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International Biodeterioration & Biodegradation 55 (2005) 55–61

INTERNATIONAL BIODETERIORATION & BIODEGRADATION

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Microalgae associated with deteriorated stonework of the fountain of Bibatauín in Granada, Spain

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Received 4 November 2003; received in revised form 29 April 2004; accepted 13 May 2004

Abstract

The fountain of Bibatauín, in Granada (Spain), is in a poor state of conservation, and biodeterioration is occurring. Colonization by microalgae and its effects on the fountain were investigated. The microorganisms from representative sampling areas were identified by optical microscopy, and the biogenic carbonate crusts they formed analysed by X-ray diffraction and field emission scanning electronic microscopy. The most representative genera found were *Cosmarium*, *Phormidium* and *Symploca*, and the main mineral was calcite.

Keywords: Ornamental fountain; Microalgae; Limestone; Biocarbonation

1. Introduction

Biodeterioration of outdoor stone monuments has been widely analysed. Nevertheless, the biodeterioration of artistic fountains has not received as much attention as it should, considering their importance in cultural heritage. Tiano et al. (1993), Ricci and Pietrini (1994) and Bolívar Galiano and García Rowe (1994) are among the few who have specifically addressed the problem.

Biodeterioration of ornamental fountains is mainly caused by the activity of microalgae, which are generally pioneers in the processes of biodeterioration (Tiano et al., 1993); their action is direct and also indirect, since they promote other mechanisms of deterioration and the growth of other communities. (Krumbein, 1991; Ascaso et al., 1998; Tomaselli et al., 2000). The alterations that they provoke are mainly related to the formation of biofilms on the substrate and this is a precursor for several mechanisms of deterioration of the underlying substrate (Gu, 2003). The stone surface loses cohesion because of the contraction and expansion of these biofilms, and the polysaccharides associated with them cause the adhesion of stone fragments to the film and eventually their detachment from the original material (Sáiz-Jiménez, 1999). By this and other exclusively biological mechanisms, microalgae create mineral crusts or stromatolites (Golubic, 1973, 1987; Borowitzka, 1989) composed primarily of calcium carbonate. The effect of these crusts on ornamental substrates is extremely harmful, for they have a different grade of porosity and specific weight; thus, their thermal contraction and expansion will be different from that of the substrate. The existence of such biofilms indicates the continuous presence of water, and therefore a potential source of physical damage (Sáiz-Jiménez, 1999), and the microorganisms themselves contribute to chemical deterioration because of the metabolic products they excrete (Sand, 1997).

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0964-8305/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibiod.2004.05.006

Fig. 1. General view of the fountain of Bibatauín and sampling areas A–E (see text for explanation).

In Granada (southern Spain) fountains are of major importance, the Arabic origin of the city explaining the huge number of ornamental fountains in its squares and streets. Among those catalogued are the famous Islamic fountains in the Alhambra, but there are others that are almost unknown. The fountain of Bibatauín is a minor fountain, constructed of Sierra Elvira stone in 1933 in the Muslim geometric style, as the revival fashion of the time dictated (Gallego y Burín, 1996). It is situated facing the palace of Bibatauín, in the shady square of the same name (Fig. 1). Sierra Elvira stone is a Liassic limestone rich in fragments of crinoids, molluscs and equinoids. Calcite is the most common mineral phase, but some dolomite and quartz can also be detected.

In its present state, the Bibatauín fountain is a good example of the biodeterioration problems mentioned above. It can be appreciated at first glance that it is in an unsatisfactory state of conservation, and that biodeterioration is occurring. This is due to the inherent characteristics of every fountain, i.e. the continuous presence of water and the fact that it is in the open air. This is responsible for the predominant growth of microalgae over other microorganisms (Tiano et al., 1995) and the subsequent formation of carbonate crusts, which can seriously damage the stone (Fig. 2a). In addition, biodeterioration has been facilitated by structural features that contribute to the development of algal biofilms (Fig. 2b). This is the reason for it being chosen for this study, the purpose of which was to describe and analyse the effects of colonisation by microalgae and the resultant alteration products on it.

