Mapping and characterization of a green biofilm inside of Vilar de Frades Church (Portugal)

A. Miller & M.F. Macedo
Departamento de Conservação e Restauro, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Monte da Caparica, 2829-516 Caparica, Portugal

ABSTRACT: Vilar de Frades church is integrated in the Vilar de Frades Monastery, located in the North part of Portugal (Barcelos). The monastery, founded in 566, suffered several architectural modifications and restoration works, the most relevant was in the XVI century. The church, in granite, has one nave and six bays, holding ten chapels with vaults of crossed ribbings. Nowadays, the chapels present a severe biological colonization characterised by an intense green biofilm, which becoming apparent in other locations inside the church. In the course of a general survey concerning the conservation state of the church, an accurate campaign was planned in order to assess the main biodeterioration agents, map biological colonization and determine the environmental conditions. Laboratory analyses were accomplished with optical microscopy and spectrofluorometry. This study presents the results of this campaign. Details on conservation or preservation works that need to be implemented are also presented.

1 INTRODUCTION

The current condition of many historical monuments clearly reveals that they are not immune to weathering factors such as biological decay or biodeterioration. In addition to physical and chemical factors, microorganisms play a contributing role in deterioration of stone monuments. Among the microorganisms dwelling on stone, the photosynthetic microorganisms possess the major ecological importance as pioneering microorganisms in the colonization of stone due to their photoautotrophic nature (Ortega-Calvo et al. 1995, Gómez-Alarcón et al. 1995, Albertano et al. 2000, Tomaselli et al. 2000b, Bellinzoni et al. 2003, Lamenti et al. 2003, Herrera et al. 2004, Crispim et al. 2005). Since they require no more than light, water and mineral ions to grow, these microorganisms colonize stone surfaces of historic monuments, regardless of whether interior or exterior, and develop a visible biofilm of enriched organic biomass, which alters the appearance of the building and serves as a substrate for the growth of other biodeteriogens (Crispim et al. 2005). The existence of such biofilms indicates the continuous presence of water, and therefore a potential source of physical damage (Saiz-Jimenez 1999), and chemical deterioration due to the metabolic products they excrete (Zurita et al. 2005). The development of biofilms formed by phototrophic microorganisms is regulated by the environmental location and climatic conditions, together with the lithotype and its surface features. The mineral composition, structure-texture, porosity and permeability of stone may influence the distribution of such organisms in the monuments. Guillitte & Dreesen (1995) reported that the bioreceptivity of building materials was controlled mainly by surface roughness, initial porosity and the mineralogical nature of the substrate. The combination of environmental factors, such as high relative humidity, temperature and light availability usually enhance the growth of microbial communities.

The purpose of this study was to identify the main phototrophic biodeteriogens and determine the environmental conditions presented inside of Vilar de Frades church, located in the North part of Portugal (city of Barcelos).

Vilar de Frades church was classified as a National Monument in 1910. It is integrated in the Vilar de Frades Monastery founded in 566 by the bishop S. Martinho de Dume. After a major destruction of the building due to Moslem invasions, a complete reconstruction was ordered by D. Godinho Viegas. It was reconstructed in Romanesque style using granite as the main building material. During its history the monastery suffered other structural and architectural modifications as well as restoration works. The most relevant change was in the XVI century, modifying substantially the look of the old Romanesque monastery. Nowadays, the interior of the church has one nave and six bays, holding ten chapels. In its present state, the church is a good ex-

ample of the biodeterioration problems. It can be seen at first glance that the chapels have a severe biological colonization characterised by an intense green biofilm.

2 MATERIALS AND METHODS

An accurate survey was carried out in April 2005 inside Vilar de Frades church. During this survey determination of environmental conditions, collection of samples and also photographic record were made. Figure 1 presents the ground level plan of the church with the numbers attributed to each chapel in this work.

