Chapter 5 Microbial Deterioration of Stone Monuments–An Updated Overview

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CHAPTER 5

Microbial Deterioration of Stone Monuments—An Updated Overview

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Abstract Cultural heritage monuments may be discolored and degraded by growth and activity of living organisms. Microorganisms form biofilms on surfaces of stone, with resulting aesthetic and structural damage. The organisms involved are bacteria (including actinomycetes and cyanobacteria), fungi, archaea, algae, and lichens. Interactions between these organisms and stone can enhance or retard the overall rate of degradation. Microorganisms within the stone structure (endoliths) also cause damage. They grow in cracks and pores and may bore into rocks. True endoliths, present within the rock, have been detected in calcareous and some siliceous stone monuments and are predominantly bacterial. The taxonomic groups differ from those found epilithically at the same sites. The nature of the stone substrate and the environmental conditions influence the extent of biofilm colonization and the biodeterioration processes. A critical review of work on microbial biofilms on buildings of historic interest, including recent innovations resulting from molecular biology, is presented and microbial activities causing degradation are discussed.

I. INTRODUCTION

A large percentage of the world's tangible cultural heritage is made from stone, and it is slowly but irreversibly disappearing. It has been calculated that, for limestone, an average of 1.5–3 mm of rock will erode away in 100 years in temperate climates, leading to the the disappearance of inscriptions on tombstones in the United Kingdom within 300 years (D. Allsopp, personal communication). The transformation of stone into sand and soil is a natural recycling process, essential to sustain life on earth. However, the deterioration of stone monuments represents a permanent loss of our cultural heritage.

Many different types of stone have been used by artists over the years. The most common are marble and limestone, of the calcareous type, sandstone (which is mainly quartz, feldspar, and iron oxide) and granite (mainly quartz and feldspar), of siliceous type. These differ in hardness, porosity, and alkalinity, properties that affect their susceptibility to biodeterioration.

These stone types are not discrete; there is an overlap between calcareous and siliceous rocks, with types such as calcareous sandstone, or siliceous limestone, existing. In addition, the materials often used to stabilize the building blocks (mortar) and to coat the surface prior to painting (plaster or stucco) could be considered. These are human-made and very variable in composition, sometimes even containing high levels of organic materials; they are generally extremely susceptible to

biodeterioration, as is the modern stone substitute, concrete. They will not be included in this review.

Damage to stone caused by microorganisms is often referred to as bioweathering but better called biodeterioration (Gorbushina and Krumbein, 2004); it is the least understood of degradation mechanisms. It was reviewed most recently by Warscheid and Braams (2000) and Gorbushina and Krumbein (2004) in general overviews on biodeterioration of stone, Kumar and Kumar (1999), who reported on biodeterioration of stone in the tropics, and Urzi (2004), who examined these processes in the Mediterranean. Only within the last two decades has it received serious attention from conservators and conservation scientists (Price, 1996; Schnabel, 1991). A thorough understanding of the factors and mechanisms involved in microbial biodeterioration is essential to develop appropriate methods for its control.

II. MICROBIAL ECOLOGY OF OUTDOOR STONE SURFACES

The microflora of external stone surfaces represents a complex ecosystem, which includes not only algae, bacteria, fungi, and lichens, but also protozoa; in addition, small animals, such as mites, may be present and lower and higher plants may develop, once the earlier colonizers have conditioned the surface.

