 DOI 10.1099/mic.0.032508-0

Review

Biodiversity of cyanobacteria and green algae on monuments in the Mediterranean Basin: an overview

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The presence and deteriorating action of micro-organisms on monuments and stone works of art have received considerable attention in the last few years. Knowledge of the microbial populations living on stone materials is the starting point for successful conservation treatment and control. This paper reviews the literature on cyanobacteria and chlorophyta that cause deterioration of stone cultural heritage (outdoor monuments and stone works of art) in European countries of the Mediterranean Basin. Some 45 case studies from 32 scientific papers published between 1976 and 2009 were analysed. Six lithotypes were considered: marble, limestone, travertine, dolomite, sandstone and granite. A wide range of stone monuments in the Mediterranean Basin support considerable colonization of cyanobacteria and chlorophyta, showing notable biodiversity. About 172 taxa have been described by different authors, including 37 genera of cyanobacteria and 48 genera of chlorophyta. The most widespread and commonly reported taxa on the stone cultural heritage in the Mediterranean Basin are, among cyanobacteria, *Gloeocapsa*, *Phormidium* and *Chroococcus* and, among chlorophyta, *Chlorella, Stichococcus* and *Chlorococcum*. The results suggest that cyanobacteria and chlorophyta colonize a wide variety of substrata and that this is related primarily to the physical characteristics of the stone surface, microclimate and environmental conditions and secondarily to the lithotype.

Introduction

Stone monuments, statues and historic buildings are exposed to the effects of physical, chemical and biological deteriorating factors. This review will focus on the damage caused by micro-organisms, in a process referred to as biodeterioration. Stone works of art can be colonized by different groups of micro-organisms, including bacteria, cyanobacteria, algae and fungi. Microbial populations present in a stone substratum are usually the result of successive colonization by different micro-organisms that has taken place over several years. It is a process that relies upon the capacity of a substratum to provide a protective niche on which micro-organisms can develop. According to several authors, cyanobacteria and chlorophyta (green algae) are considered the pioneering inhabitants in the colonization of stone (Ortega-Calvo *et al.*, 1991; Tiano *et al.*, 1995; Cecchi *et al.*, 2000; Lamenti *et al.*, 2000; Tomaselli *et al.*, 2000b; Crispim & Gaylarde, 2005). Due to their photoautotrophic nature, these micro-organisms develop easily on stone surfaces, giving rise to coloured patinas and incrustations (Fig. 1) (Tomaselli *et al.*, 2000b). Identifying the micro-organisms involved in biodeterioration is one of the most important steps in the study of the microbial ecology of monumental stones. It can help us to understand the microbial biodiversity, the phases of colonization and the relationship among populations on the surfaces and between micro-organisms and substrata.

Here we review the occurrence of cyanobacteria and green algae identified on stone monuments, statues and historic buildings in European countries of the Mediterranean Basin. Marble, limestone, travertine, dolomite, sandstone and granite were the six lithotypes considered in this work, corresponding to the lithotypes mainly used in the construction of buildings and monuments in this region.

The photosynthetic micro-organisms inventoried in this review were almost entirely found on outdoor monuments located in the Mediterranean Basin area. The prevailing Mediterranean climate in this region is characterized by mild and rainy winters, warm and dry summers and,

 032508 *Printed in Great Britain*

Fig. 1. Wall of the Palácio Nacional da Pena, Sintra (Portugal), showing extended colonization by *Trentepohlia* sp.

usually, extended periods of sunshine throughout most of the year; temperatures during winter rarely reach freezing (except in areas with a high elevation), and snow is unusual.

This review catalogues the cyanobacteria and green algae occurring on stone monuments from the Mediterranean area, together with the type of mineral substratum, occurrence locations and references. The data gathered together here will be useful for the study of biological colonization in cultural heritage buildings, and should provide a basis for laboratory experiments on stone colonization, allowing for the selection of single species or mixed communities of micro-organisms and/or stone substrata for ecological studies.

Cyanobacteria on monuments

Table 1 lists the monuments, statues and historic buildings reported in the literature covered in this review. Most of these works of art were built in limestone (32 %) and marble (30 %). Travertine (7 %) and dolomite (2 %) were less represented lithotypes (Fig. 2).

Table 2 lists the cyanobacteria detected on monuments and works of art in the Mediterranean Basin, together with the substratum. The data on cyanobacteria occurring on stone monuments and works of art is rather wide. A total of 96 taxa of cyanobacteria were found. The genus *Gloeocapsa* (Fig. 3) was the most widespread, occurring on 20 of the 45 monuments reported, on all substrata and represented by eight species. Cyanobacterial species from the genera *Phormidium* and *Chroococcus* were able to colonize five of the six stone substrata considered and they were represented by high species diversity: eight species of *Phormidium* and four species of *Chroococcus* were reported. *Chroococcus* species were detected colonizing 13 different monuments while *Phormidium* was found on 15 monuments. *Pleurocapsa* occurred on 13 monuments but only one species was identified, while the genus *Scytonema* occurred with five species and colonizing eight different monuments. Therefore, we can consider that *Gloeocapsa*, *Phormidium* and *Chroococcus* are the most common cyanobacterial genera on the monuments of the Mediterranean Basin. This is in accordance with Ortega-Calvo *et al.* (1995), who stated that the most common species found on monuments located in Europe, America and Asia belong to the genera *Gloeocapsa*, *Phormidium*, *Chroococcus* and *Microcoleus*. These genera are ubiquitous and therefore their presence is not strictly related to a specific lithic substratum or climate. Tomaselli *et al.* (2000b) also found that the data reported in the literature did not establish a clear relationship between organisms and the nature of the substratum. Nevertheless, these authors contend that *Phormidium tenue*, *Phormidium autumnale* and *Microcoleus vaginatus* prefer siliceous substrata.

Fig. 4 shows the number of cyanobacterial taxa present on each lithotype. According to the literature reviewed in this study, marble and limestone were colonized by about the same number of taxa (56 and 55, respectively). Travertine was a lithotype present only in 7 % of the monuments reported but it was colonized by a considerable number of taxa (23). Granite was colonized by a very low number of different taxa (only 10) when compared with limestone or marble. According to Ortega-Calvo *et al.* (1995), granite, with a very low porosity and pH, is an unfavourable substratum for cyanobacteria. In fact, the colonization of stones is closely correlated with porosity, roughness, hygroscopicity and capillary water absorption, which strongly influence water availability for micro-organisms (Urzì & Realini, 1998; Prieto & Silva, 2005; Miller *et al.*, 2006).

