Biodeterioration of Building Materials by Cyanobacteria and Algae

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ABSTRACT

*A study of the presence of cyanobacteria and algae in different building materials from Spain (Salamanca, Seville and Toledo Cathedrals) and Sweden (Lund Cathedral) and their possible relation to the stone decay was accomplished. Colonization of stone with the cyanobacterium *Microcoleus vaginatus* and the chlorophyte *Klebsormidium flaccidum* was also induced in the laboratory. In both field and laboratory samples, the microbial film, spontaneously detached, showed on its reverse side the presence of grains removed from the stone surface, thus causing mechanical deterioration on the colonized materials.*

INTRODUCTION

Like all humid and illuminated substrates, monument walls support the growth of diverse organisms, whose presence is related to the natural mechanisms involved in the formation and degradation of terrestrial substrates. The colonizers of such surfaces are often atmophytic cyanobacteria and algae with characteristics closely related to the pioneer ones present in non-flooded soils (Anagnostidis *et al.*, 1983), and in some cases they are the same species.

The taxonomy and ecology of soil algae have been reviewed by

Metting (1981), Starks *et al.* (1981) and recently by Hoffmann (1989). The presence of cyanobacteria and algae on a substrate (limestone, sandstone etc.) enable its disintegration (Starks & Shubert, 1982). Nevertheless, the deleterious effects of the cyanobacteria and algae on building materials could not be clarified even after an extensive review of the literature by Hueck-van der Plas (1968). According to Grant (1982) the position has remained unchanged since then, and is still, now, a matter of controversy. Their aggressive action in relation to the substrate where they develop has been considered negligible by some authors, who consider important only the aesthetic damage they cause (Dukes, 1972; Paleni & Curri, 1973; Tiano, 1986). Nevertheless, there are reports on a role of cyanobacteria and algae in the weathering of stone in nature (Pomar *et al.*, 1975; Danin *et al.*, 1983; Viles, 1987) as well as in monuments and sculptures (Hyvert, 1973; Giaccone *et al.*, 1976; Danin, 1983). The present paper consists of a study of the presence of cyanobacteria and algae in different monuments, and their possible relation to the stone decay process as viewed by scanning electron microscopy.

MATERIALS AND METHODS

Sampling

The samples were obtained from outer zones of the Cathedrals of Salamanca, Seville and Toledo (Spain) and Lund (Sweden), where green patina were apparent. The substrates where cyanobacteria and algae were growing included terracotta, sandstone, limestone, marble and granite. Samples were taken by scraping the substrate surface. Each sample was divided into subsamples for: (1) optical microscope observations; (2) inoculation of enrichment cultures and, if enough material was available, (3) for scanning electron microscope observations.

Identification

The identification of the cyanobacteria and/or algal component in the aggregates was achieved following the morphological characteristics of the taxa in the field samples as well as in enrichment and, if obtained, in monocyanobacterial and monoalgal cultures throughout the culture period.

Culture methods

The enrichment cultures were made in parafilm-sealed Petri dishes with BG11 (Rippka *et al.*, 1979) or BBM (Chantanachat & Bold, 1962) solid

medium, by placing small fragments of biomass-containing material on the agar surface. Cultures were kept at 20°C under a 12/12 h light/dark cycle (light intensity of 800 lx). The isolation procedure followed standard microbiological techniques.