Stone inhabiting microorganisms may grow on the surface (epilithic), in more protected habitats such as crevices and fissures (chasmolithic), or may penetrate some millimetres or even centimetres into the rock pore system (endolithic) (Garcia-Vallès *et al.*, 1997; Golubic *et al.*, 1975; Saiz-Jimenez *et al.*, 1990; Tiano, 2002; Wolf and Krumbein, 1996). They can be found in environments as far apart as the Antarctic (Hirsch *et al.*, 2004) and the (sub)tropics (Althukair and Golubic, 1991; Chacón *et al.*, 2006; Golubic *et al.*, 2005). Endoliths have been classified in more detail by Golubic *et al.* (1981), according to their presence in cracks (chasmoendoliths), pores (cryptoendoliths), or as euendoliths if they show a true boring ability in the stone matrix. The endolithic communities of limestone monuments have been shown, both by culture (Ortega-Morales *et al.*, 2005) and by molecular biology (McNamara *et al.*, 2006) techniques, to be different from those on the surface. These differences may be explained, at least in part, by the protective role of epilithic growth, inorganic matter, and superficial stone layers protecting against incident UV radiation (Cockell *et al.*, 2002), and varying availability of nutrients. It is likely that these microbial communities are also different at the functional level, since increased exposure to UV radiation induces the synthesis of protective pigments (Ehling-Schultz *et al.*, 1997). Warscheid

et al. (1996) considered that, whereas microorganisms in moderate climates tend to colonize the surface of stones, their tropical and subtropical counterparts prefer to penetrate deeper into the rock profile in order to protect themselves from sunlight and desiccation. Matthes-Sears *et al.* (1997), however, suggested that organisms are driven to become endolithic not for protection, but in the search for increased nutrients and space (lack of competition). As endoliths in natural carbonate rocks are rarely associated with catastrophic failure, their slow growth rates within the rock would lead them to have a relatively stable life for considerable periods (Hoppert *et al.*, 2004).

Microbial colonization generally initiates with a wide variety of phototrophic microorganisms (mainly cyanobacteria and algae). These accumulate biomass, usually embedded in a biofilm enriched with organic and inorganic substances and growth factors (Tiano, 2002; Tomaselli *et al.*, 2000b). Lichens probably follow these on the stone surface (Hoppert *et al.*, 2004). The accumulation of photosynthetic biomass provides an excellent organic nutrient base for the subsequent heterotrophic microflora. Ortega-Morales *et al.* (1999) showed that the C/N ratio of biofilm samples taken under different microclimatic conditions approximated to that of microbial cells (~4), indicating that the main source of organic matter is the biofilm itself. However, the establishment of heterotrophic communities on rocks is possible even without the pioneering participation of phototropic organisms and may in fact facilitate the subsequent growth of photosynthetic populations (Roeselers *et al.*, 2007). In this case, organic substrates from various sources are used, including airborne particles and organic vapors, organic matter naturally present in sedimentary rock (usually between 0.2% and 2%), excreted organic metabolic products and biomass from other organisms, together with synthetic or natural organic substances from previous restoration treatments (Gorbushina *et al.*, 1996; Warscheid and Braams, 2000). Highly degraded stone surfaces, with subsequent alteration of the physical condition of the rock, provide appropriate conditions (a ''proto-soil'') for the germination of reproductive structures from higher organisms such as cryptogams (mosses and ferns) and higher plants (Tiano, 2002).

Restoration treatments can, indeed, increase microbial colonization when carried out by workers with no microbiological knowledge. Caneva and Nugari (2005) showed that a consolidant made from local plant mucilaginous (carbohydrate-like) extracts (Escobilla), used at the Mayan site of Joya de Ceren, El Salvador, supported the growth of fungi and, particularly, actinomycetes; its use should be critically evaluated. Other treatments, such as simple cleaning with water, have also been shown to exacerbate microbial growth (Young, 1997).

Biodeterioration processes are rarely caused by one distinct group of microorganisms, but are rather an interaction of coexisting groups.

Table 2 shows a list of those that have been detected on stone monuments; the functional microbial groups are discussed later in more detail.

A. Molecular biology in the study of epi- and endo-lithic microorganisms

Our knowledge on the extent of the diversity of the microbial microflora is far from complete, since traditional culture techniques isolate less than 1% of the microbial community (Ward *et al.*, 1990). In recent years, molecular methods have been developed that allow the identification and, to some extent, enumeration of microorganisms in environmental samples (Amann *et al.*, 1995). Techniques such as denaturing gradient gel electrophoresis (DGGE), single strand conformational polymorphism (SSCP), and fluorescent *in situ* hybridization (FISH), point to the possibility that halophilic or alkanophilic eubacteria and archaea are also involved in stone decay (McNamara *et al.*, 2003; Ortega-Morales *et al.*, 2004; Roelleke *et al.*, 1998; Saiz-Jimenez and Laiz, 2000). These extremophiles had not previously been isolated from stone monuments and thus never considered to play a role in their biodeterioration.

