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An interdisciplinary study of biodeterioration of the external marbles of Santa Maria del Fiore Cathedral, Florence (IT)

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Abstract. Stone-built cultural heritage exposed to urban environment represents a habitat where heterogeneous microbial communities can grow causing structural and aesthetical modifications and significant biodeterioration phenomena, the most common being colored patinas and crusts. An in-depth investigation of microbial community composition and its metabolic potential is essential to take the appropriate measures to control its growth. Conventional biocides remain the most used practical solution to control microbial growth, nevertheless, they may be dangerous for human health and the environment. The Cathedral of Santa Maria del Fiore (SMFC) is a major architectural masterpiece in Florence (IT), and its conservation is a main issue of worldwide concern. The whole edifice is externally covered with polychrome stone panels consisting of white marbles, serpentinites and red limestones. Here we report a multidisciplinary investigation on the state of conservation of SMFC white marbles which show, in some extended areas, patinas and discoloration due to microbiological growth. This work provides new details on the deterioration of SMFC marble and, for the first time, on the microbial community involved. This preliminary knowledge will be used for planning field tests with innovative low environmental impact biocides, such as essential oils, to contrast biodeterioration.

1. Introduction

The conservation of historic buildings and monuments is a major issue in modern societies, both from an economical and cultural point of view. The weathering of natural stone-built cultural heritage is a problem identified since antiquity. All geomaterials at the Earth's surface, exposed as a natural outcrop or in a building, are subject to the destructive physical, chemical and biological aspects of weathering.

The decay processes of stone materials depend on several factors, both intrinsic, such as the mineralogical and textural characteristics of rocks, and extrinsic, such as the environmental conditions (climate and microclimate affecting the whole building or part of it, human activities). Among factors contributing to stone decay, biological attack is one very common and dangerous. Biodeterioration can be defined as "any undesirable change in a material brought about by the vital activities of organisms" [1]. Microorganisms are the main biological agents deteriorating cultural heritage since they can

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colonize any organic as well inorganic substrate and modify it due to their extremely wide and versatile metabolism.

The mineralogy and chemical composition as well as the porosity, surface roughness and water uptake of the material substrate are significant parameters affecting the so-called stone bioreceptivity and promoting microbial colonization [2]. Microorganisms inhabiting monumental stone exert the same biogeochemical processes as on natural stone, contributing to rocks modification. Bacteria, fungi, algae and lichens are the common colonizers of stone, growing as a complex community in biofilms adhering to the surface and playing a significant role in physical and chemical stone decay. They produce not only visible aesthetical damages, such as colored patinas, crusts or discoloration, but also severe structural modifications of materials such as fractures and material loss, due to acid corrosion and mechanical attack, since they penetrate into stone [3]. This is particularly relevant for stone-built cultural heritage exposed to outdoor urban environment, where atmospheric agents and pollution modify material surface and its bioreceptivity, facilitating microbial growth.

The knowledge of the rock characteristics, the composition and metabolic potential of the resident microbial community, as well as the interactions between microorganisms and stone, is essential to take the appropriate measures to control microbial growth and protect the material. To assess microbial community presence and diversity on deteriorated stone, culture-dependent as well culture-independent techniques can be used, depending also on the aim of the study [4,5].

Marble has been used since ancient time in the Mediterranean Basin as constructive and decorative material, in both the interior and exterior of buildings, due to its bright, white colour and translucence. However, despite its hardness, marble is subject to weathering processes that can seriously affect its durability. Deterioration phenomena associated with this degradation are numerous (e.g. bowing of façade claddings, nucleation and growth of microcracks on massive structural parts, loss of relief structure); furthermore, marble can be colonized by several kinds of microorganisms such as bacteria and cyanobacteria, algae, lichens, filamentous and meristematic fungi [2,6,7].