Colonization of stone was also induced in the laboratory. The stone used was a white-yellowish calcarenite with coarse grain (up to 4 mm) and high porosity (around 40%), from Puerto de Santa Maria (Spain) quarry. This limestone has been widely used in cathedrals, churches and palaces in south-west Spain. Before inoculation, the cubic stone samples were conditioned during 1 week by immersion in distilled water. The final pH of the leaching water after conditioning was 8.7. The stones were sterilized in an autoclave and aseptically introduced into sterile glass chambers containing liquid medium at the bottom. After 2 days, the stones were inoculated and the chamber sealed with parafilm in order to prevent evaporation. Two isolated strains were chosen for the culture of the cyanobacteria and algae on stone: *Microcoleus vaginatus* (strain SAL 3H) and *Klebsormidium flaccidum* (strain TOL 2C-2). Unicyanobacterial and unialgal cultures were inoculated from the maintenance agar slants tubes into 250 ml flasks containing 100 ml of BBM liquid medium. The flasks were incubated in a rotary shaker at 25°C and under a light-dark cycle of 12/12 h (light intensity 400 lx). After 2 weeks, 10 ml aliquots of each culture were gently spread over the surface of the stones with a sterile glass pipette. The chlorophyll *a* (chl *a*) content of the cultures was measured, after extraction with methanol in the dark and at room temperature, by the absorption at 665 nm of the extracts (specific absorption coefficient after Mackinney, 1941). It was found to be 5 µg chl *a* × ml⁻¹ for *M. vaginatus* and 14 µg chl *a* × ml⁻¹ for *K. flaccidum*. The chambers were maintained at 25°C, under a light/dark cycle (light intensity 250 lx). After 2 months, the stones that showed visible growth were washed with distilled water. Several cycles of wetness-dessication were then applied, by placing the stones in an oven at 40°C and dropping distilled water each 12 h. Samples for SEM observations were obtained by scraping stone surface.

Electron microscopy

For transmission and scanning electron microscopy, samples were treated following the methods described previously (Hernandez-Marine *et al.*, 1991). Observation was made with a Phillips 200 transmission electron microscope and with a Hitachi-600 scanning electron microscope.

The taxa present in SEM micrographs were identified through

similarities in size, shape, structure and habitat with those observed in corresponding field samples or in the cultures.

RESULTS

A total of 39 taxa have been identified, 15 of which are Cyanobacteria, 20 Chlorophyta, 1 Xanthophyta and 3 Bacillariophyta. The taxa list and the distribution are included in Table 1. The filamentous cyanobacteria *Microcoleus vaginatus* and *Phormidium autumnale* and the chlorophyte *Klebsormidium flaccidum* (Figs 1-5) were the most abundant taxa in the samples studied, comprising the majority of biomass as seen by optical and scanning electron microscopy.

The communities of cyanobacteria and algae rarely formed uniform green microbial films, localized at the surface of the material. The sampling was restricted to places with evident algal growth and was not designed to arrive at general conclusions on species distribution over the whole buildings. A general pattern of growth in place of constant water retention (cornices, holes, crevices and protected zones in general) was observed. In some cases a species segregation in very close sampling sites could be detected. For example, in Toledo Cathedral, *Plectonema* sp., *Chlorella homosphaera* and *Klebsormidium flaccidum* were present on granite, and *Nostoc* sp., *Plectonema boryanum*, *Chlorella* sp., *Chlorella reisiigii*, *Friedmannia israeliensis*, *Klebsormidium flaccidum*, and *Muriella terrestris* on the adjacent mortar. In the Cathedral of Seville, the sampling carried out in summer was repeated in the same sites in winter, after a strong rainfall period. In spite of the intense algal proliferation observed in winter, the same range of species was present, only two new taxa appearing: *Haematococcus pluvialis* and *Hantzschia amphyois*.

In some places in Lund Cathedral, the mat formed by the algal growth was several millimetres thick. This mat, dominated by *K. flaccidum*, was located up to 50 cm from the ground and spontaneously detached, showing on its reverse side the presence of grains removed from the stone surface (Figs 6-8). SEM observations showed that it consisted of a mass of entangled algal filaments held in a slimy matrix, in which were embedded particles of different sizes coming from the stone (Fig. 13) and probably from the air. In the reverse side of the mat, the presence of fungal filaments together with dry, apparently dead algal filaments was observed.