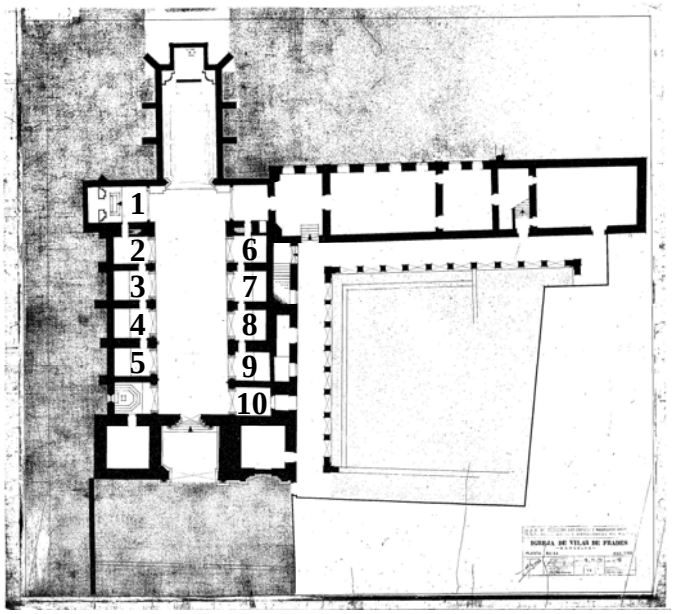

Figure 1. Ground level plan of Vilar de Frades church, presenting the chapels numbered from 1 to 10.

2.1 *Assessment of environmental conditions*

Environmental parameters were recorded in 22nd April 2005 on the interior of the Vilar de Frades church. Relative humidity of the air (RH%), temperature (TºC), visible light and ultra-violet radiation (UV) were measured using a data logger Elsec 764 Environmental Monitor. The measurements were carried out with a periodicity of 10 minutes during two hours. The values of the exterior environmental parameters reported on that day were supplied by Institute of Meteorology.

Moisture content of the substrate (%) was measured with a moisture meter Tramex Moisture Encounter Plus. The instrument measures the electrical impedance of the substrate by creating a low frequency alternating electric field between the electrodes. This field penetrates the material under test to a depth of approximately 30 mm.

2.2 *Mapping of green biofilms*

In order to map the green biological patina it was made a mapping of chapel 3 and 4 of the Vilar de Frades church, which represent the greater biological covering surface area. Mapping of biological colonization was performed through the photographic record with Photoshop CS image treatment software.

2.3 *Biological sampling*

With the permission from current authorities, biological samples were collected from columns surfaces in six chapels of the church (chapels 1 to 6). These were the chapels included in the conservation and restoration works. The biological material was collected on Rodac contact plates (∅ 5.5 cm) with BG-11 culture medium, at 1m above the ground. The plates were kept at 18ºC under a 12/12 light/dark cycle. The development of mixed cultures of phototrophs was followed using an inverted microscope, and features of individual species were observed with an optical microscope equipped with a photographic camera. After 30 days, algae and cyanobacteria colonies growing on the agar medium were isolated and identified. Determination of the microorganisms' genera and species was performed through direct observation by optical microscopy; taxonomic identification was carried out according to Bourrelly (1990) and Komárek & Anagnostidis (1999).

Biomass, calculated as the amount of chlorophyll *a* per area, was determined for the two columns on chapel 3. The samples were collected by scraping the biofilms covering the granite surface. This included scraping of fifteen areas (each of 9 cm^2) from each column until 1.50 m above the ground. The samples were taken with the help of a grid (3 x 3 cm) and using sterile materials. The amount of chlorophyll *a* of the collected samples from chapel 3 was determined spectrofluorometrically. Chlorophyll *a* from the samples was extracted with 90% aqueous acetone (v/v). Chlorophyll *a* and phaeopigments concentration was determined by the method of Yentsch & Menzel (1963) as modified by Holm-Hansen et al. (1965). The fluorescence measurements were performed in a SPEX Fluorolog F111 that was first calibrated with pure Chl*a* (SIGMA).

3 RESULTS

The climatic conditions recorded outside of Vilar de Frades church in 22nd April 2005 are shown in Table 1. Air temperature did not vary during the day, presenting a mean value of 15ºC. Relative humidity (RH) was near 100% from 00 to 06 UTC (Universal

Time Coordinated). The minimum value of relative humidity was obtained for 12 UTC (78%). Between 06 UTC and 12 UTC it rained about 6 mm.

Table 1. Air temperature, relative humidity and rainfall for 00, 06, 12 and 18 hours corresponding to universal time coordinated (UTC) in 22nd April 2005.

Hour	Temperature (ºC)	Relative humidity (%)	Rainfall in the last six hours (mm)
00 UTC	14	96	0.0
06 UTC	14	97	0.0
12 UTC	17	78	6.0
18 UTC	15	84	0.0

The environmental conditions recorded inside the church in 22nd April 2005 are presented in Figures 2,3. Relative humidity ranged from 81.5% to a maximum of 88%. Between 15:10h and 17:10h the RH remained relatively constant at about 87.5%. Note that this value of air RH inside the church is higher than the one recorded outside for that afternoon. Moreover, a relative humidity above 80% is more than sufficient to promote microorganisms growth.