A high number of taxa found on a substratum does not necessarily imply high bioreceptivity of that substratum, since many other environmental parameters play an important role in a successful colonization (solar radiation, temperature, water regime, climate, etc.). In this study we attempted to select monuments subjected to similar climatic conditions (the Mediterranean climate); nevertheless, specific microclimatic parameters (orientation, exposure to shadow, permanent capillary humidity, etc.) are generally missing in the available literature, and the extent

Table 1. Investigated monuments, statues and historic buildings in European countries from the Mediterranean Basin, and their bibliographic references

No.	Monument	Reference
1	Ajuda National Palace, Lisbon (Portugal)	Miller *et al.* (2009)
2	Bibatauín Fountain, Granada (Spain)	Zurita *et al.* (2005)
3	Boboli Garden statues, Florence (Italy)	Lamenti *et al.* (2000)
4	Brunelleschi Rotunda, Florence (Italy)	Tomaselli *et al.* (2000b)
5	Ca' d'Oro façade, Venice (Italy)	Salvadori *et al.* (1994)
6	Caestia Pyramid, Rome (Italy)	Caneva *et al.* (1992)
7	Carrascosa del Campo church, Cuenca (Spain)	Gómez-Alarcón *et al.* (1995)
8	Cathedral of Granada, Granada (Spain)	Miller *et al.* (2009)
9	Cathedral of Seville, Seville (Spain)	Saiz-Jimenez *et al.* (1991)
9a	Cathedral of Seville, Seville (Spain)	Miller *et al.* (2009)
10	Cathedral, SI (Italy)	Tomaselli *et al.* (2000a)
11	Church of St Cruz, Coimbra (Portugal)	Santos (2003)
12	Crypt, Church M. Favana, Lecce (Italy)	Tomaselli *et al.* (2000b)
13	Fontana dei Quattro Fiumi, Rome (Italy)	Ricci & Pietrini (1994)
14	Forte Belvedere, Florence (Italy)	Tomaselli *et al.* (2000b)
15	La Citadelle, Blaye (France)	Crispim *et al.* (2003)
16	La Mola quarry, Novelda, Alicante (Spain)	Ascaso *et al.* (2004)
17	Largo da Paço Building, Braga (Portugal)	Leite Magalhães & Sequeira Braga (2000)
18	Leaning Tower, Pisa (Italy)	Tomaselli *et al.* (2000b)
19	Fountains of the Alhambra, Granada (Spain)	Bolívar & Sánchez-Castillo (1997)
19a	Lions Fountain at the Alhambra Palace, Granada (Spain)	Sarro *et al.* (2006)
20	Lungotevere walls, Rome (Italy)	Bellinzoni *et al.* (2003)
21	Lutheran church, Florence (Italy)	Tomaselli *et al.* (2000b)
22	Magistral church, Alcala de Henares (Spain)	Flores *et al.* (1997)
23	Michelangelo cloister, National Roman Museum, Rome (Italy)	Pietrini *et al.* (1985)
24	Ordem de São Francisco Church, Oporto (Portugal)	Pereira de Oliveira (2008)
25	Orologio Tower, Martano (Italy)	Miller *et al.* (2009)
26	Palace of Sts George and Michael, Corfu (Greece)	Pantazidou & Theoulakis (1997)
27	Paleolithic sculptures in Angles-sur-l'Anglin (France)	Dupuy *et al.* (1976)
28	Parthenon and Propylaea acropolis, Athens (Greece)	Anagnostidis *et al.* (1991)
29	Parthenon, Athens (Greece)	Anagnostidis *et al.* (1983)
30	Roman monuments in Appia road, Rome (Italy)	Bartolini *et al.* (2004)
31	Roman Statue, Volterra (Italy)	Tomaselli *et al.* (2000b)
32	Romanic portal of the Sant Quirze de Pedret church, Berga (Spain)	Alvarez *et al.* (1994)
33	Salamanca Cathedral, Salamanca (Spain)	Ortega-Calvo *et al.* (1993a)
34	San Francisco church, Betanzos, La Coruña (Spain)	Noguerol-Seoane & Rifón-Lastra (1996)
35	San Miniato Basilica, Florence (Italy)	Tomaselli *et al.* (2000b)
36	Santa Clara-a-Velha Monastery, Coimbra (Portugal)	Miller *et al.* (2008, 2009)
37	Santiago church, Betanzos, La Coruña (Spain)	Noguerol-Seoane & Rifón-Lastra (1996)
38	Sculptures in Ostia Antica (Italy)	Giaccone *et al.* (1976)
39	St Maria church, Alcala de Henares (Spain)	Flores *et al.* (1997)
40	Tacca's Fountains, Florence (Italy)	Tomaselli *et al.* (2000a)
41	Temples of Athena, Neptune and Basilica, archaeological area of Paestum, Salerno (Italy)	Altieri *et al.* (2000)
42	The Pyramid, Florence (Italy)	Tomaselli *et al.* (2000b)
43	Toledo Cathedral, Toledo (Spain)	Ortega-Calvo *et al.* (1993a)
44	Trajan's Forum, Rome (Italy)	Caneva *et al.* (1992)
45	Vilar de Frades church, Barcelos (Portugal)	Miller & Macedo (2006)

to which microclimate determines colonization is unknown. The microclimate determines the degree of colonization, the type of community and its specific composition. Monuments can create microclimatic differences between places that are very close. Ortega-Calvo *et al.* (1993a) observed that samples taken near ground level were characterized by the absence of cyanobacteria, which were however present in samples taken from places more exposed to sunlight. Detailed observations in different monuments subjected to lower humidity suggested that the

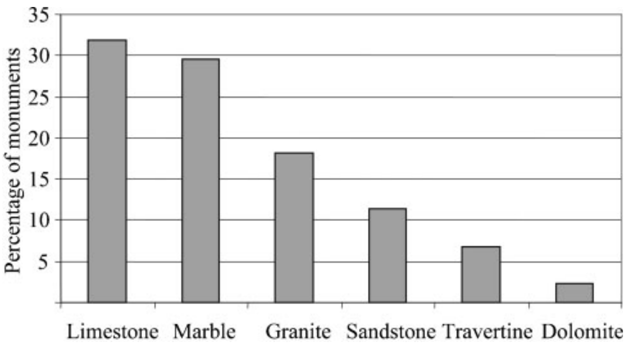

Fig. 2. Percentage of lithotypes present in the monuments, statues and historic buildings reported in this study.

formation of a photosynthetic biofilm developing on the stone surface is related substantially to the length of the period of wetness and the spatial orientation of the substratum (Fig. 5). In addition, the physico-chemical characteristics of the materials favour the establishment of photosynthetic communities at depths that also depend on external environmental factors, especially light, which influences the total biomass of the community (Saiz-Jimenez, 1995). Bellinzoni *et al.* (2003) assessed the correlation between biological growth and environmental factors on travertine. The biological colonization showed a characteristic trend with the microclimate (orientation and presence or absence of trees).