2. Materials and methods

Sampling: With permission from the current authorities, representative samples from each of five areas shown in Fig. 1, consisting exclusively of algae and their alteration products (mineral crusts), were collected in Eppendorf tubes, using scalpel and pliers. No original stone material was damaged during collection.

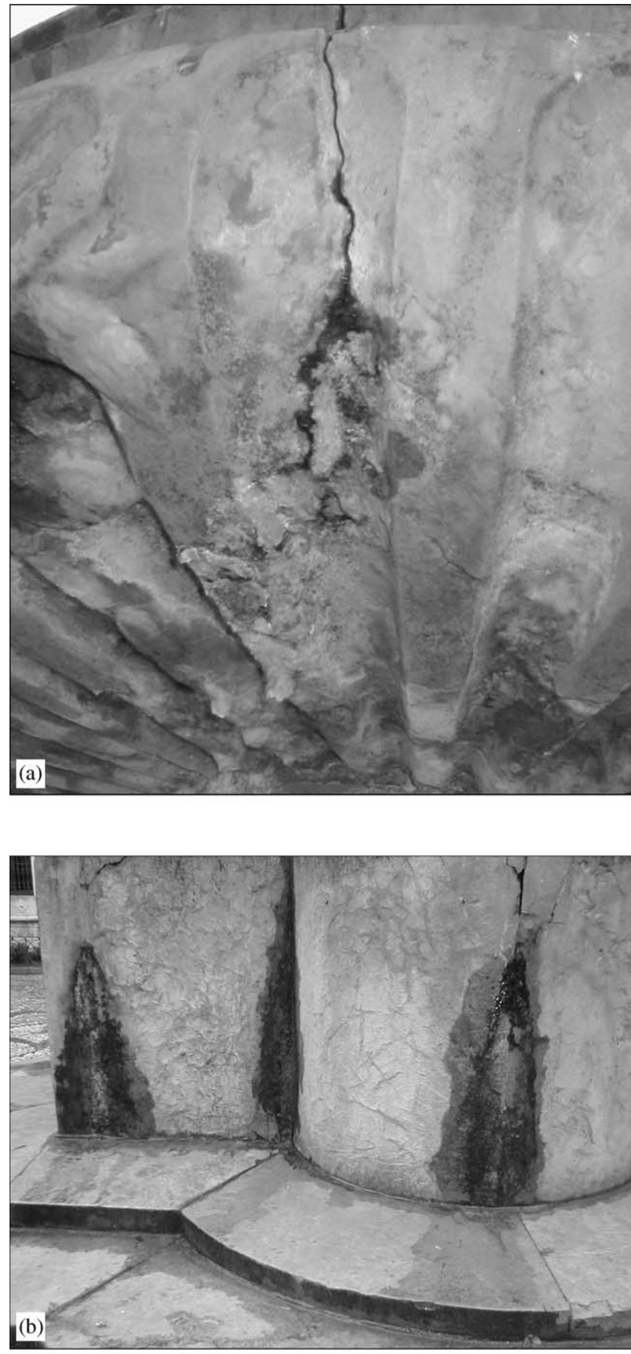

Fig. 2. Effects of biodeterioration: (a) biogenic carbonate crusts and (b) biological patinas on different surfaces of the fountain.

Alteration products: Samples of one of the most common alteration products of microalgae, microstromatolithic carbonate crusts, also known as concretions or accretions depending on their situation in relation to the water (Bolívar Galiano, 1994) were examined by stereoscopic microscopy.