Further approaches employing molecular identification techniques have resulted in the identification of previously unknown species of bacteria, including some actinobacteria, and of organisms such as the Acidobacteria, a practically unknown division of bacteria that is widely distributed in a large variety of ecosystems (Heyrman, 2003; McNamara *et al.*, 2006; Saarela *et al.*, 2004; Salazar *et al.*, 2006; Zimmermann *et al.*, 2005). In addition to the Acidobacter group, other rare microorganisms have been detected on historic buildings. Ortega-Morales *et al.* (2004), using SSCP, showed that a pink-stained area of an external wall at the Mayan site of Uxmal contained predominantly bacteria related to the Actinobacteria genus, *Rubrobacter*. These authors also showed, for the first time, putative halophiles of the genera *Halothece* and *Salinibacter*, along with photosynthetic bacteria related to the *Ectothiorhodospiraceae*. The occurrence of this latter group expands our knowledge of the microorganisms that may contribute through their autotrophic metabolism to the fixation of carbon in these terrestrial ecosystems.

Most of the bacteria identified by molecular biology have not been cultured and their role in the ecology of stone surfaces is not understood (Schabereiter-Gurtner *et al.*, 2003). Even less is known about the role of archaea in biodeterioration and conservation, but recent research sheds light on this microbial group. The archaeal species, *Halobacillus trueperi* has been shown to participate in the mineralization of carbonates *in vitro* (Rivadeneyra *et al.*, 2004), and this may be the first indication of the importance of previously uncultured microorganisms in stone deterioration.

There are many practical problems with community analysis using molecular biology methods involving DNA. These include, for example, selective extraction of DNA from different microorganisms, selective amplification in the PCR, lack of amplification of low levels of DNA in a mixture, and interference in the reaction by environmental materials such as polysaccharides or stone constituents. Nevertheless, it has been clearly demonstrated that organisms found by culture and those detected by sequencing methods are not the same. Rölleke *et al.* (1996) identified relatives of the genera *Halomonas*, *Clostridium*, and *Frankia* in an ancient mural painting; these were not detected by culture, which showed the presence of bacteria such as *Bacillus*, *Micrococcus*, and *Arthrobacter*, not detected by the molecular techniques. New strains of the actinomycete genus *Arthrobacter* were detected by a polyphasic study, including molecular analyses, in the internal biofilms on Servilia's tomb, Carmona, Spain, and St. Catherine's chapel, in the Castle of Herberstein, Austria (Heyrman *et al.*, 2005).

Laiz *et al.* (2003) showed that the majority of bacteria detected by culture from artificially inoculated building materials were spore-formers, while a much greater diversity was apparent using the culture-independent technique of DGGE and sequencing. McNamara *et al.* (2006) found a very wide range of bacteria in and on limestone from the Mayan archaeological site of Ek' Balam, Mexico, using total DNA extraction from samples, PCR with 16S rDNA primers and cloning. Although they did not attempt to culture the organisms, comparison with other publications on similar sites indicate that many more, and different, bacterial groups were detected by this method. Using a combined approach of phospholipid fatty acid markers and SSCP profiling, Ortega-Morales *et al.* (2004) determined that the main colonizers in most biofilms at another Mexican Mayan site, Uxmal, were cyanobacteria of the *Pleurocapsales* group, although *Bacillus carboniphilus* was particularly abundant in internal sites (more dense biofilms) and *Rubrobacter*-related bacteria on external surfaces (higher UV radiation). The dangers of relying on only DNA analysis for evaluation of the cyanobacterial microflora were pointed out by Gaylarde *et al.* (2004, 2005) and Chacón *et al.* (2006). Without any doubt, polyphasic detection methods are essential to determine the true nature of epilithic and endolithic communities.

