CODE 107

ASSESSING AESTHETIC AND STRUCTURAL DETERIORATION IN HISTORIC BUILDINGS - A CONTRIBUTION

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ABSTRACT

An important part of our cultural heritage assets is built of natural stone since it has always been a material of excellence due to its inherent characteristics. Despite the physical and mechanical properties that give relatively long durability as a construction material, some characteristics are less durable, namely color or eventual surface finishes. The alteration of its aesthetic features may be followed by the alteration of the physical and mechanical properties of the stone. In this way, it is crucial to characterize the material and perform regular diagnoses in the buildings classified as local cultural heritage to provide the necessary tools for future intervention campaigns of conservation and restoration.

Considering its chemical composition, each stone has a specific behavior when placed in a particular environment. Factors like humidity or temperature are strongly influenced by the weather, which will also influence the nature of microbial colonization. Keeping this in mind, the work here presented aims to demonstrate a non-invasive and non-destructive analytical methodology applied in the identification of deterioration phenomena in natural stone buildings, either by geochemical or biogenic pathways, using X-rays based techniques and through the identification of colonizing populations. This methodology was successfully performed in the marble on the main cloister of the Convent of "São João da Penitência". The Convent is located in Estremoz (Portugal), is dated from the 16th century, and presents applications of local marble. Several pathologies were identified, ranging from aesthetic damage, where the color is strongly compromised, to detachment of relatively large fragments. Impurities in the calcite matrix and the action of microbial and pollution agents were considered as the main factors that are contributing for the deterioration of the natural stone applied in this historic building.

KEYWORDS: Cultural Heritage deterioration; Natural stone; Estremoz marble; Biodeterioration.

1. INTRODUCTION

Natural stone has been manipulated for construction purposes and the manufacture of heritage assets since ancient times, and it is considered one of the most durable raw materials due to its physical and mechanical properties. However, some characteristics that input aesthetic value to this material are less stable, namely its color and surface treatments [1-3]. These characteristics must remain intact as long as possible since they are key-factors in the appreciation of a particular heritage asset as well as in contemporaneous projects. The deterioration of this type of material can be triggered and accelerated by external factors such as temperature, solar radiation, humidity, colonizing agents, pollution, and also by internal factors like the composition of the stone, biosusceptibility, among others.

Nowadays, several efforts are being made by the scientific community to know and mimic the deterioration phenomena of natural stone that occur naturally. These studies have focused mainly on the alteration of physical-chemical properties through the action of water, temperature, solar radiation, soluble salts formation, among some others [4-6]. Recently, more attention has been given to other alteration phenomena of the material based on biogenic action [7-9]. The action of the microbial colonizers can induce alteration of the aesthetic characteristics of the stone, and also compromises its physical integrity through the penetration of the hyphae of filamentous fungi in the matrix, whose growth and proliferation are highly influenced by the environmental conditions [10,11]. Additionally, other deleterious effects promoted by biocolonization must be considered, namely the secretion of acids or other compounds (e.g. carotenoids) which may contribute to the chemical dissolution and staining of stone.

1.1. Historical context

The study here presented was performed on a historical building located in Estremoz (Portugal) with local marble applications, the Convent of "São João da Penitência", founded in 1501 by the Portuguese King Manuel I. The Convent was established in 1519, being the only Monastery of the Knights of Rhodes in Portugal, and was subsequently incorporated into the Order of Malta. The church is contemporary with an 16th century structural design, exhibiting a Manueline architecture. In the XVII century, the area was enlarged with a second arcade, supported with Tuscan pillars. Each wing has ten arches, which are subdivided into four twins and two single arches.

Since antiquity (370 BC), the Anticlinal of Estremoz has been one of the main sources of marble of the Mediterranean area, and due to its historical and geological relevance, it is considered part of the Portuguese geological heritage [12]. The marble here extracted has been always recognized for its excellent physical and chemical properties, and in the Middle Ages assumed special relevance in the construction of castles, palaces and other buildings [13,14]. Later on, in the 15th century, this material becomes to be exported to other continents by Portuguese explorers.

To may prevent the gradual degradation and consequent loss of our built heritage, it is essential to create a deep knowledge about the mechanisms of the material alterability, providing the best indications for future campaigns of intervention and restoration in this type of historic buildings. With this in mind, this work aims to contribute for the development and the application of a methodology that will allow to evaluate the aesthetic and structural deterioration of heritage assets made of natural stone, using essentially non-destructive and micro invasive diagnostic techniques.