Table 1
Microorganisms present in biotypes at sampling sites A–E of Bibatauín fountain

Biotype	Microorganisms	Genera	A	B	C	D	E
Unicellular	Diatoms	*Achnanthes* sp.	+	–	+	–	–
		Navicula spp.	–	–	+	+	+ + +
		Tabellaria sp.	–	–	–	+	–
	Green algae	*Cosmarium* spp.	+	+ + + + + + +	+	+ +	–
		Chlorococcum sp.	–	–	–	–	+
Gelatinous group	Green algae	*Chlosarcinopsis* spp.	–	–	–	–	+ +
		Chroococcidiopsis sp.	+ +	–	–	–	+ + +
		Chroococcus spp.	–	–	+ +	+ +	+
	Cyanobacteria	*Chlorogloea* sp.	–	–	–	–	+
		Gloeocapsa sp.	+ +				–
Pseudo-parenchymatous	Green algae	*Pseudopleurococcus* sp.	–	–	–	+	+
		Pleurastrum sp.	–	–	–	–	+
	Cyanobacteria	*Chamaesiphon* sp.	–	–	+ +	–	–
		Pleurocapsa sp.	–	–	–	–	+
Filaments without sheath	Green algae	*Ulothrix* spp.	+	–	+ +	–	–
		Stichococcus sp.	+	–	+	–	–
Filaments with sheath	Cyanobacteria	*Phormidium* spp.	+ +	+ +	+ + + + + + +	–	+ + + + + +
		Symploca sp.	–	–	+ + +	+ + + + +	–
		Microcoleus sp.	–	–	–	–	+

Relative abundance of each taxon is indicated by the number of +; – indicates absence.

Identification of biotypes found in situ: Since biotypes develop in a particular order and provoke different phenomena (Sánchez Castillo and Bolívar Galiano, 1997), algal biotypes and genera in the samples were identified using a Zeiss inverted microscope and sometimes complemented with photomicrographs using a Zeiss Tesovar.

Mineralogical analysis of crusts: Five representative samples were chosen from each group of samples to study the mineral composition. Each was examined by powder X-ray diffraction (XRD) using a Philips PW 1710 diffractometer with an automatic slit under the following conditions: emission radiation = CuKα, voltage = 40 kV, intensity = 40 mA, goniometer speed = $0,1\ 2\theta/\mathrm{s}$. XRD goniometer calibration was performed using a silicon standard. Data were interpreted with the PLV program (Martín Ramos, 1990). Samples were ground in agar mortar, sieved to obtain a fraction of particle size $<53\,\mu\mathrm{m}$, and mounted on a siliceous support. Semiquantitative analysis of mineral phases was performed using experimentally determined reflectance power of each phase, according to methods proposed by Culliti (1956) and Rodríguez Gallego et al. (1968).

Field emission scanning electronic microscopy: To complete analysis of micro-stromatolitic crusts, eight samples selected were examined in a field emission scanning electronic microscope (FESEM) Leo Gemini 1530, coupled with INCA-200 Oxford microanalysis. FESEM secondary electron images were acquired from small pieces of carbon-coated crusts ($2 \times 4 \times 1\,\mathrm{mm}^3$ aprox.).

3. Results

An initial survey of the fountain revealed five main areas of concern (Fig. 1, A–E). Algal growth and other indicators of biodeterioration phenomena were evident. Each biotype and genus identified among the algae detected in the samples from each of the selected areas of the monument is reported in Table 1.

Area A (Fig. 1), the vertical exposed surface around the base of the pillar, was covered by a thick biofilm, and brown pustules were also found. This vertical surface was not submerged, but often wet owing to splashing and variations in water level. No mineral crusts were found, but the type of biofilm described is a preliminary step in their formation. The genera identified (Table 1) are mainly of algae ensheathed in gelatinous products, the presence of which is related to mineral fixation.

In contrast, a thin biofilm was found in area B, a vertical, permanently submerged surface on the inner face of the walls of the lower basin. Here there were no radical variations in environmental conditions, the water filtering the sunlight being relatively undisturbed. Two species of *Cosmarium* (Fig. 3) were predominant in the dark brown biofilm.