B. Effect of climate and substrate on microflora

Apart from the microorganisms present in the immediate environment, many factors influence the deterioration of stone. Physical, chemical, and biological agents act in associations ranging from synergistic to antagonistic. The physical properties of the stone influence the extent of degradation. For microbial growth, for example, rough surfaces and high

porosity favor adhesion and colonization (Caneva *et al.*, 1991; May *et al.*, 2003; Warscheid and Braams, 2000). Environmental pollution, which has increased rapidly within the last century (Wright, 2002), may influence stone degradation directly (e.g., acid rain) or indirectly, by supplying nutrients for microbial growth. It has been shown to enhance detrimental microbial activity on the stone substrate (Herrera and Videla, 2004; Mitchell and Gu, 2000; Sand *et al.*, 2002). Monuments that have survived thousands of years as relicts of extinct cultures have experienced accelerated aging in recent years (Gaylarde and Morton, 2002).

The total properties of a substrate that determine its ability to be colonized by microorganisms have been termed its bioreceptivity (Guillitte, 1995). Although this concept is more used in the engineering field, it could be of interest for heritage conservation, to allow an understanding and assessment of materials to be used in restoration. Prieto and Silva (2005) published a set of simple and well-established methods for assessing bioreceptivity; abrasion pH, bulk density, open porosity, and capillary water. The group is now working on a quantitative method to compare visual observations of surface biogenic color changes on stone (Prieto *et al.*, 2006).

Qualitative and quantitative attributes of the colonizing microflora are strongly influenced by the properties of the stone substrate (Warscheid *et al.*, 1996), and it is well known that different kinds of lichens prefer either calcareous or siliceous rocks (Allsopp *et al.*, 2003). Some empirical evidence for the effect of substrate on microbial colonization comes from studies on natural biofilms on various types of building surfaces (Gaylarde and Gaylarde, 2005). It has also been shown that pollutants deposited on the stone surface from the atmosphere can affect microbial colonization and degradation (Zanardini *et al.*, 2000).

The influence of the chemical composition of stone on general microbial colonization remains unclear; however, the condition of the stone has been said to influence the microflora. May *et al.* (2000) reported that filamentous bacteria were almost never isolated from sound stone in temperate climates, whereas actinomycetes of the genera *Streptomyces*, *Micromonospora*, and *Microphylospora* were the dominant organisms on decayed stone. There is no empirical information on whether these organisms are the cause or the result of the damage, although Mansch and Bock (1998) suggested, with indirect evidence, that colonization of sandstone by nitrifying bacteria is accelerated by chemical weathering.

Published data on the distribution of different taxa of photosynthetic microorganisms do not indicate a clear relationship between the organisms present and stone composition (Tomaselli *et al.*, 2000b) and the major influence is considered to be climate, rather than substrate (Gaylarde and Gaylarde, 2005; Tiano *et al.*, 1995). Tropical and subtropical climates enhance the destructive activity of microorganisms, while in moderate climates air pollution significantly supports microbial biodeterioration.

According to Warscheid (2003), moderate climates with regular rainfall tend to give rise to a mixed consortium of microorganisms on exposed stone surfaces, whereas semiarid climates, with less rain and higher temperatures, support the growth of more specialized microorganisms such as cyanobacteria, black yeasts, and lichens, which tend to dominate the microflora. In arid zones, the detrimental influence of microorganisms is low. Instead a ''rock-varnish'' is formed, mainly by cyanobacteria and mineral-oxidizing fungi (Gorbushina and Krumbein, 2000; Krumbein and Giele, 1979; Krumbein and Jens, 1981). The highest degree of biodeterioration occurs in the tropics, because of high humidity and temperatures. The stone microflora here is considered to be very aggressive, with a high capacity for ''biocorrosion'' (more properly called ''bioerosion'') and biofouling (Warscheid, 2003). These two terms are defined by Warscheid (2003) as: (1) microbially induced or influenced corrosion of materials, altering the structure and stability of the substrate, and (2) the presence of colloidal microbial biofilms on or inside materials, leading to visual impairment and potentially altering the physiochemical characteristics of the substrate. The production of pigments, thick walls, or capsules protects microorganisms from adverse climates; however, their aesthetic damage is severe. Deeply-colored coccoid and filamentous cyanobacteria, which predominate in biofilms on buildings in the hot and humid climates of Latin America (Gaylarde and Gaylarde, 2005), are more frequently present on surfaces of buildings at high altitude in the tropics and subtropics than at lower altitudes (Gaylarde and Englert, 2006; Gaylarde and Gaylarde, 2005; Gaylarde *et al.*, 2004; Fig. 5.1).