Air temperature was almost constant with a mean value of 13.4ºC. This temperature allows microorganisms to growth but is not an optimum value. With the recorded values of air temperature and relative humidity the average dew point is 11.2 ºC. This implicates that if temperature decreases about 2ºC, the condensation of water in the interior walls of the church would occur. This situation is very liked to happen during the night.

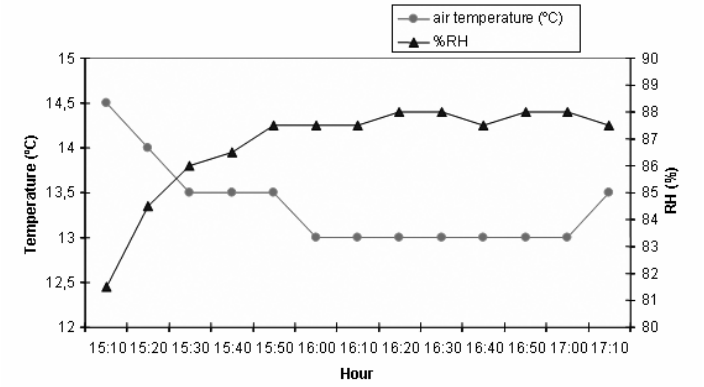

Figure 2. Temperature and relative humidity recorded inside the Vilar de Frades church in 22nd April 2005.

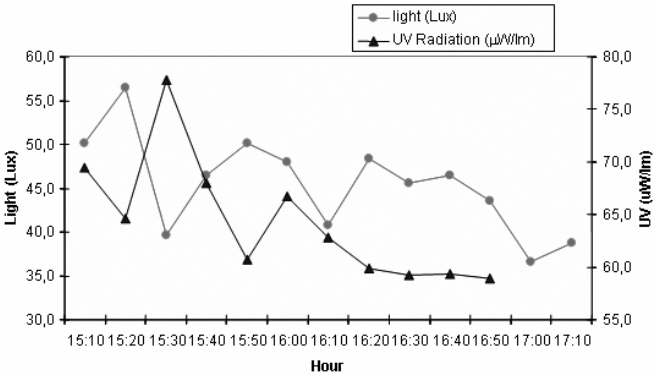

Figure 3. Light and UV radiation recorded inside the Vilar de Frades church in 22nd April 2005.

The interior of the church is illuminated by natural and artificial light source. During the day, the light irradiance is mainly from natural origin provided by several windows located on the first level of the building. Light irradiance presented values below 50 Lux, with the exception of one obtained at 3:20h that reached 56.4 Lux (Fig. 3). The average value for light irradiance recorded was 45.5 Lux. The UV radiation ranged from 54.4 µW/lm to 77.8 µW/lm presenting an average value of 64.3 µW/lm.

Moisture content of the substrate (%) was measured in the columns of the six chapels under study until 3 m high. The results showed that water content of the substrate was always around 100% at all measured areas. This is in agreement with the temperature and relative humidity measured in the air.

The granite surfaces of the chapels were abundantly covered with a green biological colonization. Biodeterioration phenomena were evident through these extensive and thick green biofilms. The biofilms from each chapel present different extension and distribution, and were firmly bounded to the stone surfaces (Fig. 4). Mapping of green biological patina, in chapels 3 and 4, is presented in Figures 5a, b, 6a, b.

Figure 4. Green biofilm covering the granite surface of chapel 3 left column.