Samples with less severe algal growth than that observed in the Cathedral of Lund also presented an intimate relation of filaments with the substrate, as observed in Seville Cathedral (Figs 9-12). SEM observation revealed that biomass was more abundant in flat sites or in

	A	B	C	D
CYANOBACTERIA				
<i>Hyella</i> sp.				+
<i>Microcoleus vaginatus</i> (Vauch.) Gom.	+	+	+	
<i>Myxosarcina concinna</i> Printz	+			+
<i>Nostoc</i> sp.		+		+
<i>Nostoc punctiforme</i> (Kutz.) Hariot	+			
<i>Oscillatoria amphigranulata</i> Van Goor	+			
<i>Phormidium autumnale</i> (Ag.) Gom.	+	+	+	
<i>Phormidium fragile</i> (Menegh.) Gom.		+		
<i>Phormidium subfuscum</i> Kutz.		+	+	
<i>Phormidium tenue</i> (Menegh.) Gom.	+		+	
<i>Plectonema</i> sp.		+	+	
<i>Plectonema boryanum</i> Gom.			+	
<i>P. boryanum</i> f. <i>hollerbachianum</i> Elenk.	+			
<i>Synechocystis</i> sp.	+			
<i>Tolypothrix hyssoides</i> (Berk.) Kirchn.	+			
CHLOROPHYTA				
<i>Apatococcus lobatus</i> (Chod.) Boye-Pet		+		
<i>Bracteacoccus</i> cf. <i>minor</i> (Chod.) Petrova	+		+	
<i>Chlorella</i> sp.	+	+	+	
<i>Chlorella homosphaera</i> (Skuja) Handa et Nakano	+	+	+	
<i>Chlorella reisi</i> Wat.		+		
<i>Chlorella vulgaris</i> Beij.	+			
<i>Chlorella zoofingensis</i> Donz	+			
<i>Chlorokybus atmophyticus</i> Geitler		+		
<i>Chlorosarcinopsis</i> sp.	+			
<i>Ecdysichlamys obliqua</i> G. S. West	+	+		
<i>Haematococcus pluvialis</i> Flotow	+			
<i>Friedmannia israeliensis</i> Chantanachat et Bold				+
<i>Klebsormidium flaccidum</i> Silva, Mattox et Blackwell	+	+	+	+
<i>Muriella terrestris</i> Boye-Pet.	+	+	+	
<i>Neochloris bilobata</i> Vinatz.	+			
<i>Pseudodendroclonium printzii</i> (Vischer) Bourr.	+			
<i>Scenedesmus quadricauda</i> (Turp.) Breb. sensu Chod.	+			
<i>Stichococcus bacillaris</i> Naeg.	+	+	+	
<i>Trebouxia</i> sp.				+
<i>Trebouxia decolorans</i> Ahmadjian		+		
XANTHOPHYTA				
<i>Chlorellidium tetrahotrys</i> Vischer et Pascher	+			
BACILLARIOPHYTA				
<i>Achnantes coarctata</i> (Breb.) Grun.	+			
<i>Hantzschia amphioxys</i> (Ehr.) Grun.	+			
<i>Navicula mutica</i> Kutz.	+			

A: Pardon Gate, Cathedral of Sevilla; B: Cathedral of Salamanca; C: Cathedral of Toledo; D: Cathedral of Lund.

Fig. 1. *Microcoleus vaginatus*, showing confluent sheaths and capitata end cells (arrows).

Fig. 2. *Phormidium autumnale*.

Fig. 3. Transmission electron micrograph (TEM) of a longitudinal section of *Phormidium autumnale*, showing the general morphology, the individual sheath and the attenuated end cells. Irregular thylakoid arrangement with abundant phycobilisomes can be observed.

small, protected crevices, where the filaments penetrated following the fissures (Fig. 9). The filamentous cyanobacteria were found either alone (Figs 11 and 12) or with other taxa, forming a beginning of stratification. In this case, the upper layer was formed by cyanobacteria while, in

Fig. 4. TEM of the multilayered sheath of *Phormidium autumnale*. In some cases, as shown in the figure, the sheath reaches considerable thickness.

Fig. 5. *Klebsormidium flaccidum* from Cathedral of Lund.

general. *K. flaccidum* was in the layer below. In sites with higher humidity or more protected against desiccation, *K. flaccidum* was found growing alone or with sarcinoid or coccoid algae, such as species of *Chlorella* and *Muriella*. In some humid places, association with fungi could also be observed.