The Cathedral of Santa Maria del Fiore (SMFC) is a is one of the greatest masterpieces of Gothic art and the first Italian Renaissance in Florence (IT), whose conservation is a main issue of worldwide concern. It was begun in 1296 in the Gothic style on a project of Arnolfo di Cambio and was structurally completed, by 1436, with the dome designed by Filippo Brunelleschi. The exterior of the cathedral is faced with polychrome stone panels consisting of white marbles, serpentinites and red limestones [8,9,10] and has an elaborate 19th-century neo-Gothic façade by Emilio de Fabris. The external marbles show, in some extended areas, patinas and discoloration due to microbial growth and periodic cleaning interventions are needed. Conventional biocides remain the most used practical solution to control microbial growth, nevertheless, they may be dangerous for human health and the environment. Extensive research is now ongoing to find alternative and eco-friendly substances or methods to cope with biodeterioration.

Here we report a multidisciplinary investigation on the state of conservation of SMFC white marbles and the microbial community involved in their deterioration. The knowledge acquired by this work will be used to test innovative methods to contrast microbial growth.

2. Materials and Methods

2.1. Macroscopic description. The marbles utilized in the revetment of the Santa Maria del Fiore Cathedral come from the mining areas of the Apuan Alps, in the Carrara district [8], known as the world largest marble mining basin, both for the number of active quarries (much more than a hundred), and for the historical significance of the stone resource. The Apuan marbles represent the best known stone materials, used by architects and artists throughout the world. Microstructural characteristics of Apuan Marbles vary depending on the different extraction site and inside the same quarry, according to the complex tectono-metamorphic history that they have undergone [11].

The SMFC white marbles exhibit extended forms of decay (figure 1), such as deposits, crusts, erosion, mechanical damage and biological colonization (patinas and discoloration).

Figure 1. Different form of decay in the SMFC marble: a),c),d),e) fractures and cracks; a),d),e) discoloration; a),d),f) deposits and patinas; b) black crusts; c) granular disaggregation; e) differential erosion; a),c),d),f) biological colonization.

2.2. Site description and sampling. External white marbles were studied in two different areas, North-West and South-East exposed, of the outside walls around the apse, accessible from the continuous gallery on the upper part of SMFC (figure 2).

Figure 2. SMFC sites of study: a) South walls; b) a particular of the gallery of the South-East walls; c),d) the inside face of the openwork parapet of the studied North-West and South-East areas, respectively.

For mineralogical and petrographic analyses, marble samples were collected in a non-invasive way removing small chips of material from areas affected by fractures, cracks and detachments.

For microbiological analysis, sampling was done on the inside face marble surface, showing biological growth, of the openwork parapet of the gallery (figure 2b). Surficial particulate was gently scraped with a sterile spatula (micro-invasive method) and collected into sterile tubes. Three samples of about tens of mg were taken from the North (N1, N2, N3; figure 2c) and three from the South-East (S1, S2, S3) areas (figure 2d). Samples were immediately brought to laboratory and processed.

2.3. Mineralogical and petrographic characterization. The mineralogical and petrographic analyses were carried out using X-ray Diffraction (XRD) and a polarizing microscope. XRD analysis was conducted using a Philips PW 1050/37 powder diffractometer with radiation CuKα1 (λ=1.545 Å) and graphite monochromator, operating at 40 kV, 20 mA, investigated range 2θ=5–70°, software X'PertPRO and High Score for data acquisition and data interpretation of the mineralogical composition; the textures of the marbles were studied on thin sections (30 microns thickness) using a Zeiss Axio Scope.A1 polarising microscope, equipped with a camera (resolution 5 megapixel).

2.4. Cultivation of microorganisms. 40 mg of marble particulate were suspended in 1 ml of PBS (8 g/l NaCl, 0.2 g/l KCl, 1.44 g/l Na_2HPO_4, g/l 0.24 KH_2PO_4, pH 7.4) added with Tween 80 0.001% and vortexed. 0.05 or 0.1 ml of undiluted and 10^{-1} diluted suspension were plated in duplicate on nutrient media and then incubated up to seven days. Viable titer was calculated as mean value of the number of Colony Formant Units (CFUs) per gram of marble particulate. Statistical analysis of data has been performed with one-way ANOVA and Tukey post-hoc comparison by using Past [12].