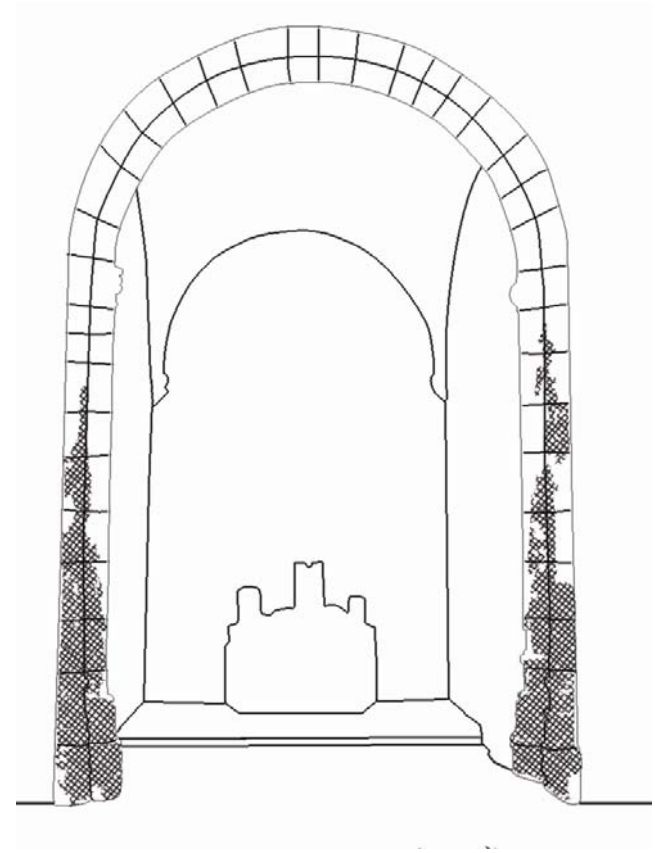

Figure 5. a) Mapping of biological colonization from chapel 3.

b) Chapel 3.

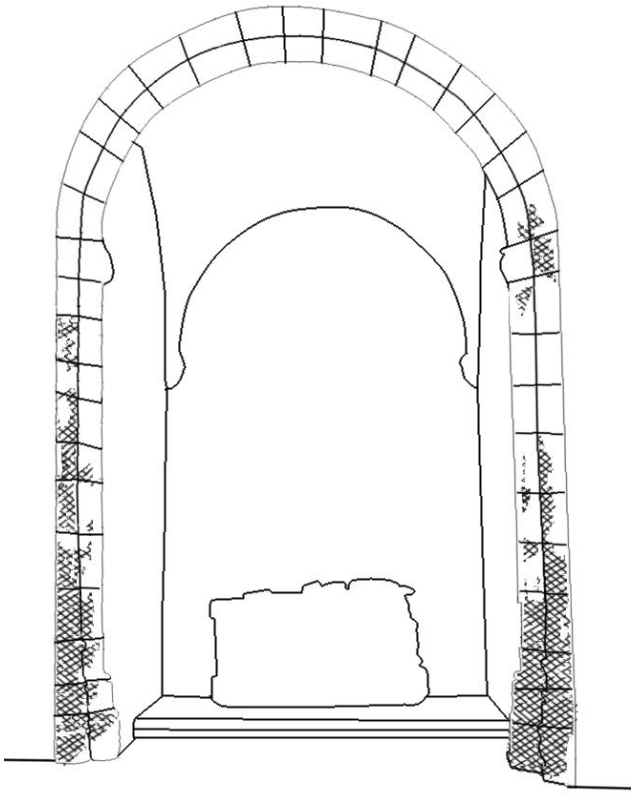

Figure 6. a) Mapping of biological colonization from chapel 4.

b) Chapel 4.

Concentration of chlorophyll *a* was determined for the two columns of chapel 3 (Table 2). The left column of the chapel presented superior values of concentration of chlorophyll *a* in relation to the right column.

Table 2. Chlorophyll *a* concentration (g Chl*a* m^{-2}) determined from the right and left column of chapel 3.

Chapel 3	Left	Right
Mean (g Chl*a* m^{-2})	4.22	2.61
Standard Deviation (g Chl*a* m^{-2})	0.57	0.62
Minimum (g Chl*a* m^{-2})	3.58	2.25
Maximum (g Chl*a* m^{-2})	4.65	3.32

Regarding species identification, one genus of cyanobacteria and seven genera of microalgae were found. Green biofilms consisted mainly of cyanobacteria *Gloeocapsa* sp. that was present in all the collected areas. The microalgae found on this green biofilms were essential Chlorophyta coccoid morphotypes. The main microalgae identified were: *Chlamydocapsa* sp., *Chlorella ellipsoidea*, *Chlorococcum* sp., *Desmococcus* sp., *Tetracystis* sp., *Trebouxia* sp. and *Trentepohlia* sp..

4 DISCUSSION

The presence of green biofilms inside Vilar de Frades church is not a new problem, it remains for many years. The authorities in charge of that monument have tried different approaches (cleaning, use of biocides, etc.) but the problem still persists. These green biofilms not only affect drastically the aesthetic value of the church as they also promote the deterioration of stone due to their photosynthetic nature and retention of water.

