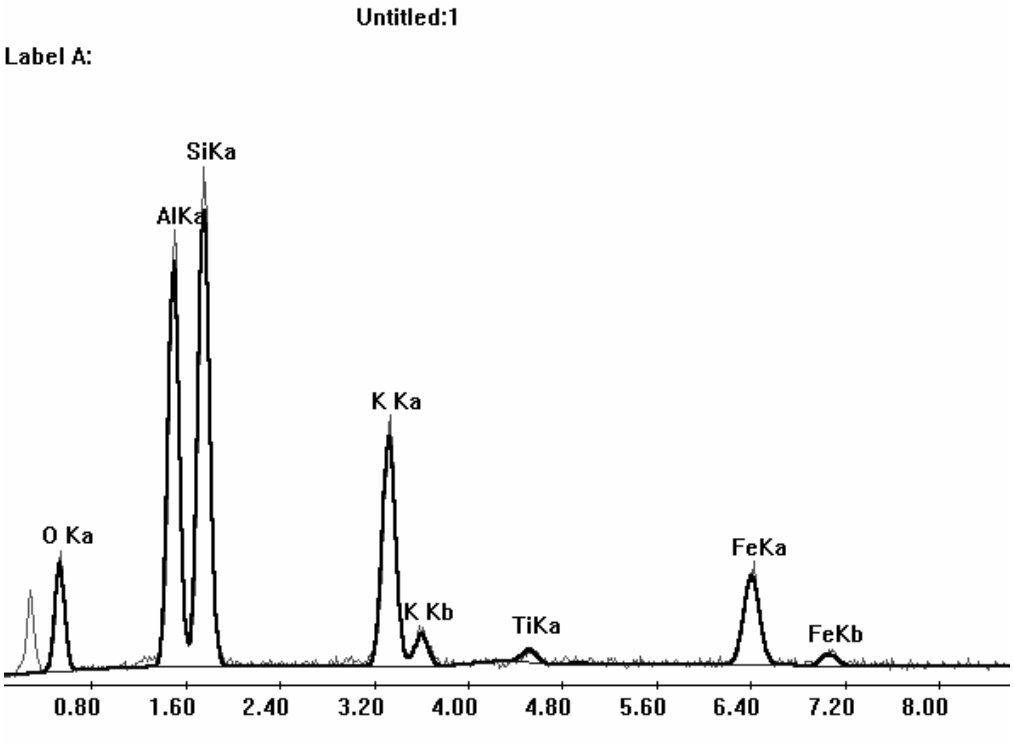

Figure 3 : EDS elemental analysis of black patina

Numerous filamentous and a few spherical structures are seen in the SEM. These have the appearance of biological organisms and this is confirmed by light microscopy, as described in the following section.

3.2 Microbiological and molecular analyses

Low power light microscopy of the black surface before any manipulation showed the presence of a thick mat of dark brown filaments that was quite easily detached by scraping. After incubation on mineral medium, it was possible to identify these as the filamentous cyanobacterium, *Stigonema ocellatum* (Geitler 1932). Figure 4 shows light (a) and fluorescence (b) micrographs of the incubated biofilm. Bright areas of fluorescence show the chlorophyll-packed cells. Filaments of the organism were used in the PCR reaction with specific primers and the resulting product sequenced. Comparison with DNA sequences in the public data bases showed a 96% similarity with *Stigonema ocellatum* SAG 48.90, confirming the identity of the principal organism forming the black patina.