Several genera of filamentous cyanobacteria with sheaths were identified in areas C and D. Both were wet exposed surfaces, the former under the upper basin and the latter by the jets in this basin. Area C was characterized by thick mineral concretions, cracks in the stone and dried-out algae (Fig. 2a), and area D by thin mineral concretions, patches and pustules. *Phormidium*

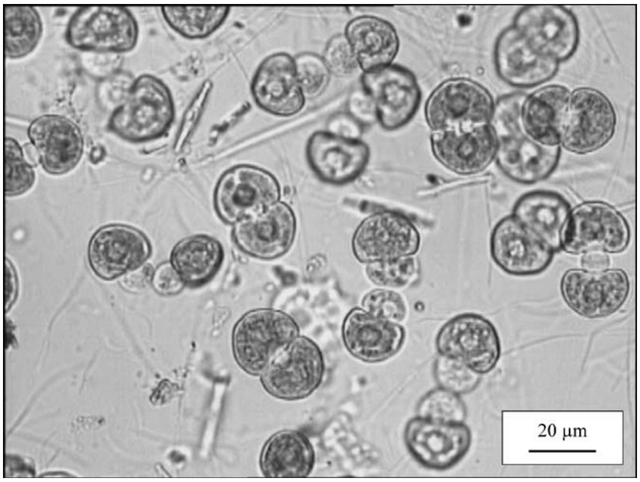

Fig. 3. *Cosmarium* sp. recovered from sample B2.

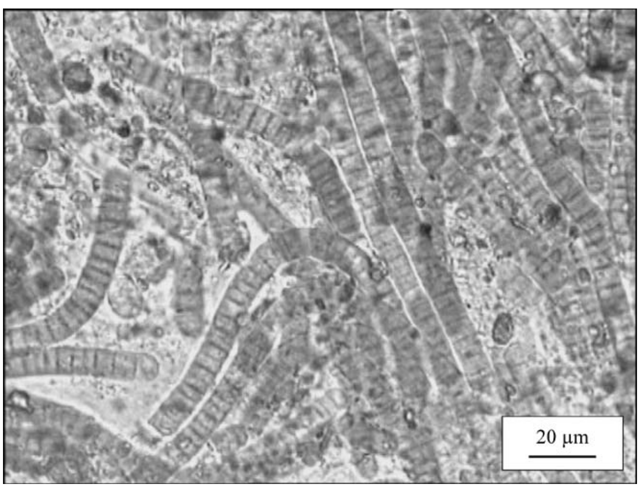

Fig. 4. *Phormidium* sp. hormogonia recovered from sample E4.

(Fig. 4) was present in C and *Symploca* in both areas. Water from the upper basin continually ran down over those areas, contributing to the formation of mineral stalactites, and cracking of the stone. Microalgae beside the cracks and mineral structures contributed to crust formation (Fig. 2a).

The wet vertical exposed surface of area E, on the outer face of the walls of the lower basin, by the joints between the blocks, had the greatest range of different biotypes, although filamentous cyanobacteria with sheaths were predominant as in areas C and D. The development of thin algal biofilms and incipient mineralization beside the joints on this external face was attributable to the poor condition of the joints allowing seepage of water to the outside.

This study of algal communities and alterations related to them supports the hypothesis of a biological origin for the crusts. Stereoscopic examination of the crusts in many cases revealed a stratified structure, where sometimes several layers could be clearly distinguished Fig. 5a). Other samples showed an amorphous structure that was less lithified. Although carbonation was more advanced in areas C and D (Table 2), all samples had a mineral component, with calcite being most frequent. Other varieties of calcitic salts, such as dolomite and ankerite, were also detected, as were silica minerals, most probably owing to the prominence of diatoms in samples, especially from areas D and E (Table 2). The FESEM images (Figs. 5a–d) show the close relationship between microalgae and development of mineral crystals.