Under the most extreme terrestrial climates, such as hot and cold deserts, endolithic cyanobacterial growth can occur, and the cyanobacteria commonly inhabit the outer millimetres to inner centimetres of rocks exposed to such environments (Walker *et al.*, 2005). The endolithic microhabitat gives protection from intense solar radiation and desiccation, and it provides mineral nutrients, rock moisture and growth surfaces (Friedmann, 1982; Bell, 1993; Walker *et al.*, 2005). However, very few studies report the presence of endolithic cyanobacteria in the monuments of the Mediterranean Basin. Pentecost (1992) observed that endolithic growth was often obscured by superficial algal growths, and consequently overlooked. In our study, we noted endolithic growth of *Phormidium*, *Plectonema*, *Chroococcopsis*, *Synechocystis* and *Synechococcus* on Ordem de São Francisco Church, Portugal. These cyanobacteria were found growing under a black patina in granite (Pereira de Oliveira, 2008; Pereira de Oliveira *et al.*, 2008). *Phormidium* was also found growing under a black sulphated crust developed on limestone in the Cathedral of Seville, Spain (Saiz-Jimenez *et al.*, 1991). Endolithic growth of *Hyella fontana* was also observed in marble statues in Rome, Italy (Giaccone *et al.*, 1976).

Cyanobacteria can live in rock fissures and cracks and in cavities occurring in porous transparent rocks such as sandstones and marble, but not in dense dark volcanic rocks. Chasmo- and endolithic cyanobacteria and chlorophyta were present in all samples examined from the marbles of the Parthenon and Propylaea Acropolis in Athens, Greece (Anagnostidis *et al.*, 1991). Cryptoendolithic cyanobacteria such as *Chroococcidiopsis* live beneath rock surfaces together with cryptoendolithic lichens, fungi and bacteria. *Chroococcidiopsis* can survive extreme cold, heat and arid conditions and it may be the single autotrophic organism most tolerant to environmental extremes (Graham & Wilcox, 2000).

The cyanobacterium *Borzia periklei,* a rare aerophytic species – first described and found on the marbles of the Parthenon, Greece (Anagnostidis & Komarek, 1988) – is of particular interest. The second record was in the stuccos of the Roman town of Baelo Claudia, South Spain (Hernandez-Marine *et al.*, 1997) (Fig. 6). The growth of this cyanobacterium on mortars suggests a good adaptation to carbonate environments. Mortars, not considered in this review, can provide niches which are suitable for relatively rare species or species peculiar to other, very specific, ecological niches. The high porosity and the mobilization of salts, together with the humidity retained in the inner layers of the wall, facilitate the colonization and growth of such organisms.

Most of the cyanobacteria mentioned in this review (e.g. *Gloeocapsa*, *Gloeothece*, *Phormidium*, *Chroococcus*, *Plectonema*, *Scytonema*, *Lyngbya* and *Microcoleus*) have a gelatinous sheath that acts as a reservoir of water, where it is bound through strong molecular forces, allowing these cyanobacteria to colonize stone even when dry conditions prevail (Ortega-Calvo *et al.*, 1991). The sheath can also play an important role in adhesion to the substratum (Fig. 7). Sometimes sheaths may be pigmented. This is particularly evident in some cyanobacterial genera such as *Gloeocapsa* or *Scytonema* that have thick sheaths with intense colours, being the expression of different ecological stages and environmental adaptations. Cyanobacteria can take on a yellow-brown colour under low-nitrogen conditions due to a reduction in chlorophyll and phycocyanin and an increase in carotenoids. In other cases, it has been shown that pigmentation changes in response to environmental factors including light intensity, light quality, nutrient availability, temperature and the age of cells. Bartolini *et al.* (2004), in a study on monuments located on the Appia Antica road (Rome), observed that grey-black patinas were widespread on marble and travertine stoneworks exposed to sun irradiation, and that green patinas were more frequent on tufaceous materials and mortar in shaded areas. These coloured patinas cause aesthetic damage, giving an unsightly appearance of neglect to buildings, statues and monuments.

Green algae on monuments

A considerable number of green algae (chlorophyta) have adapted to life on land. The chlorophyta constitute the most common group of algae colonizing stone cultural heritage (Ortega-Calvo *et al.*, 1993b). In Table 3, 76 taxa of

Table 2. Cyanobacteria reported on stone monuments, statues and historic buildings in European countries from the Mediterranean Basin, on different substrata

Cyanobacterium	Substratum	Monument no. (Table 1)
Aphanocapsa sp.	Marble, limestone	26, 27, 28
Aphanocapsa grevillei (Berkeley) Rabenhorst	Travertine	13
Aphanocapsa roeseana De Bary	Marble	6, 44
Aphanothece sp.	Sandstone, limestone	27, 32
Aphanothece saxicola Nägeli	Marble, limestone	19
Borzia periklei Anagn.	Marble	28
Borzia trilocularis Cohn ex Gomont	Marble	29
Calothrix sp.	Sandstone, limestone	26, 32
Calothrix braunii Bornet & Flahault	Limestone	38
Calothrix marchica Lemmermann	Marble	6, 44
Calothrix parietina (Nägeli) Thuret	Marble	38
Chamaesiphon sp.	Limestone	2
Chamaesiphon incrustans	Marble, limestone	19
Chlorogloea sp.	Limestone, travertine	2, 41
Chlorogloea microcystoides Geitler	Travertine, marble, limestone	19, 41
Chlorogloea purpurea Geitler	Marble, limestone	19
Chroococcidiopsis sp.	Limestone, marble, granite, sandstone	1, 2, 3, 8, 9a, 14, 18, 21, 40, 42
Chroococcopsis sp.	Granite	24
Chroococcus sp.	Limestone, travertine, sandstone, dolomite	2, 16, 32, 38, 41
Chroococcus lithophilus Ercegović	Travertine, marble	20, 30
Chroococcus minor (Kützing) Nägeli	Marble, limestone	6, 26, 28, 29, 44
Chroococcus minutus (Kützing) Nägeli	Travertine, limestone	13, 26
Chroococcus tenax (Kirchner) Hieronymus	Limestone	38
Cyanosarcina sp.	Marble, limestone	26, 28
Cyanosarcina parthenonensis Anagnostidis	Marble	28
Cylindrospermopsis sp.	Limestone	1
Dermocarpa kerneri (Hansgirg) Bourr.	Marble	29
Geitlerinema sp.	Marble	3, 10, 31, 35, 40
Gloeocapsa sp.	Limestone, marble, travertine, sandstone, dolomite, granite	2, 3, 5, 11, 15, 16, 27, 32, 35, 41, 42, 45
Gloeocapsa alpina (Nägeli) Brand	Limestone	45
Gloeocapsa biformis Ercegovic	Travertine, marble	6, 20, 26, 27, 28, 30, 44
Gloeocapsa calcarea Tilden	Marble	6, 44
Gloeocapsa compacta Kützing	Marble, travertine	30
Gloeocapsa decorticans (A. Braun) Richter	Marble	29
Gloeocapsa kuetzingiana Nägeli	Limestone	11, 26
Gloeocapsa novacekii Komárek & Anagnostidis	Limestone	11
Gloeocapsa sanguinea (Agardh) Kützing	Limestone	26
Gloeothece sp.	Sandstone	32
Gomphosphaeria sp.	Sandstone	32
Hydrocoleus homeotrichus Kützing ex Gomont	Marble	29
Hyella fontana Huber & Jadini	Marble, limestone	19, 38
Hyella fontana var. maxima Geitler	Limestone	38
Leptolyngbya sp.	Marble, limestone	3, 9a, 18, 26, 28, 35, 36
Leptolyngbya boryanum (Gomont) Anagnostidis & Komárek	Marble, limestone	26, 28
Lyngbya sp.	Travertine, limestone	27, 41
Lyngbya spiralis Geitler	Travertine	20
Merismopedia sp.	Sandstone	32
Microcoleus sp.	Limestone	1, 2
Microcoleus chthonoplastes Gomont	Limestone	26
Microcoleus lacustris (Rabenh.) Farlow ex Gomont	Marble	29
Microcoleus vaginatus (Vaucher) Gomont	Marble, sandstone	7, 29, 33, 43
Microcystis sp.	Marble, sandstone	6, 32, 44
Myxosarcina sp.	Limestone, marble, travertine	5, 10, 11, 19, 26, 41
Myxosarcina chroococcoides Geitler	Limestone, marble	19, 38
Myxosarcina concinna Printz	Marble	6, 29, 44