The main biodeterioration agents identified were the cyanobacterium *Gloeocapsa* sp. and several microalgae. *Gloeocapsa* sp. was the genus that most occurred in all samples. Cyanobacteria are generally adapted to resist adverse conditions because of their thick outer envelopes and the presence of protective pigments (Crispim et al. 2005). Gaylarde & Gaylarde (2005) found that, in laboratory experiments, the wall-inhabiting organisms tolerant to the highest salt levels were the cyanobacteria *Gloeocapsa* sp.. This may be an explanation for the large amount found on the granite surfaces of Vilar de Frades church.

The microalgae found on this green biofilms were essential Chlorophyta. *Chlamydocapsa* can be found in very inhospitable places like the sub-Antarctic lakes (Hansson & Tranvik 1997). *Chlorella ellipsoidea* was also identified on a limestone monument in the central part of Portugal, city of Coimbra (Santos 2003). *Chlorococcum* has been reported by several authors on granite, marble and limestone monuments (e.g. Leite Magalhães & Braga 2000, Tomaselli et al. 2000b, Santos 2003). *Desmococcus* is able to habitat tree bark and desert crusts (Hu et al. 2003). *Tetracystis* is usually isolated from soils; therefore is expected to be also found in stones surfaces. The genus *Trebouxia* was identified on granite from Cathedral of Lund by Ortega-Calvo et al. (1991), and *Trentepohlia* has also found on another granite monument in Ourense, NW Spain (Noguerol-Seoane & Rifon-Lastra 1997). In summary, the microorganisms present on this biofilm are adaptable to a wide range of extreme environmental conditions.

The results of the present work show that environmental conditions inside the church promote the growth of microorganisms. These results also indicate that photoautotrophic microorganisms constitute the main portion of the biomass on these granite surfaces. Photosynthetic microorganisms are common inhabitants of monuments and their growth represents a significant input of organic matter to the stone, as estimated through chlorophyll *a* quantification. High relative humidity, surface moisture and the present of light are the main factors affecting the growth of photoautotrophic microorganisms inside the church. The light irradiance reported in this work was not very intense; however, photoautotrophic microorganisms were growing on the stone. Some cyanobacteria and green algae have lower light requirements in order to compete with other phototrophic organisms. These phototrophic microorganisms are able to withstand the low photon flux densities available in the environment with dim light like caves, necropolis and catacombs. Mapping results (Figs 5a, 6a) showed that near the floor there was a higher development of the green biofilms. This can be explained by the retention of moisture near the floor. In fact, very humid environment contributes to the thriving of phototrophic organisms (Ariño et al. 2002). The establishment of phototrophic microorganisms on stone is also regulated by their intrinsic properties, which are the capacity of adhesion, oligotrophy, metabolic flexibility and tolerance to adverse conditions. Oligotrophy allows for initial substrate colonization without immediate utilization of the substrate, although this may follow once the organism has become established. An important factor in the adhesion and proliferation of microorganisms on stone surfaces is the hydrophobicity of their cell surface. Cell hydrophobicity is not the sole mechanism determining the adhesion of microorganisms. Excreted polymeric substances like, sheaths, capsules and slimes, assure the adhesion. The extracellular substances, in addition to joining organism and substrate, participate in the formation of microbial aggregates, which contribute to the development of firm biofilms (Tomaselli et al. 2000a).

This study shows that stone-degrading cyanobacteria and microalgae are present on the interior walls of Vilar de Frades church. The visible deterioration of the interior of Vilar de Frades church is mainly due to biological phenomena and it is strictly related to the environmental conditions. Therefore, in order to improve the conservation state of interior walls of the church the environmental conditions have to be altered. First, it would be recommended to avoid sunlight inside the church. In this way, there would not be enough light for the photosynthetic organisms growth, since artificial light are usually turned off. Secondly, the relative humidity and temperature of the church should be controlled to avoid the growing of heterotrophic microorganisms (fungi and bacteria). Values of relative humidity around 55 – 65% and temperature between 18ºC and 20ºC can be suggested. However, special care is necessary in order to avoid fast environmental changes that could damage the materials inside the church. The biofilms should be cleaned with a vacuum cleaner and a soft brush. Afterwards the moisture content of the walls should decrease. Meanwhile, different hydrophobic protective mixed with different biocide should be tested *in situ*, in order to select the mixture to be applied. After the application of the selected treatment monitoring plan should be implemented. This plan should include an hourly determination of air relative humidity, temperature and light. And a survey with photographic records, samples collection and surface moisture content determination, on a monthly basis.

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