Nutrient media used for bacteria growth were: Nutrient Agar (NA, Oxoid), Luria Bertani Agar (LB Broth, Sigma,15 g/l agar if solid), R2A Agar (Oxoid). R2A Agar was also used added with 0.1% and 1% marble powder (obtained by a marble workshop), and with 1% aqueous marble extract (starting from a 50% sterile water solution of marble extract). Plates were incubated at 30°C in aerobic conditions and growing colonies counted after 2 and 7 days of incubation.

Nutrient media used for fungi growth were: Malt Extract Agar (MEA, Oxoid), Sabouraud Dextrose (Oxoid, 15 g/l agar if solid), Rose-Bengal Choramphenicol Agar (Oxoid,). MEA was also used added with 0.1% and 1% marble powder, and with 1% aqueous marble extract. Plates were incubated at 30°C in aerobic conditions and growing colonies counted after 7 days of incubation.

2.5. Optical Microscopy. Marble particulate was suspended in physiological solution (9 g/l NaCl) and suspension observed at the optical microscope Axioscope 5 (Zeiss) equipped with the digital camera Axiocam 208 color.

3. Results

3.1. Mineralogy and Petrography. XRD analysis clearly indicated calcite as the dominant constituent of the studied marbles; small amounts of quartz are limited to a few rock samples. The microscopic observations confirm the presence of prevailing calcite which displays the typical polysynthetic twinning; the calcite crystals show heterogeneous grain-size, generally in the range 150-200 μm, up to 500 μm; the accessory mineral is essentially quartz, as identified also via XRD analyses. The SMFC marbles are characterized by a prevalent heteroblastic or, in some cases, homeoblastic mosaic texture with grain boundary shapes from straight to lobate-curved and sutured; no preferred orientation of the crystals is visible; limited to a few samples, fine-grained calcite crystals can be observed along the grain boundary. Under thin-section microscope observation, the marbles show low porosity; however, where the crystals boundary is prevalently straight, a slight grain detachment is observed.

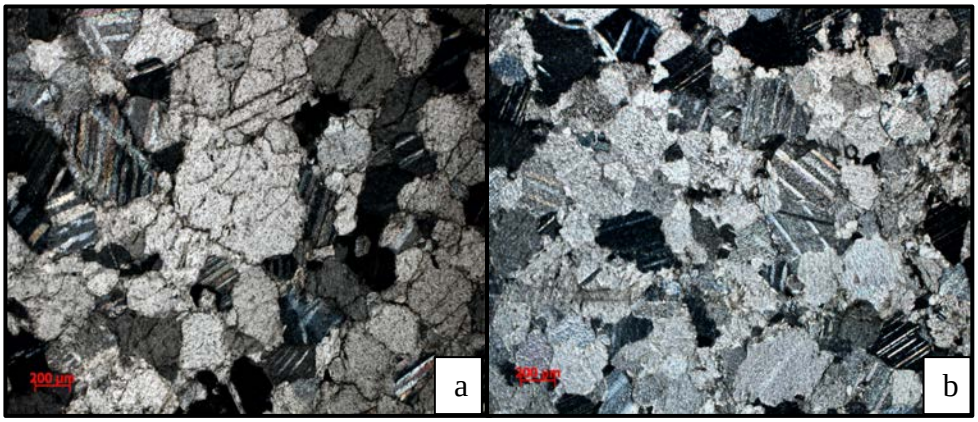

Figure 3. Thin section photomicrographs of South-East (a) and North (b) exposed SMFC marbles showing the different grain boundaries shapes.