Table 2. cont.

Cyanobacterium	Substratum	Monument no. (Table 1)
Myxosarcina spectabilis Geitler	Travertine, marble	6, 20, 44
Nodularia harveyana (Thwaites) Thuret	Marble	29
Nostoc sp.	Limestone, marble, travertine	1, 3, 10, 11, 19, 26, 27, 28, 38, 41
Nostoc punctiforme Hariot	Travertine	30
Oscillatoria sp.	Limestone	27
Oscillatoria lacustris (Klebahn) Geitler	Travertine	13
Phormidium sp.	Limestone, granite	2, 9, 11, 24
Phormidium ambiguum Gomont	Limestone, marble	19
Phormidium autumnale Gomont	Marble, sandstone, travertine	7, 13, 33, 43
Phormidium favosum (Bory) Gomont	Limestone, marble	19
Phormidium foveolarum Gomont	Marble, travertine, limestone	6, 13, 26, 28, 29, 44
Phormidium fragile Gomont	Sandstone, granite	33, 34
Phormidium retzii Gomont	Travertine, limestone, marble	13, 19
Phormidium subfuscum Kützing	Marble, sandstone	33, 43
Phormidium tenue (Meneghini) Gomont	Marble, granite, sandstone, limestone	7, 26, 43
Plectonema sp.	Granite, marble, limestone, sandstone	3, 12, 14, 21, 24, 42, 43
Plectonema boryanum Gomont	Granite	34, 37, 43
Plectonema boryanum f. *hollerbachianum* Elenk.	Marble	29
Plectonema radiosum (Schiedermann) Gomont	Marble	6, 44
Pleurocapsa sp.	Limestone, marble, granite	1, 2, 3, 8, 9a, 10, 14, 18, 25, 28, 35, 36
Pleurocapsa minor Hansgirg	Limestone, marble	19
Schizothrix sp.	Limestone, marble	27, 19
Schizothrix bosniaca (Hansgirg) Geitler emend. Claus	Marble	29
Schizothrix coriacea Gomont	Limestone	26
Schizothrix tenuis Woronichin	Limestone, marble	19
Scytonema sp.	Limestone, marble	3, 11, 12, 21, 35
Scytonema crustaceum (Agardh) Bornet & Flahault	Limestone	26
Scytonema hofmanni Agardh	Limestone	26
Scytonema javanicum Bornet	Limestone	11
Scytonema julianum Meneghini	Travertine	20
Scytonema myochrous (Dillwyn) Agardh ex Bornet & Flahault	Limestone, marble and travertine	26, 30
Stigonema sp.	Limestone	27
Symploca sp.	Limestone	2
Symploca elegans Kützing ex Gomont	Limestone, marble	19
Synechococcus sp.	Marble, granite	3, 6, 18, 24, 44
Synechococcus aeruginosus Nägeli	Travertine	20
Synechococcus elongatus (Nägeli) Nägeli	Marble	28, 29
Synechocystis sp.	Sandstone, limestone, granite	7, 15, 20, 24, 30, 37
Synechocystis pevalekii Ercegovic	Travertine	20
Tolypothrix sp.	Limestone, sandstone	11, 32
Tolypothrix byssoidea (Agardh) Kirchner	Marble, travertine	6, 20, 30

chlorophyta found on monuments and works of art in the Mediterranean Basin are listed, together with the substratum on which they occurred. Most of these genera are soil algae. This is predictable, since the main source of biological colonization of stone is the surrounding soil, containing large numbers of many different types of bacteria, algae and fungi, which can contaminate the stone shortly after quarrying. Windblown detritus or rising groundwater infiltration may also be a source of stone inoculation (Koestler, 2000).

From Table 3 we can see that chlorophyta were not found on dolomite. The genus *Chlorella* was the most widespread, occurring on 20 of the monuments reported, represented by four species and occurring on four different substrata. *Stichococcus* was found on 17 monuments and was also present on four substrata. Members of the chlorophyte genus *Chlorococcum* were detected on all the stone substrata considered, with the exception of dolomite, and this genus occurred on 15 distinct monuments. Therefore, we can consider that *Chlorella*, *Stichococcus* and *Chlorococcum* are

Fig. 3. *Gloeocapsa* sp. observed by optical microscopy. Scale bar, 20 μm.

Fig. 5. *Klebsormidium flaccidum* biofilm on a terracotta statue, Cathedral of Seville.

the green algal genera most abundant on the monuments of the Mediterranean Basin. This is in fair agreement with Ortega-Calvo *et al.* (1995), who stated that *Chlorella*, *Chlorococcum Klebsormidium* and *Trentepohlia* can be readily observed in monuments located in Europe, America and Asia. However, it was not possible to establish a correlation between these genera and a specific substratum or climate.