2. MATERIALS AND METHODS

The Convent of "São João da Penitência" is located on the eastern side of the town's main square (coordinates 38º 50' 44" N 7º 35' 30" O), where the climate is dry and temperate, and the yearly average temperature and rainfall are 15.6 ºC and 699 mm, respectively. The main cluster, built in marble, has been subject to deterioration phenomena over the years, which has led to the appearance of aesthetically unacceptable patterns. Six different areas, representative of the alteration phenomena occurring in these

marble materials were selected, such as white (SJP1) and yellow (SJP2) patinas, red stains (SJP3) and fissures (SJP4), and the formation of red (SJP5) and blackish (SJP6) biofilms (fig. 1). Two areas of non-altered stone were also selected to have a comparative basis. Therefore, this study intended to characterize the stone, detect the presence of alteration products and evaluate the colonization of microbial agents, using mainly non-destructive and micro invasive methods.

Figure 1. Main pathologies present in the Convent. Formation of white (SJP1) and yellow (SJP2) patinas (a, b), red stains (SJP3 and SJP4) (c, d), and formation of red (SJP5) and blackish (SJP6) biofilms (e, f).

To minimize the aesthetic impact of the building, an in-situ approach was initially made, applying the methodology following described.

2.1. In-situ approach

Imaging was performed using a digital microscopy (Dinolite, 430 nm, Anmo Electronics Corporation) at 45-75x magnification in reflected light and UV light mode. The measurement of the color was performed with a portable spectrophotometer (DataColor CheckPlusII, equipped with an integrating sphere, diffuse illumination 8º, and Standard Illuminant/Observe D65/10º) through the determination of the parameters of the CIELAB color space. The changes in these parameters were determined by calculating the parameter ΔE (total difference in color), through the following equation:

$$\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2}$$

Where $\Delta L = (L_2-L_1)$; $\Delta a = (a_2-a_1)$; $\Delta b = (b_2-b_1)$.

The color of each area was measured in 5 different points with 5 mm each, whose the data showed represents the mean value. On the other hand, the elemental composition was characterized through X-ray fluorescence using a handheld Bruker Tracer III/IV-SD operated with an XFlash® SDD with 145eV of resolution. The acquisition time was 120 s, operating the tube at 40 kV and 30 μA current under low vacuum conditions. Data were processed with the software ARTAX 7.4.0, and the peak areas were calculated after its normalization with the RhKα, and represents a mean value of nine measurements.

2.2. Micro-sampling process

The sampling process was performed under restricted conditions to minimize the aesthetic and structural impact on the building. Micro-fragments were collected, taking into account their detachment in specific locations and that proved to be representative of the areas analyzed. This process was closely monitored by a conservator-restorers team.

On the other hand, sterile swabs were used to characterize the microbial population proliferating on the stone, which were placed in a Maximum Recovery Diluent (MRD) transport solution and stored at 4 ºC until processing.

2.3. Material characterization and detection of alteration products

The mineralogical composition was determined by X-ray microdiffraction, using a Bruker Discover D8 Discover diffractometer. A CuKα radiation tube operating at 40 kV and 40 mA was used. Diffraction patterns of the micro-fragments were measured between 3º-75º 2Θ with 0.05º step size and 1s of recording time per step, without any previous sample preparation. The crystalline phases were identified with the PDF-ICDD Database, using the Bruker EVA software (version 3.0).
The complementary details of the chemical composition were given by scanning electron microscopy coupled to energy dispersive spectrometry. A HITACHI S-3700N microscope coupled to a microanalysis system equipped with an X-ray detector Bruker XFlash® SDD with 129eV of spectral resolution at the FWHM/Mn Kα was used. The micro-fragments were analyzed without any previous preparation, using the variable pressure analysis mode (40 Pa).

2.4. Microbial population assessment

2.4.1. Evaluation of biological contamination

The micro-fragments were coated with Au/Pd for 60 s and observed in the scanning electron microscope in high vacuum mode, using 10 kV of accelerating voltage and 10 mm of working distance to assess the presence of microbial communities.