Particularly sheltered areas on historic buildings in the United Kingdom have been shown to give rise to rich and homogenous biofilms consisting

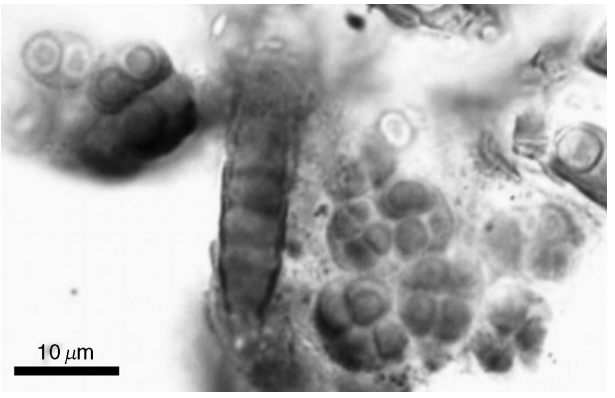

FIGURE 5.1 Mixed coccoid and filamentous cyanobacteria on the external surface of a church in Minas Gerais, Brazil, showing intense pigmentation. This photo is from a rehydrated biofilm. (See Color Plate Section in the back of the book.)

mainly of bacterial rods (May *et al.*, 2003). Biofilms exposed to salt from marine aerosols were of heterogeneous structure with coagulated cells entangling stone particles; whether salting or microbial activity was the main cause of decay is not clear. Exposure to high levels of solar radiation in these temperate climates, with subsequent drying of the substrate, leads to preferential growth of spore forming bacteria, such as *Bacillus* and heat tolerant actinomycetes, over gram-negative bacteria (May *et al.*, 2000). Actinomycetes are frequently found in the more temperate climates of Europe (Palla *et al.*, 2002; Warscheid *et al.*, 1995). Gaylarde and Gaylarde (2005) suggested that they are more common on external surfaces in these milder conditions, whereas in the hot and humid tropics and semitropics, they seem to prefer the interiors of buildings, or to grow as endoliths. However, actinomycetes have also been reported on surfaces in areas of hot climate (Hyvert, 1966; Ortega-Morales *et al.*, 2004), the genus *Geodermatophilus* apparently being common in calcareous stone (Eppard *et al.*, 1996).

Tayler and May (1991) reported seasonal changes in the microbial community of sandstone from ancient monuments in the United Kingdom, with higher bacterial numbers in winter and early spring than in summer and early autumn. Seasonal climate changes tend to result in higher numbers and greater diversity of gram-positive bacteria during summer months in temperate climates. In the warmer Mediterranean climate of Crete, no seasonal changes were observed in heterotrophic bacteria. In this geographical area, the location of the exposed surface (sheltered or not) seemed to play a more significant role than climate change (May *et al.*, 2000). Tomaselli *et al.* (2000a) also reported that there were few seasonal changes in the composition of photosynthetic populations on marble statues from different locations in Italy. Quantitative, rather than qualitative, differences were found, higher numbers of photosynthetic microorganisms being detected during summer months.