4. Discussion

Although diatoms, green algae and cyanobacteria were identified in this study, diatoms were not encountered as frequently as the other microorganisms, possibly because they are usually pioneers (Wetherbee et al., 1998), however, here colonization of the substrate was advanced. The diatoms found in several samples (Fig. 5c), especially *Navicula* and *Nitzschia*, are very common genera in freshwater environments, and have been identified in previous studies of monumental fountains (Foged, 1983; Bolívar and Sánchez-Castillo, 1995; Peraza Zurita and Bolívar Galiano, 2002). Although proportionally they were minor components, there were enough for the XRD to detect traces of silica minerals in many samples, probably from the diatom frustules. Their greater occurrence in samples from area E may also be attributed to this, but the presence of particles from the concrete in the joints by the sampling point, or particles brought by wind, might also have contributed.

Among the green algae and cyanobacteria, "sticky" biotypes were predominant. In fact, both gelatinous groups and filaments with sheaths were very frequent at all sampling areas. The presence of other groups was only occasional, except for the green alga *Cosmarium*, which was abundant and almost the only taxon found on the face in contact with the water in the basin of some samples from area B, i.e. the submerged inner wall of the fountain. *Cosmarium* is a sensitive genus that is rarely mentioned in relevant literature and does not threaten the integrity of the substrate. It reacts adversely to chemical and temperature changes, but the submerged surface of area B would have provided conditions favourable for its development.

With reference to "sticky" algae, several genera belonging to gelatinous groups were identified, all of them frequently mentioned in other studies (Caneva et al., 1992; Young and Wainwright, 1995; Urzí and de Leo, 2001). The filamentous genera *Phormidium* and *Symploca*, both in the order *Nostocales*, were particularly prominent where carbonate crusts were more