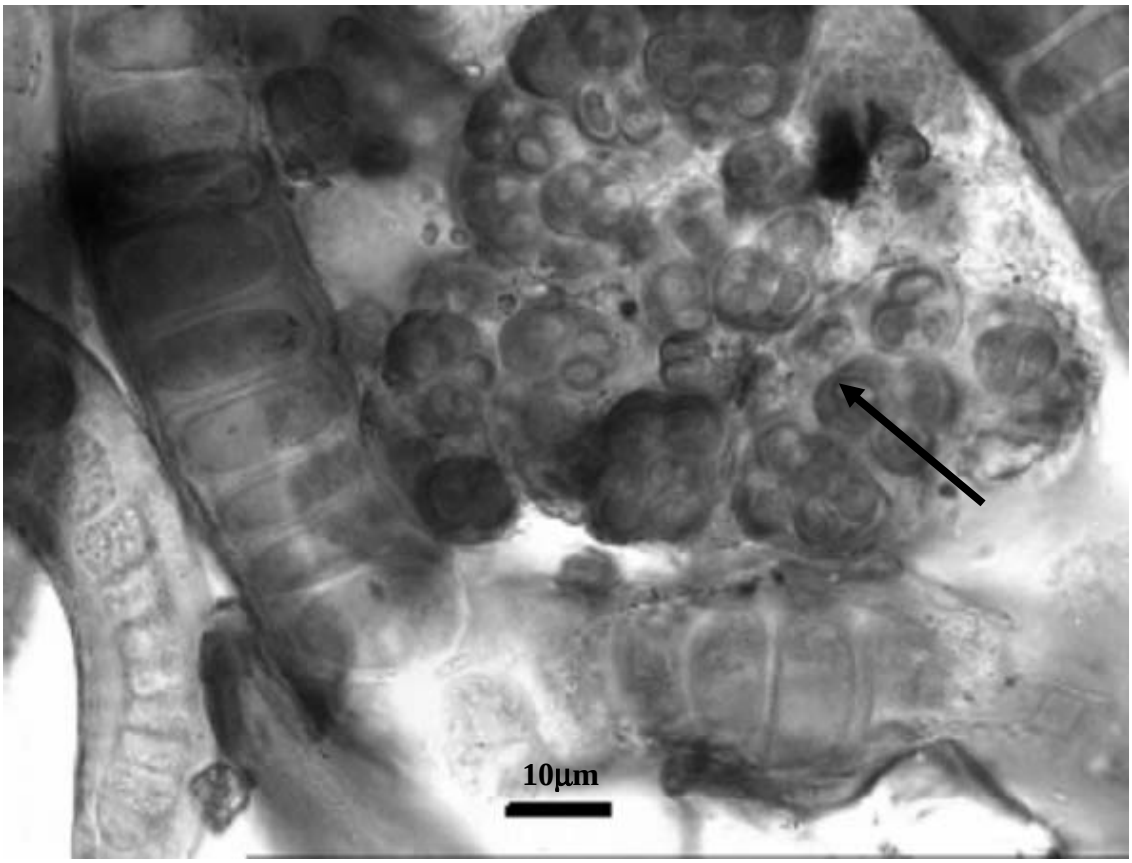

Figure 4a : Light micrograph of cyanobacteria forming black patina on the quartzite parapet. Arrow indicates groups of coccoid cells in dark red capsules.

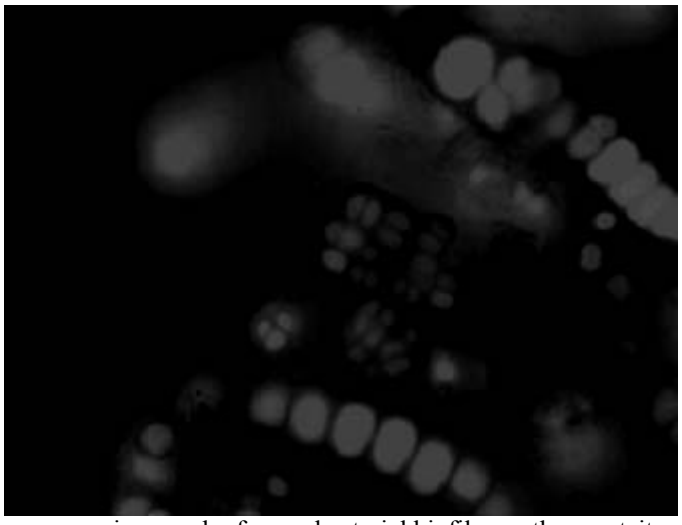

Figure 4b : Epifluorescence micrograph of cyanobacterial biofilm on the quartzite surface. Bright areas are chlorophyll-containing cells within spherical or filamentous sheaths.

In addition to *Stigonema* and its brown sheath, groups of ovoid cells in red capsules are seen in Fig. 4a (arrow). These belong to the cyanobacterial genus *Gloeocapsa*, probably Cluster 2 (Herdman et al. 2001). Although the filamentous organism was the main component of the biofilm, it is obvious that the red pigment of *Gloeocapsa* adds to the dark colouration of the patina.

Closer observation of the scanning electron micrographs showed that spherical structures were sitting in similar sized pits on the stone surface (Fig. 2, arrow). This indicates that *Gloeocapsa* can penetrate into the stone, causing degradation. This genus has previously been stated to have the ability to penetrate into limestone (Ferris and Lowson 1997, Ortega-Morales et al. 2005), but this is the first time that it has been shown to produce pits in siliceous stone. Both organisms have outer sheaths or capsules that facilitate their adhesion to surfaces and also allow dirt and pollutants to become integrated readily into the patina. This is seen in the EDS analysis, where iron and aluminium levels are higher than in quartzite itself because of the incorporation of aerial pollutants.

4 CONCLUSIONS

1. The black patina on the quartzite parapet of the church was composed solely of cyanobacteria (previously called blue-green algae).
2. The filamentous organism, *Stigonema ocellatum*, was the principal organism present and was mainly responsible for the black colouration. It did not penetrate into the stone and thus did not cause degradation directly, although its mere presence would lead to differential heating and moisture effects and hence damage.
3. The other organism present was the coccoid cyanobacterium *Gloeocapsa*, which was embedded in red capsules. These added to the discoloration, but, in addition, penetrated into the stone producing pits.
4. The identification of these two organisms as being responsible for patina formation and potential destruction of the quartzite allows preventative steps to be taken to prevent rapid reoccurrence after the surface has been cleaned.

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