Klebsormidium, *Trebouxia* and *Trentepohlia* (Fig. 1) also showed a significant representation among the chlorophyte genera colonizing stone substrata in the Mediterranean Basin. The occurrence of *Trebouxia* and *Trentepohlia* indicates that these microalgae could be involved in the lichenization process leading to colonization by lichens. In fact, the genus *Trebouxia* occurs in approximately 20 % of all lichens and has rarely been found free-living. Regarding endolithic growth of green algae, *Trentepohlia*, *Chorella* and *Klebsormidium* were found growing under a black patina, probably a cryptoendolithic niche, in the Ordem de São Francisco Church, Portugal (Pereira de Oliveira, 2008; Pereira de Oliveira *et al.*, 2008). Cryptoendolithic growth of *Stichococcus bacillaris* was also observed in granite from the Cathedral of Toledo, Spain (Ortega-Calvo *et al.*, 1995).

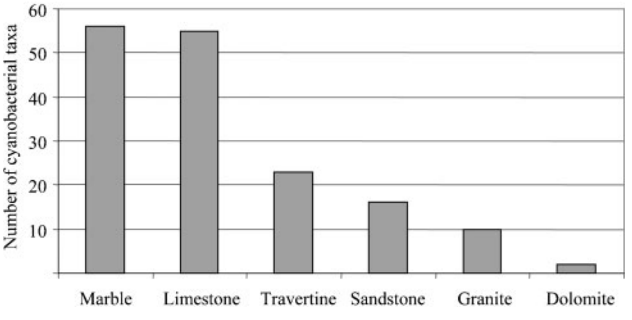

Fig. 4. Number of cyanobacterial taxa found on each lithotype.

Fig. 8 shows the number of chlorophyta taxa present in each lithotype. Limestone was colonized by the highest number of taxa (34), followed by marble (27). Travertine and granite were colonized by about the same number of taxa (21 and 22, respectively), although the number of monuments built of travertine (7 %) was considerably lower than the number made of granite (18 %). From Table 3 we can see that *Oocystis*, *Cosmarium* and *Staurastrum* species appear almost exclusively on travertine. Nevertheless, the majority of the results suggest that green algae can colonize a wide variety of substrata and this is primarily related to the physical characteristics of the stone surface (porosity, roughness and permeability) and secondarily to the nature of the substratum. This is in accordance with Tiano *et al.* (1995). These authors carried out a laboratory experiment using two photosynthetic strains: a green alga (*Pleurococcus*) and a cyanobacterium (*Lyngbya*), inoculated on 12 different lithotypes exposed to constant climatic conditions. They demonstrated that the

Fig. 6. Light micrograph of trichomes of *Borzia periklei*, composed of 4–8 cells. Cells are 5–6 μm wide and 5–11 μm long.

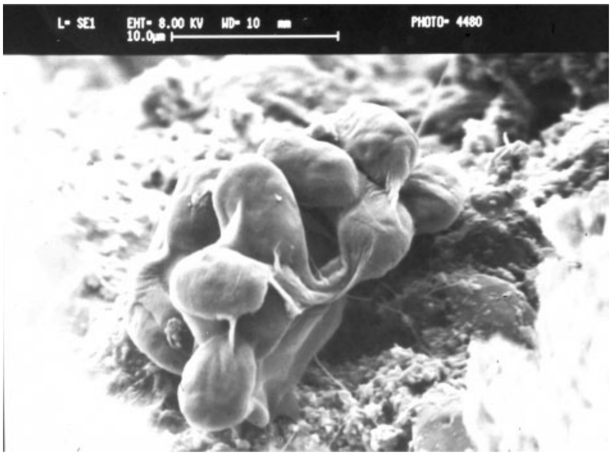

Fig. 7. SEM micrograph of a biofilm induced *in vitro* on a sandstone, showing *Gloeothece* cells and sheath. Scale bar, 10 μm. Reproduced from Ariño (1996) with permission.

preferential colonization (percentage of stone surface coverage) was correlated mainly with physical characteristics of the stone (roughness and porosity) while the chemical composition had low influence (Tiano *et al.*, 1995).

Survival strategies of cyanobacteria and algae on stone

As mentioned above, environmental conditions, climate, microclimate and other site-specific characteristics may have a stronger influence on community development than stone lithotype. However, green algal and cyanobacterial diversity as well as abundance is clearly dependent on the availability of water, allowing micro-organisms to form subaerial biofilms on virtually any surface (Gorbushina, 2007). This leads to species characteristic of very different habitats and ecological requirements. Moreover, it was observed that the most representative genera occurring in unfavourable environments develop different strategies to survive in such conditions; these include the production of a sheath composed of extracellular polymeric substances (EPS) outside the cyanobacterial cells (Fig. 7) as a protection against desiccation (Urzì & Realini, 1998; Tomaselli, 2003). Due to the presence of these colloidal polymeric substances, the biofilm incorporates large amounts of water into its structure, ensuring the maintenance of moisture by balancing changes in humidity and temperature, which permits cyanobacteria and algae to resist drought periods (Gómez-Alarcón *et al.*, 1995; Saiz-Jimenez, 1999; Schumann *et al.*, 2005). These biofilms are composed of populations or communities of different micro-organisms (microalgae, cyanobacteria, bacteria and fungi) immobilized on the stone surface (substratum) and frequently embedded in an organic polymer matrix formed by EPS, such as polysaccharides, lipopolysaccharides, proteins, glycoproteins, lipids, glycolipids, fatty acids and enzymes (Young *et al.*, 2008). EPS are also responsible for binding cells and other particulate matter together (cohesion) and to the substratum (adhesion) (Cecchi *et al.*, 2000; Warscheid & Braams, 2000) (Fig. 9).

The stone surface may have also several characteristics that are important in the attachment process. The extent of microbial colonization appears to increase as the surface roughness increases. This is because shear forces are diminished, and total surface area is higher on rougher surfaces (Morton *et al.*, 1998; Donlan, 2002). Tomaselli *et al.* (2000a) showed that high stone porosity and rough surface, together with environmental factors, played a greater role than mineral composition in promoting microbial establishment. The most important of the environmental factors is water availability. Adequate temperature and solar irradiance, and type of atmospheric deposition, are also relevant factors (Tomaselli *et al.*, 2000a).

Endolithic colonization is also a successful survival strategy when surface environmental conditions are adverse for life on stone. The protection provided by the rock leads to the abundance of endolithic micro-organisms in extreme environments, such as cold and hot deserts, semiarid lands and even polar regions (Friedmann, 1982; Bell, 1993; Walker *et al.*, 2005).