2.4.2. Characterization of the microbial population

The samples collected with the swabs were mechanically agitated for 24 h. After that, inoculation with 100 µL of each sample was performed in NA and MEA (Nutrient Agar and Malt Extract Agar) culture media for the growth of prokaryotic and eukaryotic communities, respectively. Microbial isolates were obtained, stained with methylene blue and characterized using an optical microscope Motic BA410E equipped with a MoticamPro 282B camera. Obtaining and storing microbial isolates in this type of approach is essential since it enables their use for future studies in laboratory, such as susceptibility to biocolonization.

To characterize the total microbial population (i.e. cultivable and non-cultivable) present in the sampled areas, a metagenomic approach was carried out based on Next Generation Sequencing technique, as described at Dias et. al. (2018) [2].

3. RESULTS AND DISCUSSION

The methodological strategy here presented aims to diagnose the alteration phenomena occurring in the Convent “São João da Penitência”, which has a strong cultural history. This historic building contains applications of a local marble that is recognized for its excellent properties. The study intends to contribute to the implementation of a methodology that favors non-invasive or micro invasive methods which can provide key indicators for a future conservation and restoration campaign, which can be adopted for the study of other types of building materials.
Regarding the pathologies identified in the marble areas of the main cloister of the Convent, 3 broad categories compose the research work – patina formation (areas SJP1 and SJP2), staining (areas SJP3 and SJP4), and biofilm formation (areas SJP5 and SJP6).

3.1. Measurement and characterization of the color

The alteration of the original color of the stone was measured through the calculation of the parameter ΔE (table 1). The high ΔE values (>16 units) demonstrate that the original color of the surfaces of the stone in these areas is seriously compromised. The areas with biofilm development on their surface presented the greater deleterious alteration of the color, which proves to be the major responsible for the aesthetic damage in this Convent.

Table 1. Measurement of the colorimetric parameters on the selected areas, using the CIELAB system and ΔE determination.

Area I.D.	L*	a*	b*	ΔE
Non-altered	72.74 ± 4.64	0.6 ± 1.34	5.33 ± 3.01	-
SJP1	91.52 ± 2.41	1.84 ± 0.58	6.40 ± 2.04	18.85
SJP2	66.98 ± 3.95	8.69 ± 3.53	25.00 ± 6.03	22.03
SJP3	41.58 ± 8.57	10.94 ± 1.16	17.21 ± 2.69	34.91
SJP4	63.82 ± 5.49	8.77 ± 1.73	16.50 ± 2.13	16.46
SJP5	36.05 ± 4.33	13.46 ± 3.02	15.55 ± 2.21	40.19
SJP6	27.64 ± 1.68	1.82 ± 0.51	4.25 ± 1.34	45.13

3.2. Characterization of the material and alteration products

Deterioration of carbonate stone is frequently associated with the deposition and/or formation of compounds on its surface. These deposits not only contribute to change its appearance but also increase the degradation rate (e.g. by increasing water retention). The microfragments collected from the areas containing patina formation revealed the presence of calcium oxalates (fig. 2), namely weddellite ($CaC_2O_4 \cdot 2H_2O$) and whewellite ($CaC_2O_4 \cdot H_2O$). These compounds may have a biogenic or chemical origin since they can be a result of the microbial activity or the dissolution of calcium, which the latter is a relatively common process that occurs in carbonate rocks.

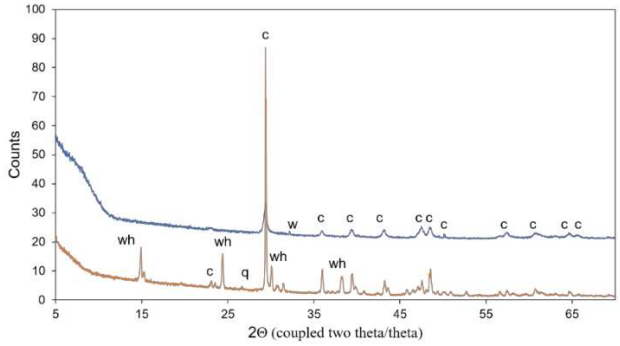

Figure 2. Micro-diffractograms obtained on the microfragments collected in the areas SJP1 (—) and SJP2 (—). Abbreviations: c – calcite; wh – whewellite; q – quartz; w – weddellite.