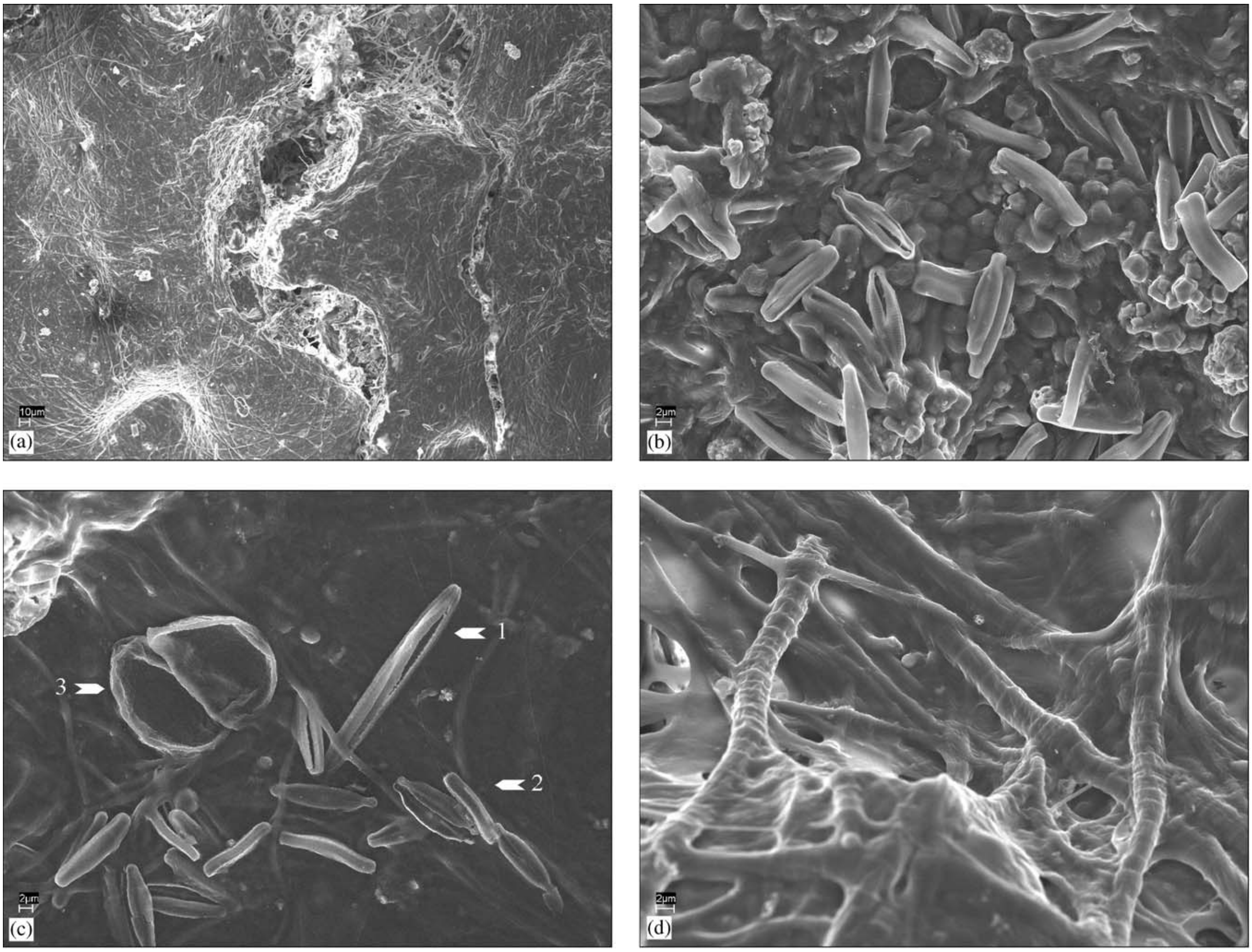

Fig. 5. FESEM images: (a) general view of the surface of a carbonate crust from area C; (b) *Achnantes* sp. frustules and carbonate crystals from sample A1; (c) *Nizstchia* sp. (1), *Achnantes* sp. (2) and *Cosmarium* sp. (3) from sample A4; and (d) cyanobacterial filaments covered by carbonates from sample C0.

Table 2
Semiquantitative analysis of crusts at sites A–E on stonework of Bibatauín fountain

Minerals	A	B	C	D	E
Calcite	+ + + +	+ + + +	+ + + +	+ + +	+ + +
Dolomite	tr	–	–	–	+
Ankerite	–	–	–	–	+ +
Silica	tr	–	tr	+	+ +

The concentration of each mineral type is indicated by number of +; tr, traces quantities; - in, absence.

developed (areas C and D, Figs. 5a,d). Both genera were also frequently found in previous studies of stone monuments (Ortega-Calvo et al., 1991; Caneva et al., 1992; Young and Wainwright, 1995; Ariño et al., 1997, Sáiz-Jiménez, 1999; Urzí et al., 2001). They are protected by the polymeric secretions they produce, and this allows them to grow in a variety of conditions, some of which may be extreme. Because of the covering sheath in the latter, and the gelatinous binding in the former, these organisms contribute to biodeterioration by provoking chemical interactions with the substrate and by fixing carbonates (Lowenstam, 1981; Borowitzka, 1989; Sand, 1997). Communities of these microorganisms were frequently found to be established at the inner boundary of the crusts in many areas of the fountain indicating that the origin of these crusts would have had an important biological component. In support of this, XRD analysis of the samples showed a large amount of carbonate, mainly calcite. FESEM analysis revealed mineral layers both covered by and covering organic structures (Figs. 5a, d).

It can be concluded that the visible deterioration of the ornamental fountain of Bibatauín is mainly due to biological phenomena, the effects of which have been enhanced by neglect. The main agents are evidently microalgae, especially those producing gelatinous binding or protective structures. Communities of these have widely colonised the fountain, with consequent formation of carbonate crusts, this biocarbonation seriously damaging the stonework. Elimination would be a difficult task, since the algae are protected under the crusts that they have formed. The state of the fountain could be improved by thorough and deep cleaning, accompanied by biocide treatment, but only if followed up by measures to prevent a re-colonization, i.e. treatment of surface roughness or water pH. Many recent examples show the futility of restoring an outdoor monument if it is not cared for afterwards (Krumbein et al., 1993; Tiano et al., 1993, 1995; Dornieden et al., 2000).