3.2. Cultivation of microorganisms from marble

Multiple cultivation experiments of bacteria and fungi from marble suspensions were performed, with the aim to find suitable conditions for microbial growth for evaluation of future *in situ* trial biocide treatments.

3.2.1. Bacteria. The first cultivation test was performed by plating marble suspensions from each sampling points of the North-West (N) and South-East (S) areas on LB plates. Results after 6 days of incubation are shown in Table 1. The whole CFUs data of N (N1+N2+N3) and S (S1+S2+S3) samples showed no statistically significant differences (figure 4).

Table 1. Bacterial viable titer of marble samples of North-West (N) and South-East (S) areas on LB medium

Sample	Viable titer (CFU/g ± Sd)
N1	$7.25 \times 10^{3} \pm 5.30 \times 10^{3}$
N2	$3.00 \times 10^{5} \pm 8.45 \times 10^{4}$
N3	$3.87 \times 10^{4} \pm 1.16 \times 10^{4}$
S1	$1.21 \times 10^{4} \pm 5.05 \times 10^{3}$
S2	$1.66 \times 10^{6} \pm 7.07 \times 10^{4}$
S3	$2.35 \times 10^{5} \pm 9.75 \times 10^{4}$

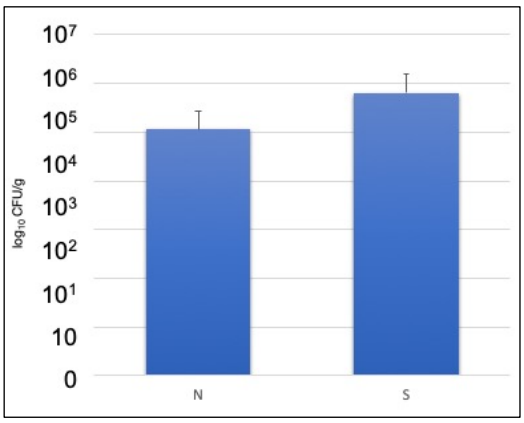

Figure 4. Bacterial viable titers on LB medium of marble samples from Nort-Westh (N) and South-East (S) areas. Reported values indicate average from 3 replicates. Error bars indicate standard deviation. No significant differences (p-values > 0.05) between inoculants have been detected (one-way ANOVA).

Only few colonies grew on NA medium. Bacteria cultivation was also tested on R2A medium. Comparison of viable titers obtained on LB and R2A from the North sampling area is reported in figure 5.

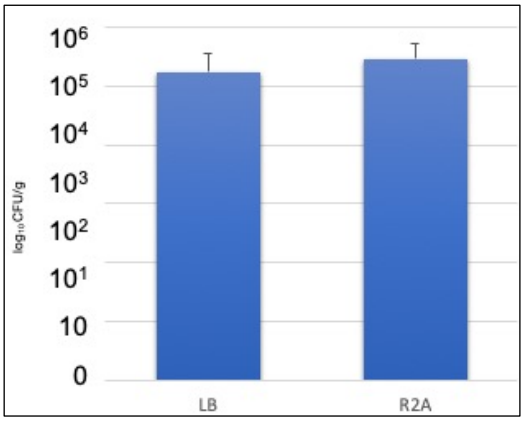

Figure 5. Barplots of the bacterial viable titers of North-West samples on LB and R2A media. *Values indicate average from 3 replicates. Error bars indicate standard deviation. No significant differences (p-values > 0.05) between inoculants have been detected (one-way ANOVA).*

No statistically significant differences between LB and R2A cultivation were detected. To verify if marble powder could have any factors helping growth of microorganisms isolated from the same material, suspensions of samples S and N were plated on the complex media supplemented with marble powder and aqueous marble extract. No significant differences were found in viable titers obtained with and without any marble addition.