However, these compounds have been used as a strong indicator for the presence of microorganisms [15-17] since oxalic acid is a common metabolite resulting from microbial activity. Additionally, in some cases, the formation of these compounds seems to be triggered after conservation actions [16]. In this case study, their origin is not completely clear, but considering the presence of microbial colonizers (below detailed), both hypotheses are strong possibilities by acting together. In these same areas, sulfate compounds were also identified (calcium sulfates, determined by SEM-EDS – data not shown), which suggest that atmospheric SO_2 may also have an important contribution to the deterioration of the stone [18].

Regarding the two areas presenting reddish stains, although they are spatially close, seem to be originated by two different ways. The staining observed on the area SJP3 is characterized as red stains in a porous area (fig. 1c), while the staining observed on the area SJP4 is characterized by the accumulation of reddish pigmentation inside cracks (fig. 1d). The spectra here obtained by X-ray fluorescence (data not shown) reveal a higher enrichment in iron, mainly in the area SJP3, which may indicate that iron is present in the calcite as an impurity, since Fe^{3+} ion can replace Ca^{2+} or directly enter in its matrix [19]. Complementarily, elemental maps were obtained, showing the coexistence of Fe, Al, Si, and K in some particles, which is compatible with the presence of "Terra Rossa" or red soil. It refers to clay-rich soil that is commonly derived from calcareous rocks and is typical of Mediterranean regions

[20]. It is a result of the chemical degradation of layers that typically cover the bedrock or limestone deposits with a terrestrial or marine origin, and the preferential formation of hematite over goethite gives them a reddish color.

3.3. Microbiological assessment

The areas SJP5 and SJP6 present the development of red and blackish biofilms, respectively, that are covering the entire surface of the stone. Thus, the microbial communities here proliferating were characterized. SEM micrographs allowed a broad vision of it and to determine how the biofilms are developing and their impact on the surface of the stone. In the area SJP5 (reddish biofilm) it was possible to state the ability of filamentous fungi to involve and penetrate the stone through its pores (fig. 3). The deleterious effects of the development of these structures in this area are quite evident, where large pieces of rock are being completely detached from the rest of the structure. On the other hand, the area SJP6 (blackish biofilm) revealed the presence of diatoms, microalgae, and filamentous fungi (fig. 4), which suggests a greater diversity of the microbial community here present when compared with the area SJP5.

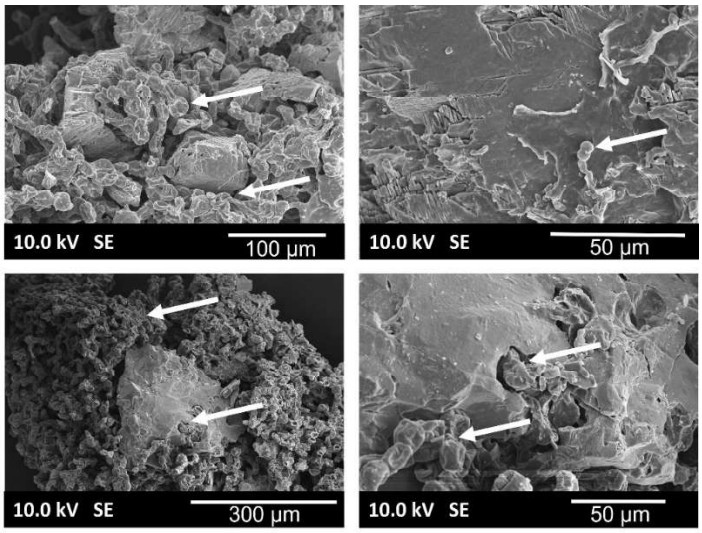

Figure 3. Micrographs obtained on the microfragment collected on the area SJP5 showing the ability of filamentous fungi to surround and penetrate the porous of the stone.

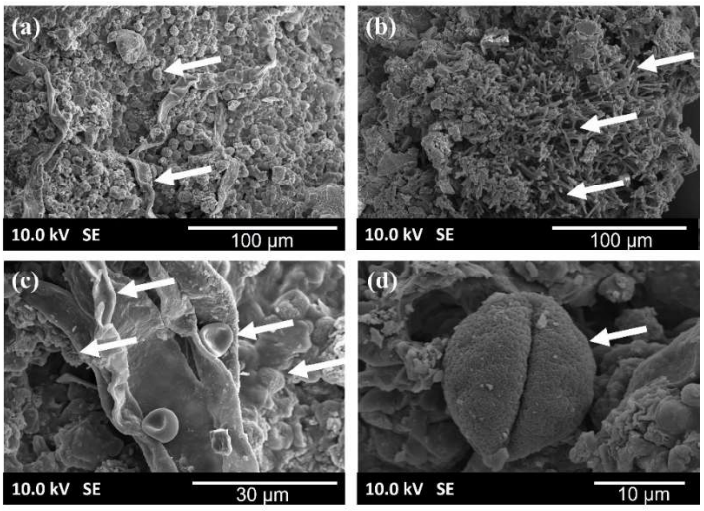

Figure 4. Micrographs obtained on the microfragment collected on the area SJP6 showing the microscopic aspect of the biofilm (a, b), presence of microalgae (c), and some unidentified structures (d).