Wollenzien *et al.* (1995) showed qualitative differences in fungi occurring on calcareous stone in the Mediterranean. During periods of higher humidity and less sunshine, rapidly growing mycelial fungi, particularly of the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Phoma*, and *Ulocladium*, were dominant, but they were rarely found during the dry season. Nitrifying bacteria, which are known to be highly dependent on the water regime, have been found to be more abundant in indoor environments and during the rainy season at the archaeological site of Uxmal (Ortega-Morales, 1999).

III. MECHANISMS OF MICROBIAL BIODETERIORATION

The detrimental effects of microorganisms may be aesthetic, biogeochemical, and/or biogeophysical. Microbial cells may contribute directly to the deterioration of stone by using it as a substrate or indirectly by imposing

physical stress, serving as nutrients for other organisms, or providing compounds for secondary chemical reactions (Sand, 1996). May (2003) stated that the intimate association of microorganisms with the mineral substrate may reach more than 3 cm deep into the stone, while Wolf and Krumbein (1996) reported microbial contamination in highly degraded, fine grained marble to a depth of 20 cm. These may not have been active boring microorganisms, but such organisms do exist, although their mechanisms of penetration are not fully understood (Salvadori, 2000).

A. Biofilms

Surface biofilms are microbial cells embedded in extracellular polymeric substances (EPS). The simple presence of a biofilm has aesthetic, chemical, and physical effects on the stone. EPS, produced by the cells to allow their adhesion to a given surface, facilitate entrapment of airborne particles, aerosols, minerals, and organic compounds, increasing the dirty appearance of the substrate (Kemmling *et al.*, 2003). Biofilms are areas of high metabolic activity, where digestive enzymes excreted by microorganisms are concentrated. Kemmling *et al.* (2003) found that the EPS in a biofilm from the Market Gate of Miletus (Pergamon Museum) protected cell enzymes against repeated desiccation and rehydration cycles, thus offering the organisms within the biofilm a distinct advantage over nonembedded cells on external surfaces.

Microbial EPS are polymers containing predominantly a range of mainly anionic sugar molecules (but also pigments, proteins, nucleic acids, and lipids) exhibiting several types of functional groups, some of which are capable of binding cations in solution (Moran and Ljungh, 2003). Calcium can be leached from limestone surfaces, or chelated, once solubilized from the matrix, by hexuronic acids, carbonyl, and hydroxyl groups (Ortega-Morales *et al.*, 2001; Perry *et al.*, 2004). Ortega-Morales *et al.* (2001) found higher levels of hexuronic acids in EPS directly extracted from degraded limestone surfaces at Uxmal than on sound stone blocks that were heavily colonized by cyanobacterial biofilms. These molecules may have mediated the deposition of carbonate minerals around coccoid cells, previously demonstrated by SEM (Ortega-Morales *et al.*, 2000).

EPS also act as a physical barrier that protects microorganisms from detrimental substances, such as biocides, and prevents the penetration of conservation materials. The formation of biofilms intensifies microbial attack by weakening the mineral lattice through repeated wetting and drying cycles and subsequent expansion and contraction (Warscheid *et al.*, 1996). They may change the pore size, dry density, water content, surface hardness, and weight of the stone and act as a permeability block for the evaporation of humidity within the stone (May, 2003; Papida *et al.*, 2000).

Biofilms have a lower thermal conductivity than stone, which may lead to uneven heat transfer within the artifact (Dornieden *et al.*, 1997, 2000; Warscheid and Braams, 2000). However, EPS from biofilms have also been reported to have a certain protective nature, owing to a consolidation effect (Kurtz, 2002). Microbial polysaccharides and other naturally occurring biopolymers of various chemical compositions have been shown to inhibit dissolution under certain conditions (Papida *et al.*, 2000; Welch and Vandevivere, 1994).

B. Discoloration

Discoloration is mainly an aesthetic problem (Fig. 5.2). It may be caused by pigments released from, or contained within, the microorganisms (melanins, carotenes, and photosynthetic pigments). Figure 5.3 shows carotene-packed cells of the alga *Trentepohlia umbrina* on a pink-stained limestone surface at the Mayan site of Edzna, Mexico. Crushed samples of stone showed that the calcite crystals had taken up the orange stain (Gaylarde *et al.*, 2006).