Attachment of the filaments to the rigid surfaces, specially in the cyanobacteria, was mediated by the extracellular sheath material, clearly visible with optical and transmission electron microscopy (Figs 1-4). This material caused the close union observed in some samples, making it difficult sometimes to differentiate biological from non-biological structures. SEM showed that some cyanobacterial hormogonia, such as in the case of *Phormidium autumnale*, produced empty sheaths left behind in their movement (figure not shown).

Basically, the colonization induced in the laboratory under conditions of high humidity and nutrient abundance showed the same characteristics as the field samples (Figs 14-19). Clear differences in growth

Fig. 6. Macroscopic aspect of the outer face of an algal mat growing on sandstone from the Cathedral of Lund.

Fig. 7. The same as Fig. 6, but of the reverse face, showing the stone grains attached to the mat.

Fig. 8. Scanning electron micrograph (SEM) of the mat.

Fig. 9. SEM of the degraded surface of a terracotta statue from Pardon Gate, Cathedral of Seville, showing colonization within fissures.

pattern could be observed between the cyanobacterium and the green alga. *Microcoleus vaginatus* grew as a layer firmly bound to the stone surface by mucilage, which in some places was seen clearly cementing the stone grains (Figs 14 and 15). When stone preparations comprising the first few surface millimetres were observed upside-down, cyanobacterial trichomes could be observed, indicating their penetration in the

Fig. 10. SEM showing *Klebsormidium flaccidum* growing under *Phormidium autumnale*.

Fig. 11. *Phormidium autumnale* as seen by SEM.

stone allowed by the high porosity. These trichomes were interestingly devoid of sheaths (Fig. 16). In contrast, *K. flaccidum* grew forming a net of filaments over the stone surface, attached by specific points (Fig. 17). Also a slight penetration was observed from the inner face of stone preparations. The green alga showed a faster growth and a higher degree of stone colonization than the cyanobacterium under the growth conditions used. Cyanobacterial and algal aggregates could be detached easily from the stone surface, containing stone grains in clear analogy with the samples from the Cathedral of Lund (Fig. 18).

DISCUSSION

The range of species found in our study is similar to those observed in other studies on historic buildings and monuments (Raistrick & Gilbert,

Fig. 12. SEM of cyanobacterial filaments growing intimately attached to stone surface through sheath material, in a sample from Pardon Gate, Cathedral of Seville.

Fig. 13. SEM of stone grain held by *Klebsormidium flaccidum* filaments on the reverse of the mat shown in Figs 6 and 7.

Fig. 14. SEM of *Microcoleus vaginatus* growth pattern induced in the laboratory.

Fig. 15. Detail showing particle cementing by cyanobacterial sheath.

1963; Lefevre, 1974; Schlichting, 1975; Tomaselli *et al.*, 1982; Anagnostidis *et al.*, 1983; Rands *et al.*, 1984). With few exceptions, they are frequent in soils and corticolous habitats, not being characteristic in any particular region.

In our study only two heterocystous cyanobacteria, *Nostoc* sp. and *Nostoc punctiforme*, were found. The presence on the buildings of nitrogen-fixing cyanobacteria has been considered as possibly relevant

Fig. 16. SEM of *Microcoleus vaginatus* trichomes growing inside the stone.

Fig. 17. SEM of *Klebsormidium flaccidum* growth pattern induced in the laboratory.

for the establishment and development of other organisms (Grant, 1982), although it is not clear whether in polluted environments the probable presence of combined nitrogen favours the presence of nitrogen fixers. Tomaselli *et al.* (1982) found abundance of species of *Nostoc* in samples from different wall paintings in Italy. The measured level of acetylene reduction (used to estimate the nitrogenase activity) was similar to that reported for *Nostoc* in another terrestrial environment (Whitton *et al.*, 1979). In contrast, samples from stone monuments presented greater predominance of green algae (Tomaselli *et al.*, 1982).

The persistence of the species observed during the year in the Cathedral of Seville indicates their survival in drought periods even in the high temperatures reached in summer (up to 45°C). An unfavourable

Fig. 18. SEM of an algal aggregate detached from the cultured stone.