For a precise characterization of the population that is colonizing the Convent, both culture-dependent and High Throughput Sequencing (HTS) methods were applied, where it was possible to state that the eukaryotic are the major colonizers. Regarding the prokaryotic population, 8 strains were isolated, predominantly with bacillus and coccus morphology. The data obtained through the HTS approach show that the prokaryotic population that is colonizing the Convent is mostly composed of phyla Proteobacteria, Actinobacteria and Cyanobacteria, while the most representative genera are *Pseudomonas*, *Rubrobacter*, *Geodermatophilus*, *Erwinia* and *Anabaena*. Previous studies have already been reported the identification of these kinds of microorganisms in stone-built monuments [9, 21-23].
On the other hand, the cultivable fungi (10 strains) are composed of black yeasts and microorganisms of the genera *Rhodotorula*, *Aspergillus*, *Mucor*, *Penicillium*, *Alternaria* and *Cladosporium*. The details obtained through the HTS approach allowed to state that the eukaryotic population colonizing the areas SJP1-4 is mostly composed of phyla Ascomycota and Basidiomycota, while the most representative genera are *Cladosporium*, *Alternaria*, *Stagonosporopsis*, *Mycosphaerella*, *Stemphylium*, *Aureobasidium* and *Vishniacozyma* (fig. 5).

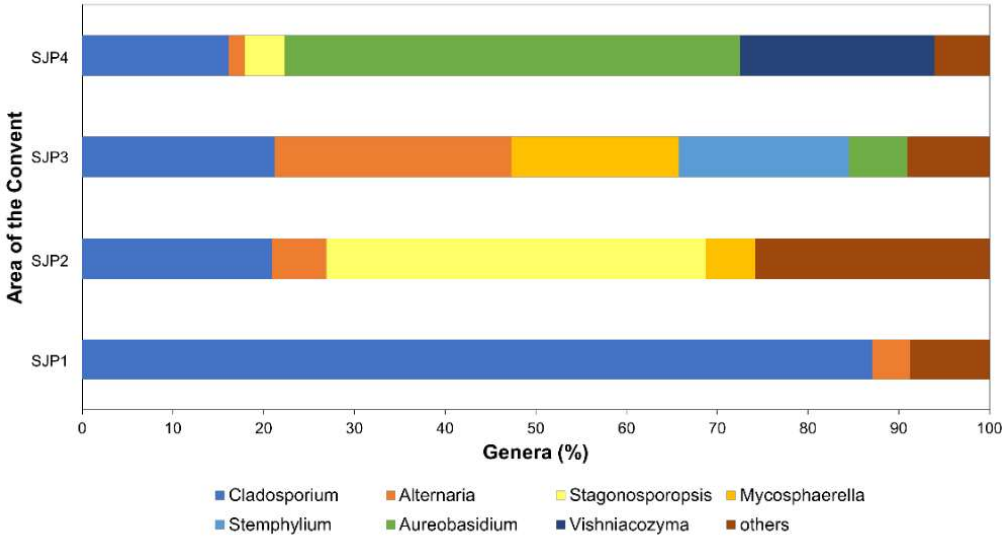

Figure 5. Major eukaryotic populations present on the areas SJP1-4, at genera level.

Fungi can excrete acids like oxalic, glyoxalic, citric, acetic, formic, or fumaric acids which can react with the carbonates of the stone. *Penicillium* sp. and *Cladosporium* sp. are the most common fungi isolated from cultural heritage materials, both responsible for soiling [24]. Grote in 1986 suggested that microorganisms of the genera *Alternaria*, *Cladosporium*, *Fusarium*, and *Penicillium* can oxidize iron and manganese on stone substrates [25], which enlarge the list of microorganisms that can induce deleterious effects on the surface of the stone. Regarding the areas SJP1 and SJP2, the presence of calcium oxalates can be a strong indicator of microbial activity on the stone applied in the Convent, as suggested in previous studies [15-17]. The oxalic acid excreted by microorganisms reacts with the calcitic matrix, leading to the formation of a thin calcium oxalate membrane (patina).