Acknowledgements

This research has been supported by the Research Group RNM179 of the Junta de Andalucía and by the Research Projects REN 2002-04397-C03-01 and DGI-MAT-2000-1457. We thank the Centro de Instrumentación Científica of the University of Granada for technical assistance during FESEM analyses.

References

Ariño, X., Hernández-Mariné, M.C., Sáiz-Jiménez, C., 1997. Colonization of Roman tombs by calcifying cyanobacteria. Phycologia 36, 366–373.

Ascaso, C., Wierzchos, J., Castello, R., 1998. Study on the biogenic deterioration on calcarenite stone caused by endolitic lychens and microorganisms. International Biodeterioration and Biodegradation 42, 29–38.

Bolívar Galiano, F.C., 1994. Diagnosis and treatment of the deterioration by microalgae within the Nazarí Palaces in la Alhambra. Ph.D. Thesis, University of Granada.

Bolívar Galiano, F.C., García Rowe, J., 1994. Biodeterioration within the Fountain in the Main Cloister of the Hospital San Juan de Dios (Granada, Spain). Cuadernos de Arte de la Universidad de Granada 24, 197–203.

Bolívar, F.C., Sánchez-Castillo, P.M., 1995. Preliminary results on the study of the algae biodeterioration within the Alhambra. In: Bosher, A., Chandra, M., Edyvean, R. (Eds.), Biodeterioration and Biodegradation, the 9th International Biodeterioration and Biodegradation Symposium, Institution of Chemical Engineers, Rugby, pp. 210–217.

Borowitzka, M.A., 1989. Carbonate calcification in Algae: initiation and control. In: Mann, S., Webb, J., Williams, R.J.P. (Eds.), Biomineralization, Chemical and Biochemical Perspectives. VHC Verlag, Berlin, pp. 63–94.

Caneva, G., Nugari, M.P., Ricci, S., Salvadori, O., 1992. Pitting of marble Roman monuments and the related microflora. In: Delgado Rodrigues, J., Henriques, F., Telmo Jeremias, F. (Eds.), Proceedings of the 7th International Congress on Deterioration and Conservation of Stone, Lisbon, pp. 521–529.

Culliti, B.D., 1956. Elements of X-ray Diffraction. Addison-Wesley, Reading, MA.

Dornieden, T., Gorbushina, A., Krumbein, W.E., 2000. Patina: physical and chemical interactions of sub-aerial biofilms with objects of art. In: Ciferri, O., Tiano, P., Mastromei, G. (Eds.), Of Microbes and Art. Kluwer/Plenum, New York, pp. 105–120.

Foged, N., 1983. Diatoms of fountains, reservoirs and some other humid and dry localities in Rome (Italy). Nova Hedwigia 38, 433–470.

Gallego y Burín, A., 1996. Granada: An Artistic and Historical Guide to the City. Comares, Granada.

Golubic, S., 1973. The relationship between blue-green algae and carbonate deposits. In: Carr, N.G., Whitten, B.A. (Eds.), The Biology of Blue Green Algae. Blackwell, London, pp. 434–472.

Golubic, S., 1987. The role of diatoms in stromatolite growth from modern freshwater settings. Journal of Phycology 23, 195–201.

Gu, J.D., 2003. Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances. International Biodeterioration and Biodegradation 52, 69–91.

Krumbein, W.E., 1991. Studies on the biodeterioration of marbles and colour changes related to it. In: International Symposium on Biodeterioration, Madrid, 25–27 September 1991, Abstracts, p. 33.