Fig. 19. Detail showing cohesion between particles and *Klebsormidium flaccidum* filaments.

microclimate in the stone may induce the formation of resistant algal forms. This is a common mechanism in survival strategies (Mattox, 1971; Coleman, 1983). It was seen by Pietrini *et al.* (1985) in marble remains in Rome. The reddish-orange coloured patina present on the marble was produced mainly by the presence of cysts of *Haematococcus pluvialis*, which accumulated cytoplasmic carotenoids.

Most of the cyanobacteria identified and the filamentous chlorophyte *K. flaccidum* possess sheaths. The sheath in terrestrial cyanobacteria and algae acts as a reservoir of water, where it is bound through strong molecular forces (Campbell, 1979; Shephard, 1987). These organisms

react to a scarcity of biologically available water by diminishing metabolic activity. Activity is quickly restored upon wetting (Potts & Friedman, 1981). De Winder *et al.* (1989a) attributed part of the ability of metabolic activity recovery to the water retention properties of the substrate. Thus, the sheath contributes to their survival during drought periods (Trainor, 1985). In addition, a role of the sheath in substrate adhesion has been proposed (Robins *et al.*, 1986). Furthermore, sheath production by the cyanobacteria and algae has been considered of importance for soil stability, acting as a cementing agent (Campbell, 1979; Starks *et al.*, 1981; De Winder *et al.*, 1989b). The empty sheaths produced by *Microcoleus* gliding movement was considered to be responsible for the increase in thickness of desert crusts (Campbell, 1979).

Due to its water retention properties, the sheath of cyanobacteria and algae undergoes large volume changes, collapsing when a critical amount of water is removed (Campbell, 1979). The volume changes of the sheath exert considerable force through repeated shrinking and relaxation when they are going through cycles of drying and moistening, loosening rock grains and constituting a possible factor in the gradual destruction of the rocks (Jaag, 1945; Friedmann, 1971; Golubic, 1973; Anagnostidis *et al.*, 1983). This may well be the cause of the mechanical degradation observed in our samples, which clearly showed the rock grains attached to the algal mats (Figs 6-8 and 13). The fact that this process is also effective in the case of less severe growth, was confirmed by laboratory experiences, involving the use of culture techniques.

The formation of crusts induced by cyanobacterial and algal growth also results in a longer moisture retention at the stone surface, increasing the mechanical damage produced by the freezing and thawing of water present in the pores of the stone (Hueck-van der Plas, 1968). A role in the calcium carbonate dissolution system, proposed for the weathering of rocks by epilithic cyanobacteria in nature (Viles, 1987), should not be discarded.

The association with fungi, detected by our SEM observations, may have relevance in the weathering of the stone. Cyanobacteria and algae may play an indirect role by supporting the growth of heterotrophic organisms such as fungi or bacteria, with higher destructive potential (Strzelczyk, 1981; Saiz-Jimenez, 1984; Koestler *et al.*, 1985; Andreoli *et al.*, 1988; Kuroczkin *et al.*, 1988; De la Torre *et al.*, 1991). In fact, the cyanobacteria and algae, when dead, become a nutrient source for heterotrophic microorganisms. Furthermore, heterotrophs can live at the expense of the extracellular organic matter synthesized by living cyanobacteria or algae (as in the case of bacteria thriving on the

cyanobacterial sheath). If the biological layer is deep enough, the algae in contact with the stone surface lack enough light and die, becoming the substrate for the growth of fungi and bacteria at that level, where their chemically aggressive action through organic acid production can be more efficient. The water retention properties of the algal layer assures biological activity.