The eukaryotic population that is colonizing the area SJP5 (reddish biofilm) is mostly composed of Ascomycota (76.48 %) and Basidiomycota (7.56 %), while the most representative genera are *Sordaria*, *Cladosporium*, *Aureobasidium*, *Guehomyces*, *Mycosphaerella*, and *Vishniacozyma* (fig. 6a). On the other hand, the population that is colonizing the area SJP6 (blackish biofilm) are predominantly composed of Ascomycota (78.51 %) and Basidiomycota (3.62 %), while the most representative genera are *Cladosporium*, *Thelebolus*, *Aureobasidium*, *Alternaria*, *Vishniacozyma*, *Neodevriesia* and *Gibberella* (fig. 6b).

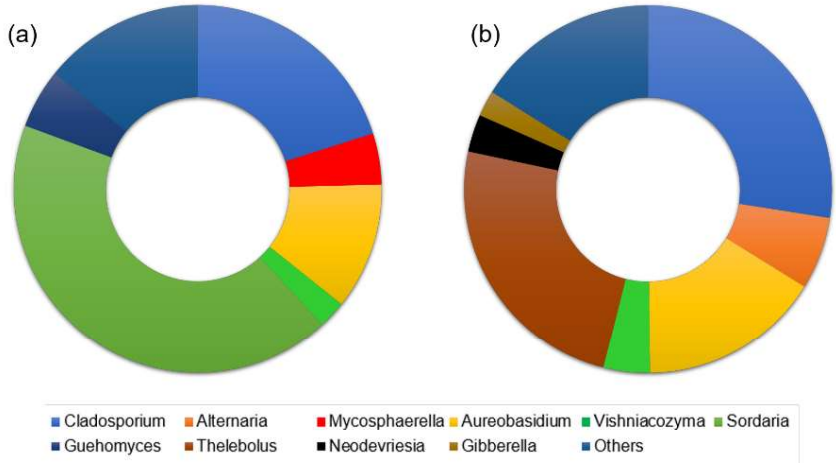

Figure 6. Major eukaryotic populations identified on the red (a) and black (b) biofilms, at genera level.

The reddish coloration of the biofilm formed in the area SJP5 is likely achieved by the proliferation of microorganisms and/or excretion of their metabolites. Here we can refer to microorganisms of the genera *Sordaria*, as some phenotypes exhibit ascopores with a red color [26], *Cladosporium*, as some colonies exhibit a red pigmentation due to the excretion of a large amount of perylenequinone [27], *Aureobasidium*, since there are colonies that can acquire a red pigmentation [28] or *Rubrobacter*, as it has already been associated with red pigmentation in stone [9].

On the other hand, strains of the genera *Cladosporium*, *Aureobasidium*, and *Alternaria* can form black-colored biofilms [29, 30]. Particularly, the species *Aureobasidium pollulans* that was identified in the area SJP6 belongs to the group of the so-called black yeasts [31, 32] that is strongly associated with deterioration processes such as biofouling and bioweathering. Therefore, the results obtained suggest that microorganisms of the genera *Aureobasidium*, *Cladosporium*, and *Alternaria* are the major responsible strains for the coloration of the biofilm thriving in this area.

4. CONCLUSIONS

In this study, a multi-analytical approach was carried out, privileging the application of non-destructive and micro invasive techniques. Among the pathologies existing in the marble applied in this historic Convent, three main categories composed the research work – patina formation, staining, and biofilm formation – which assessment of its possible origins, either geochemical and/or biogenic, were enabled. This research also aims to aware the appropriate entities for the real needs of a regular application of diagnostic and conservation-restoration campaigns in this type of structures. With such a rich cultural history, it is of utmost importance to preserve its aesthetic and structural integrity for the next generations. In this way, monitoring and preventive plans should be envisaged and complemented with mitigation strategies and conservation-intervention processes, using new consolidant solutions and natural mitigation products, which are emerging in the market (e.g. natural biocides). These solutions are human and environment-friendly, which should be strongly considered as an alternative to the conventional products usually applied.

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