Sulfur cycle bacteria can convert limestone into gypsum, common especially in sulfur-polluted environments. This can lead to the formation of dark surface colorations, even when the normally responsible fungi and cyanobacteria are not present. Dark discolorations may also be due to airborne particles trapped in EPS.

FIGURE 5.2 Black biofilms on the surface of the Ambar Fort in Jaipur, India.

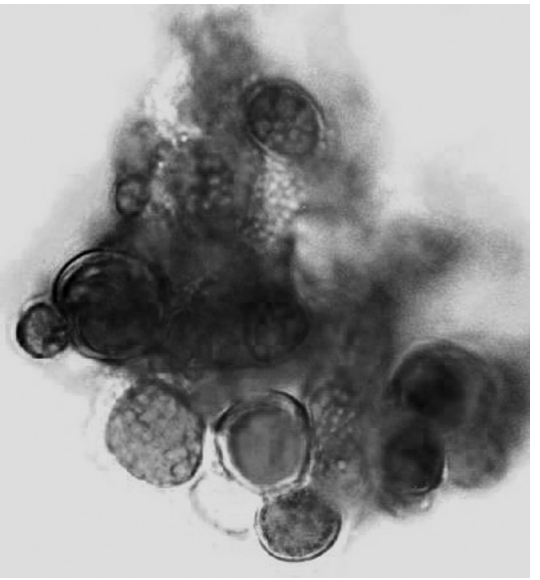

FIGURE 5.3 A pink stained limestone surface in Edzna, Mexico (a). The microscopic image shows bright orange (carotene-packed) cells of *T. umbrina*. (See Color Plate Section in the back of the book.)

Discoloration is not, however, purely aesthetic. Discolored areas may absorb more sunlight, which increases physical stress by expansion and contraction caused by temperature changes (Sand *et al.*, 2002; Warscheid, 2000). Temperatures on darkly stained areas of stone have been shown by Garty (1990) to differ by as much as 8 °C from lighter-colored areas. This effect has been shown experimentally by Carter and Viles (2004), using limestone blocks with and without a lichen covering. Surface temperature change and thermal gradients were greater below the lichen. The darkening of the stone surface decreases its albedo, so that it experiences increased heating/cooling and wetting/drying cycles, causing stresses within the stone (Warke *et al.*, 1996).

A special example of this is the so-called ''black crust.'' Thick (several mm) crusts, such as those found on buildings in Aberdeen, Scotland (Urquart *et al.*, 1996), or on Seville cathedral, Spain (Saiz-Jimenez, 1995), are rich in Ca, Si, and C (Wright, 2002). The carbon component may be composed of hydrocarbons, deposited from vehicle exhausts, and microorganisms within the biofilm may be able to utilize these molecules as nutrients (Saiz-Jimenez, 1995). On the other hand, the more commonly seen thin black crusts (Fig. 5.4) seem to be predominantly cyanobacterial (subtropics/tropics) or fungal (moderate climate) in composition (Gaylarde and Englert, 2006; Gaylarde *et al.*, 2007; Pattanaik and Adhikary, 2002), and are enriched in Si and Fe (Gaylarde and Englert,

FIGURE 5.4 A thin black crust on Campeche cathedral, Mexico. Smaller photo shows close-up. Dark brown branching filamentous cyanobacteria were the main component (authors' unpublished observations).

2006; Wright, 2002). Apart from their aesthetic effects, these crusts also block pores within the stone. This can result in water retention and subsequent spalling of the surface, although it is also possible that they protect the stone by reducing water infiltration (Garcia-Vallès *et al.*, 1997).