Combination of the observations in both field and laboratory materials may let us describe the process of colonization and development of cyanobacterial-algal communities on the stone. The filamentous cyanobacteria may well be the first colonizers due to their resistance to dessication provided by their developed sheaths. The mobility of their trichomes, as in the case of *M. vaginatus*, lets them spread over the surface; if the porosity of the substrate is high enough, the trichomes can even go inside the first few millimetres of the stone, where they can still live due to their low light requirements. The trichomes escape from their sheaths in their gliding movements, thus leaving empty sheaths that contribute to increased water retention properties of the stone surface. This makes the surface suitable for the colonization of the less drought-resistant algae such as *K. flaccidum*. It forms a surface network of filaments in which a more complex microbial community is developed, where heterotrophic organisms can degrade the organic material produced by primary productivity. The slimy surfaces of these communities favour the adherence of environmental particles (dust, pollen, spores, oil- and coal-fired particles). The inclusion of these materials, together with those derived from the substrate (quartz, calcium carbonate, silicates) and detritus (dead cells, microbial by-products, etc.), into the biogenic layer, gives rise to complex crusts and patinas.

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- Q. In your abstract you state that '... the microbial films ... cause(d) a mechanical deterioration on the colonized materials.' While the result is apparently true that filamentous microbes mechanically removed stone grains, it seems difficult to attribute this as the sole cause. Chemical deterioration, e.g. enzymatic or water dissolution, may be a contributing factor. (Your Fig. 15, from culture experiments, seems to show etched crystals.) What led you to attribute the deterioration to mechanical causes and not chemical?
- A. In fact, there is some evidence of etched crystals in our limestone samples cultured in the laboratory. However, because the experimental procedure (cycles of wetness-dessication) could induce water dissolution of crystals, we cannot attribute it exclusively to microbial deterioration. On the other hand, we know, from culture observations,

that the filaments contract when they are dry. If the cohesion between the filaments and the substrate is higher than the rupture resistance of the filaments, they will break down. In the case of growth on a substrate formed by particles, and the adherence of the substrate layers being lower than the traction tension created by the dry filaments, the grains will be progressively loosened.

- Q. Is the sheath of the microorganisms reported here composed of 'sticky' molecules? If so this could certainly aid in mechanical degradation.
- A. The adhesive properties of the extracellular sheath or mucilage produced by these organisms could be clearly observed by their tendency to adhere to the walls of the flasks where they were cultured. In fact, this material is responsible for soil stability by aggregating soil particles. However, chemical studies of extracellular mucilages of this type of organism are very rare. Flaibani *et al.* (1989) have identified two main components in this material: a polypeptide moiety and a complex glycuronoglycan. The hydrophilic protein-free glycuronoglycans are not adhesive unless substituted with hydrophobic groups. The polypeptide moieties diminished the solubilities of the glycuronoglycans to which they were attached, and they contained 35–40% of hydrophobic aminoacid residues. Thus, these authors considered that the presence of hydrophobic polypeptide moieties played a major role in binding the hydrophilic glycuronoglycans to inert surfaces [Flaibani, A., Olsen, Y. & Painter, T. J. (1989). Polysaccharides in desert reclamation: compositions of exocellular proteoglycan complexes produced by filamentous blue-green and unicellular green edaphic algae. *Carbohydrate Research*, **190**, 235–481.
- Q. If you were to try and remove or control the microbes on these stones would you target treatments to certain taxa? If so which?
- A. In preliminary laboratory experiments we have observed that algae can use biocide formulations based on urea-derivatives as a nitrogen source. The algae *Coccomyxa*, *Bracteacoccus* and *Klebsormidium* grew better with very low doses of biocides (Diuron, Metabenzatiazuron and Simazine) than the controls. This means that, if the treatment is not repeated, these compounds may stimulate recolonization, when a favourable concentration is reached on the stone surface. Nevertheless, the response of different species to a given treatment may be different, as, for example, not all cyanobacteria are capable of using urea as nitrogen source. Therefore the choice of appropriate treatments should be made after considering different factors, such as the specific response of the taxa to the treatment, the type of

building material, etc. There are no universal treatments with optimal results in all cases, one of the reasons being the different responses of the taxa.

- Q. Were you able to determine a rate of stone loss in your stone + micro-organism culture experiments?
- A. The systematic removal of microorganisms and loosened grains from the colonized stones is a rather difficult technical problem, if a certain degree of reproducibility is desired when estimating stone loss. We are currently working on standardization of the method.