Krumbein, W.E., Diakumaku, Peterson, Warscheid, Urzí, C., 1993. Interactions of microbes with consolidants and biocides used in the conservation of rocks and mural paintings. In: Thiel, M.J. (Ed.), Conservation of Stone and Other Materials, Vol . 2, Prevention and treatment. RILEM/UNESCO, Paris, pp. 589–596.

Lowenstam, H.A., 1981. Minerals formed by organisms. Science 211, 1126–1131.

Martín Ramos, J.D., 1990. Programme for the control and analysis of RX diffractometer. DL: M-11719.

Ortega-Calvo, J.J., Hernández-Mariné, M.C., Sáiz-Jiménez, C., 1991. Biodeterioration of historic buildings by Cyanobacteria and Algae. In: International Symposium on Biodeterioration, Madrid, 25–27 September 1991, Abstracts, p. 61.

Peraza Zurita, Y., Bolívar Galiano, F.C., 2002. Monumental fountains within the Alhambra in Granada (Spain): biodeterioration problems. Kermes 48, 63–68.

Ricci, S., Pietrini, A.M., 1994. Characterization of the microfloral algae present on the Fountain of Four Rivers, Rome. In: Fascina, V., Ott, H., Zezza, F. (Eds.), Third International Symposium on the Conservation of Monuments in the Mediterranean Basin, 22–25 June 1994, Venice, pp. 353–357.

Rodríguez Gallego, M., Martín-Pozas, J.M., Martín Vivaldi, J.L., 1968. Análisis cuantitativo de filosilicatos de la arcilla por difracción de rayos X: Influencia de las sustituciones isomórficas y cristalinidad. Anuario de la Real Sociedad Española Física y Química 65, 25–29.

Sáiz-Jiménez, C., 1999. Biogeochemistry of weathering processes in monuments. Geomicrobiology Journal 16, 27–37.

Sánchez Castillo, P.M., Bolívar Galiano, F.C., 1997. Characterization of epilithic algal communities within monumental fountains and application on diagnosis of biodeterioration. Limnética 13, 31–46.

Sand, W., 1997. Microbial mechanisms of deterioration of inorganic substrates—a general mechanistic overview. International Biodeterioration and Biodegradation 40, 183–190.

Tiano, P., Acolla, P., Tomaselli, L., 1993. Effectiveness of some biocides against algal biodeterioration. In: Thiel, M.J. (Ed.), Conservation of Stone and Other Materials, Vol. 2, Prevention and treatment. RILEM/UNESCO, Paris, pp. 573–580.

Tiano, P., Camaiti, M., Acolla, P., 1995. Methods for the evaluation of products against algal biocoenosis of monumental fountains. In:

ICCROM International Colloquium on Methods of Evaluating Products for the Conservation of Porous Building Materials in Monuments, Rome, pp. 75–86.

Tomaselli, L., Tiano, P., Lamenti, G., 2000. Occurrence and fluctuation in photosynthetic biocoenoses dwelling on stone monuments. In: Ciferri, O., Tiano, P., Mastromei, G. (Eds.), Of Microbes and Art. Kluwer/Plenum, New York, pp. 63–76.

Urzí, C., de Leo, F., 2001. Sampling with adhesive tape strips: an easy and rapid meted to monitor microbial colonization on monument surfaces. Journal of Microbiological Methods 44, 1–11.

Urzí, C., Brusetti, L., Salamone, P., Sorlini, C., Stackebrandt, E., Daffomchio, D., 2001. Biodiversity of Geodermatophilaceae isolated from altered stones and monuments in the Mediterranean basin. Environmental Microbiology 3, 471–479.

Wetherbee, R., Lind, J.L., Burke, J., Quatrano, R.S., 1998. The first kiss: establishment and control of initial adhesion by raphid diatoms. Journal of Phycology 34, 9–15.

Young, G.S., Wainwright, I.N.M., 1995. The control of algal biodeterioration of a marble petroglyph site. Studies in Conservation 40, 82–92.