3.2.2. Fungi. Cultivation of marble suspensions on Sabouraud and Bengal-Rose media gave no growth of fungal colonies. Better results were obtained on MEA medium, which showed growth of fungal filamentous colonies. The viable titer of molds on MEA was up to 1,49 x 10^4 ± 3,15 x 10^3 CFU/g (N sample). No significant differences were found in viable titers obtained on MEA with and without any marble addition.

3.3. Microscopical observation of marble

Dispersed composite aggregates of complex microbial communities constituted by phototrophic (algae, lichens, cyanobacteria) and non-phototrophic (fungi and bacteria) microorganisms were observed on marble samples, from the South-East as well as North site, by optical microscopy (figure 6).

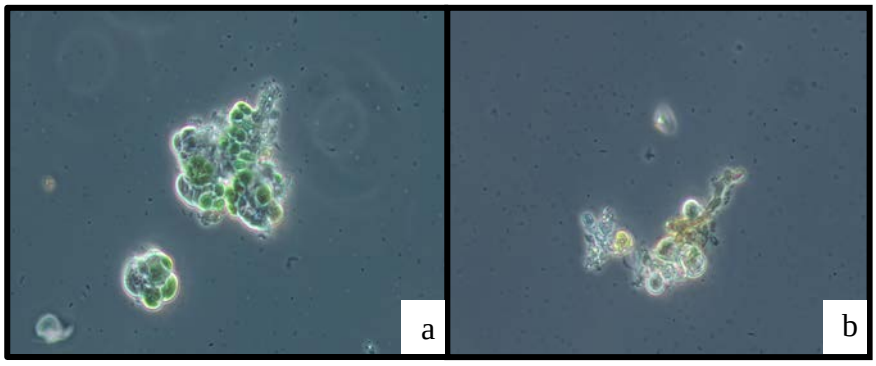

Figure 6. Microbial aggregates from patinas covering white marbles observed at the light microscope (400X). a) aggregates of cyanobacteria from the South-East site sample; b) aggregate of algae and fungi from the North-West site sample. Free bacteria are visible in a) and b).

4. Discussion and concluding remarks

The present study focuses on the diagnostic of the external white marbles of the Cathedral of Santa Maria del Fiore (SMFC), a World Heritage site in Florence (Italy).

At naked eye observations, the SMFC white marbles exhibit extended forms of decay, such as deposits, crusts, erosion, mechanical damage and biological colonization (patinas and discoloration).

Two different sites, North-West and South-East exposed, of the outside walls around the apse of SMFC, were selected to study external white marbles deterioration. Marbles samples coming from the two areas were subjected to microbiological, microscopic, mineralogical and petrographic analyses.

The different marble samples showed overall similar mineralogy and petrographic characteristics. However, some differences in boundary grain shapes have been observed; according to several authors [e.g. 11,13], the microstructure has a strong influence on physical parameters such as the open porosity and the water absorption thus affecting the marble resistance to physical weathering by change of temperature, humidity, freeze–thaw cycles; a microstructure showing straight grain boundaries results in more regular shape of intercrystalline pores and easier grain detachment, thus causing an easier water absorption in respect to a lobate-sutured grain boundary microstructure [14].

Preliminary tests were carried out to find the best condition for cultivation of bacteria and fungi, in order to use microbial viable count to assess the effectiveness of innovative biocides in future field trials. The media R2A and MEA were chosen for future cultivation of chemoorganotrophic bacteria and fungi, respectively. On the other hand, they have already been used for successful cultivation of bacteria [15] and fungi [6,7] from stone.

The microscopic observation of biodeteriorated marble showed the presence of a complex biofilm constituted of multikingdom microorganisms with a great potential in causing severe physico-chemical damage.

Here, preliminary data on the state of conservation of SMFC white marbles were reported. A more in-depth investigation is ongoing to get a better knowledge of biodeterioration phenomena affecting marble also with a view to testing innovative and low-impact biocides to control microbial growth in *in situ* trials. In our opinion, the multidisciplinary approach used in this work can be applied to other stone-built historical buildings to better cope with specific biodeterioration phenomena.

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