Biodeterioration of stone

The presence of cyanobacterial and algal biofilms on stone surfaces can be considered biodeteriogenic, simply because of the aesthetic damage they cause, producing variously coloured patinas (Ortega-Calvo *et al.*, 1995). These micro-organisms have an important role in the disfigurement of monuments and stone works of art (Figs 1 and 5). Moreover, there are several references in the literature that point to direct decay mechanisms caused by these photosynthetic micro-organisms (Anagnostidis *et al.*, 1991; Griffin *et al.*, 1991; Krumbein & Urzì, 1991; Ortega-Calvo *et al.*, 1992, 1993b; Wakefield & Jones, 1998; Saiz-Jimenez, 1999; Warscheid & Braams, 2000; Crispim & Gaylarde, 2005; Zurita *et al.*, 2005). In fact, cyanobacteria and green algae living in rocks can enhance soil formation and water retention. It has been estimated that 20–30 % of stone deterioration is a result of biological activity (Wakefield & Jones, 1998). Two types of biodeterioration can be considered: biogeophysical and biogeochemical. While the effects and extent of biogeochemical deterioration processes are controlled and determined by the chemistry of minerals and the binding cement of each rock, biogeophysical mechanisms are mostly regulated by the porosity and shape of the interior surface of the rocks (Warscheid & Braams, 2000).

Biogeophysical deterioration can be defined as the mechanical damage caused by exerted pressure during biological growth, resulting in surface detachment, superficial losses, or penetration and increased porosity (Griffin

Table 3. Chlorophyta reported on stone monuments, statues and historic buildings in European countries from the Mediterranean Basin, on different substrata

Chlorophyte taxon	Substratum	Monument no. (Table 1)
Apatococcus sp.	Limestone, granite, marble	3, 10, 19, 21, 22, 35, 39, 40
Apatococcus lobatus (Chodat) Petersen	Marble, limestone	30, 19
Bracteacoccus sp.	Limestone, marble	11, 43
Chaetophorales species	Marble	19a
Chlamydocapsa sp.	Granite	45
Chlorella sp.	Granite, sandstone, marble, limestone	3, 4, 5, 7, 10, 14, 17, 19a, 24, 26, 21, 33, 35, 36, 40
Chlorella ellipsoidea Gerneck	Limestone, granite	11, 45
Chlorella homosphaera Skuja	Granite	34, 43
Chlorella reisiglii (Reisigl) Watanabe	Limestone	11
Chlorella vulgaris Beijerinck	Granite, limestone, sandstone	33, 34, 36, 37
Chlorococcum sp.	Granite, limestone, sandstone, marble, travertine	2, 3, 6, 17, 19a, 20, 26, 28, 32, 38, 40, 41, 42, 44, 45
Chlorococcum wimmeri Rabenhorst	Marble	38
Chlorokybus atmophyticus Geitler	Sandstone, limestone	33, 11
Chlorosarcina sp.	Marble	28
Chlorosarcinopsis sp.	Limestone, marble	2, 19, 28
Chlorosarcinopsis minor (Gerneck) Herndon	Granite, marble, limestone	34, 38, 19
Choricystis chodatii (Jaag.) Fott	Granite	38
Cladophora sp.	Marble	19a
Coccomyxa sp.	Granite, marble	3, 14
Cosmarium sp.	Limestone	2, 11
Cosmarium depressum (Nägeli) Lundell	Travertine	13
Cosmarium granatum Brébisson	Travertine	13
Cosmarium reniforme (Ralfs) Archer	Travertine	13
Crucigenia quadrata Morren	Travertine	13
Cylindrocystis brebissonii (Meneghini) De Bary	Limestone	11
Desmococcus sp.	Sandstone, granite	32, 45
Desmococcus vulgaris Brand	Limestone, travertine	11, 20
Ecdysichlamys obliqua G.S. West	Sandstone	33
Euastrum insulare (Wittrock) Roy	Travertine	13
Friedmannia israeliensis (Chantanachat & Bold) Friedl	Sandstone	7
Geminella terricola Petersen	Limestone	11
Gongrosira sp.	Marble, limestone	38, 19
Haematococcus pluvialis Flotow	Marble	23, 44
Klebsormidium sp.	Granite, limestone	11, 17, 24
Klebsormidium flaccidum (Kützing) Silva, Mattox & Blackwell	Limestone, granite, marble, sandstone	7, 11, 26, 33, 34, 37, 43
Monoraphidium sp.	Granite	17
Muriella terrestris Boye-Peterson	Sandstone, travertine, granite	20, 33, 34, 37, 43
Myrmecia sp.	Limestone	11, 36
Nannochloris sp.	Sandstone	33
Oocystis crassa Wittrock	Travertine	13
Oocystis lacustris Chodat	Travertine	13
Oocystis solitaria Wittrock	Travertine	13
Pediastrum boryanum (Turpin) Meneghini	Travertine	13
Pleurastrum sp.	Limestone	2
Pleurastrum terrestre Fritsch & John	Limestone	11
Podohedra bicaudata Geitler	Limestone	11
Poloidion didymos Pascher	Limestone, marble	19
Protococcus sp.	Limestone	15
Pseudococcomyxa simplex (Mainx) Fott	Limestone	11
Pseudodendoclonium basiliense Vischer	Marble	38
Pseudopleurococcus sp.	Limestone, marble	2, 19

Table 3. cont.

Chlorophyte taxon	Substratum	Monument no. (Table 1)
Pseudopleurococcus printzii Vischer	Marble	38
Pseudosphaerocystis lacustris (Lemmermann) Novàkovà	Travertine	13
Rhizothallus sp.	Marble	3
Scenedesmus sp.	Travertine, granite	41, 17
Scenedesmus ecornis (Ehrenberg) Chodat	Marble, limestone	19
Scenedesmus obliquus (Turp.) Kütz.	Marble, limestone	19
Scenedesmus quadricauda (Turpin) Brébisson	Sandstone	33
Scenedesmus smithii S.S. Wang	Marble, limestone	19
Scotiellopsis terrestris (Reisigl) Hanagata	Limestone, marble	11, 19
Staurastrum boreale West	Travertine	13
Staurastrum lunatum Ralfs	Travertine	13
Staurastrum lunatum var. *planctonicum* West & West	Travertine	13
Staurastrum manfeldtii Delponte	Travertine	13
Staurastrum pingue Teiling	Travertine	13
Stichococcus sp.	Granite, limestone, marble	2, 3, 10, 15, 17, 18, 21, 31, 36
Stichococcus bacillaris Nägeli	Limestone, granite, marble, sandstone	7, 11, 26, 33, 43, 44
Stichococcus minutus Grintzesco & Pterfi	Granite	34, 37
Trebouxia sp.	Limestone	11, 30, 34, 36, 37, 38, 45
Trebouxia decolorans Ahmadjian	Sandstone	33
Trentepohlia sp.	Marble, sandstone, granite	10, 24, 31, 32, 45
Trentepohlia aurea (Linnaeus) Martius	Granite	34, 37
Tetracystis sp.	Granite, marble, limestone	45, 19
Tetraedron muticum (Braun) Hansgirg	Travertine	13
Tetraspora gelatinosa (Vaucher) Desvaux	Travertine	13
Ulothrix sp.	Limestone, marble, sandstone	2, 3, 6, 21, 30, 42

et al., 1991). This kind of deterioration also occurs due to the presence of cyanobacterial and algal biofilms that undergo large volume changes and exert considerable force through cycles of drying and moistening, loosening rock grains (Saiz-Jimenez, 1999). This can lead to the alteration of the stone's pore-size distribution and result in changes of moisture circulation patterns and temperature response (Saiz-Jimenez, 1999; Warscheid & Braams, 2000). Furthermore, the formation of crusts induced by cyanobacterial and algal growth also results in longer moisture retention at the stone surface, increasing the stone colonization potential.

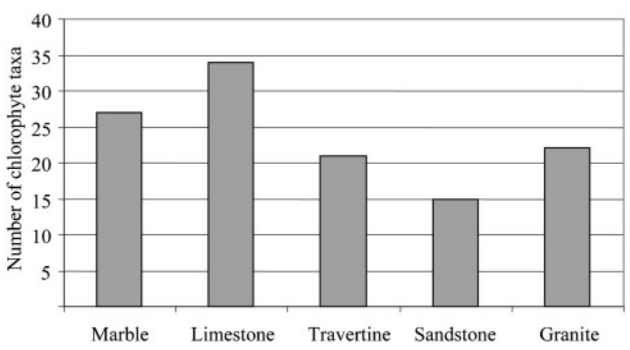

Fig. 8. Number of chlorophyte taxa found on each lithotype.

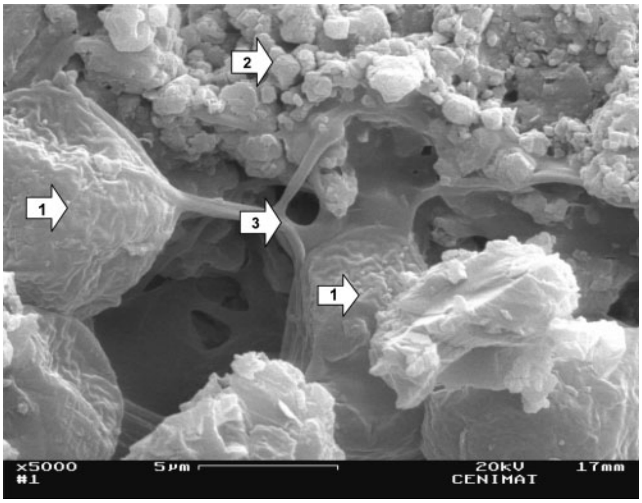

Fig. 9. SEM image showing green algae cells (arrow 1) adhered to a limestone surface (arrow 2) embedded in matrix of extracellular polymeric substances (arrow 3). Scale bar, 5 µm.

Biogeochemical deterioration is the direct action caused by the metabolic processes of organisms on the substratum. The biogenic release of corrosive acids is probably the best known and most commonly investigated biogeochemical damage mechanism in inorganic materials. The process, known as biocorrosion, involves the release of organic acids which can etch or solubilize stone, the exudation of organic chelating agents which sequester metallic cations from stone, or the conversion of inorganic substances by redox reactions which form acids that etch stone and contribute to salt formation (Griffin *et al.*, 1991; Fernandes, 2006). For instance, aerobic micro-organisms produce respiratory carbon dioxide which becomes carbonic acid and contributes to dissolution of stone and soluble salt formation (Griffin *et al.*, 1991; Wakefield & Jones, 1998). The precipitation of calcium salts on cyanobacterial cells growing on limestone suggests the migration of calcium from neighbouring sites (Fig. 10) (Ariño *et al.*, 1997; Crispim & Gaylarde, 2005). In addition, the production of organic acids such as lactic, oxalic, succinic, acetic, glycolic and pyruvic has been found and associated with the dissolution of calcite in calcareous stones (Danin & Caneva, 1990; Caneva *et al.*, 1992). Endolithic photosynthetic micro-organisms actively dissolve carbonates to enable penetration into the stone, enhancing stone porosity (Griffin *et al.*, 1991; Fernandes, 2006). Furthermore, the slimy surfaces of microbial biofilms favour the adherence of airborne particles (dust, pollen, spores, carbonaceous particles from combustion of oil and coal), giving rise to hard crusts and patinas (Saiz-Jimenez, 1999).

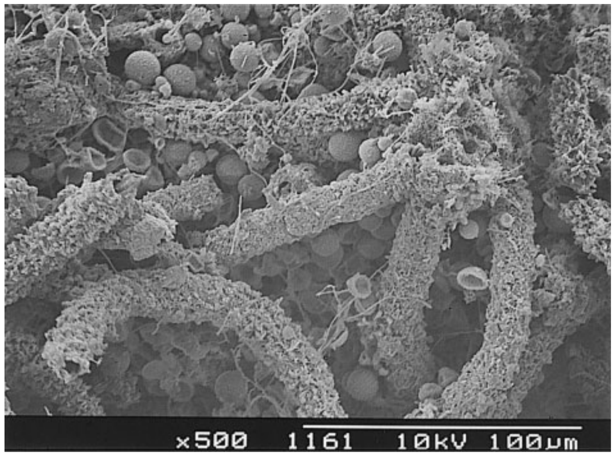

Fig. 10. SEM micrograph of *Scytonema julianum* mat. The calcified sheaths are composed of triradiate calcite crystals. Scale bar, 100 μm. Reproduced from Ortega-Calvo *et al.* (1995) with permission.

Sophisticated tools, consisting mainly of microscopy techniques, have been applied to the study of the stone biodeterioration process by photosynthetic micro-organisms. Microscopy techniques provide direct evidence of biofilm formation by imaging actual cells. The most common are scanning electron microscopy with back-scattered electron imaging (SEM-BSE), confocal scanning laser microscopy (CSLM) coupled with fluorescent probes (Fig. 11), low-temperature SEM (LTSEM) and environmental SEM (ESEM) (Roldán *et al.*, 2004; Ascaso *et al.*, 2004; De los Ríos *et al.*, 2004; Wierzchos *et al.*, 2004; De los Ríos & Ascaso, 2005). CSLM studies revealed that cells in biofilms are organized within a complex exopolymeric matrix, and the biofilm consists of a heterogeneous distribution of cells and cellular aggregates with void spaces or water channels (Costerton *et al.*, 1995; Kawaguchi & Decho, 2002). The use of microscopy techniques to study microbial colonies in their natural microhabitat has also demonstrated that EPS penetrate small pore spaces of the substratum and may facilitate subsequent penetration of the micro-organisms into the material, increasing stone biodeterioration. These studies have shown that penetration of growing organisms into rock and the diffusion of their excreted products may occur to depths of several millimetres (Saiz-Jimenez, 1999; Koestler, 2000; Salvadori, 2000; Pohl & Schneider, 2002; Young *et al.*, 2008). Pohl & Schneider (2002) applied computerized image analysis to detect and quantify the biomass and depth of penetration of endolithic micro-organisms into carbonate rock surfaces. The natural carbonate rocks investigated were endolithically colonized by lichens, cyanobacteria, algae and fungi. Photosynthetic micro-organisms from intensely insolated dry sites retreated to depths of 150-250 μm below

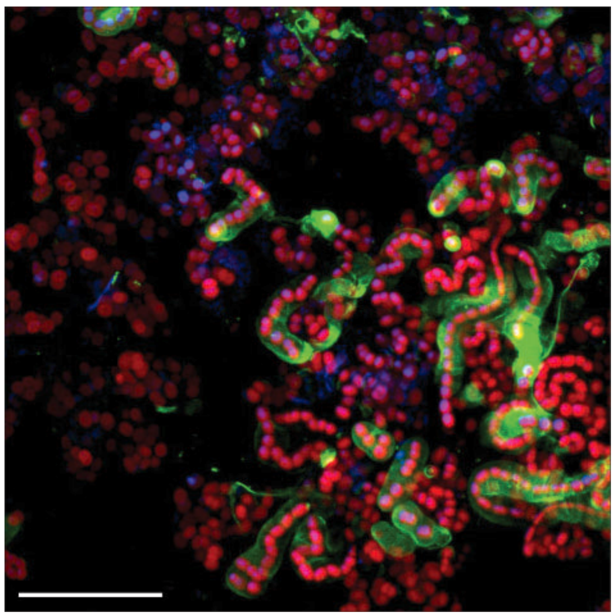

Fig. 11. Confocal microscope image of filaments of *Nostoc punctiforme* in the vegetative growth state (biofilm sample from Nerja Cave, Málaga, Spain). Pigment fluorescence is shown as red colour, polysaccharides labelled with Con-A-AlexaFluor 488 as green colour, and nucleic acids stained with Hoechst 33258 as blue colour. Scale bar, 50 μm.

the rock surface and showed 'cushions' of EPS oriented toward the surface, which protect them against intense light and provide water retention. In addition, the authors demonstrated that as soon as the endolithic biofilm was established it exerted an overall protective effect on the carbonate rocks. Salvadori (2000) examined by SEM the endolithic communities inhabiting Italian stone monuments and observed that euendolithic cyanobacteria and fungi could easily penetrate the calcite crystals of marble. The periodic contraction and expansion of EPS induces mechanical stress on the stone surface, particularly when the polymer penetrates into the pores, stimulating defoliation of the biofilm and the underlying substratum, evident at macroscopic scale (Saiz-Jimenez, 1999; Young *et al.*, 2008; Kemmling *et al.*, 2004). Krumbein & Urzì (1991) reported the physical action on and within marble through biofilms and microbial growth, demonstrating that stability and activity of water in polyionic gel matrix was crucial for the physical reactions and interactions between microbiota and rock and rock porosity, giving rise to decomposition and detachment of grains, chips or scales in marbles. Apart from direct deterioration of stone substrata, microbial biofilms, especially those formed by photosynthetic micro-organisms, play an indirect role in stone biodeterioration as described by Ortega-Calvo *et al.* (1992). It was concluded that photosynthetic micro-organisms contribute directly to stone deterioration through physical and chemical action and indirectly through the synergistic interactions with heterotrophic micro-organisms such as fungi and bacteria. The accumulation of photosynthetic biomass on stone surfaces provides nutrients for the growth of other communities which graze on cyanobacterial and algal polysaccharides and cell debris, contributing to accelerating the deterioration of the stone (Tiano, 1998; Crispim & Gaylarde, 2005; McNamara & Mitchell, 2005; Fernandes, 2006). Therefore, the role of cyanobacteria and green algae in the degradation of cultural heritage cannot be neglected. The detection and identification of these micro-organisms is extremely important for future studies of the biodeteriogenic process and the development of prevention and control methods.

Conclusion

A wide range of stone monuments from the Mediterranean Basin are colonized by cyanobacteria and green algae, showing notable biodiversity. A total of 96 taxa of cyanobacteria and 76 taxa of chlorophyta were found. The most widespread commonly reported taxa in the stone cultural heritage in the Mediterranean Basin are, among cyanobacteria, *Gloeocapsa*, *Phormidium* and *Chroococcus* and, among chlorophyta, *Chlorella*, *Stichococcus* and *Chlorococcum*. These genera were found associated with all lithotypes. Although an extensive literature survey was performed, the preference of the cyanobacteria and chlorophyta for a specific stone substratum was more complicated to correlate than expected. The majority of the results suggest that green algae and cyanobacteria can colonize a wide variety of substrata and this is primarily related to the physical characteristics of the stone surface (porosity, roughness and permeability) and secondarily to the nature of the substratum. Most cyanobacteria and chlorophyta did not show a clear relationship with the nature of the substratum, suggesting that environmental variables and site-specific characteristics (e.g. exposure to light, special architectural features) together with secondary, tertiary and/or extrinsic stone bioreceptivity have a stronger influence on community development than the substratum itself. In this complex amalgam of factors, it is often difficult to determine the influence of each factor alone; the evaluation of their combined effects, their synergy and dynamics is complex, and probably all factors are relevant. In order to ascertain a correlation between stone substratum and organisms, we need more detailed data about lithotype properties, and the microclimatic and environmental conditions of the monuments studied.

Cyanobacteria and green algae play an important role in the deterioration of monuments and other stone works of art, being responsible for aesthetic, biogeophysical and biogeochemical damage. Future work should focus on ecological and physiological studies of specific species of these micro-organisms in order to gain a better understanding of their role in stone colonization and biodeterioration processes. Moreover, an interdisciplinary team working on the same 'case study' is necessary in order to simultaneously investigate all the factors involved in the biodeterioration process such as mineralogical-petrographic, physico-chemical and climatic (and microclimatic) parameters.

Acknowledgements

This work was supported by the Ministério da Ciência, Tecnologia e Ensino Superior, Portugal, with a doctoral grant (SFRH/BD/21481/2005), and has been partially financed by the CEPGIST FCT subproject DECASTONE. The work also received support from the Programa de Financiamento Plurianual da Unidade de Investigação da FCT, financed by the European Union FEDER and the national budget of the Portuguese Republic. This is also a TCP CSD2007-00058 paper. The authors thank Dr Xavier Ariño for the permission to reproduce one of the figures of his doctoral thesis. The authors are also very grateful to Dr M. Hernandez-Marine (University of Barcelona, Spain) and Professor Rui Silva for the permission to use a confocal microscope image and a SEM image, respectively.

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