C. Salting

Salting, the production of efflorescences, involves secondary minerals produced through reaction of anions from excreted acids with cations from the stone (Fig. 5.5); this mechanism is related to that discussed above. The damage caused by such salts is mainly of a physical nature, leading to blistering, flaking, scaling, and granular disintegration, and this may often be the main mechanism of stone decay (Wright, 2002). Hydration and subsequent swelling of a salt molecule within a small stone pore may cause cracking. During desiccation, the salts crystallize, leading to an increase in volume (Ortega-Morales *et al.*, 2005). At low temperatures, hydration of salts increases the water content and may lead to mechanical damage through ice crystal formation. Salts of biotic or abiotic origin may also increase the production of EPS and biofilm density

FIGURE 5.5 Salting on the internal walls of the tomb of Servilia, Carmona, Spain.

(May, 2003; Papida *et al.*, 2000; Sand, 1996; Sand *et al.*, 2002). Areas of efflorescence present a niche for halophilic/tolerant microorganisms, for example, several Archaea. These specialized microorganisms may have synergistic action with the salts and therefore severely enhance the physical and chemical deterioration processes (May, 2003; Papida *et al.*, 2000).

D. Physical damage

Physical damage may be caused by penetration of filamentous microorganisms (particularly fungal hyphae) into the stone (Hirsch *et al.*, 1995a). Many cyanobacteria, not necessarily filamentous, have also been shown to have this ability. Weakened areas of the stone will be affected first. Danin and Caneva (1990) proposed that calcareous stone is decayed by cyanobacteria by attachment of cyanobacterial cells in small fissures and growth within these fissures. This is followed by water uptake and expansion of cell mass, exerting pressure within the structure, precipitation of carbonates and oxalates around the cells, opening of the fissure with subsequent entry of dust, pollen, grains, etc. and death of some cyanobacterial cells, allowing the establishment of heterotrophic bacteria, fungi, and small animals such as mites. The final increased internal pressure on the superficial layer of the structure leads to its detachment (spalling).

E. Inorganic acids

Inorganic acids, mainly nitric acid (HNO_3) and sulfuric acid (H_2SO_4), but also carbonic acid (H_2CO_3), sulfurous acid (H_2SO_3), and nitrous acid (HNO_2) may dissolve acid-susceptible materials, with the production of

substances more soluble in water (calcium sulfate, nitrates, and calcium hydrogen carbonate). This leads to weakening of the stone matrix. This dissolution action proceeds through the depth of the stone (Sand, 1996; Sand *et al.*, 2002). Sulfuric and sulfurous acids are predominantly produced by *Thiobacillus*, as well as by *Thiothrix*, *Beggiatoa*, and some fungi. The involvement of sulfur-oxidizing bacteria in the degradation of sandstone was first proposed in 1904, but later workers, in Germany, did not find these bacteria on natural stone buildings (Bock and Krumbein, 1989), leading to doubts about their role in biodegradation.

Nitric and nitrous acids are produced by ammonia and nitrite oxidizers, heterotrophic nitrifiers, and some fungi. Endolithic nitrifying bacteria were the first microorganisms to be proposed as the cause of stone decay, in 1890 (Muntz, 1890). However, since most natural stone is alkaline in reaction, nitrification will result in the production of nitrate and not nitric acid; thus acid attack is unlikely (Gaylarde and Gaylarde, 2004) and, indeed, Mansch and Bock (1998) suggest that acidic breakdown of stone is required prior to colonization by these bacteria.

Carbonic acid is produced by all living organisms as an end product of energy metabolism after the reaction of CO_2 with water (Sand, 1996). It is a weak acid, however, and unlikely to contribute greatly to stone degradation, especially in calcareous stone, where it will react to form calcium bicarbonate, a weak alkali buffer.

F. Organic acids

Organic acids (e.g., oxalic, citric, acidic, gluconic, malic, succinic acid, but also amino acids, nucleic acids, uronic acids, etc.) may react with the stone, solubilizing it via salt formation and complexation (Sand *et al.*, 2002; Torre de la *et al.*, 1993). Complexation, or chelation, is not, of course, ''acid degradation'' and pH values may not be reduced. Almost all microorganisms can excrete organic acids, especially when growth is unbalanced (Sand, 1996); perhaps the most frequently mentioned is oxalic acid, produced by fungi and lichens, mainly as the monohydrate form (whewellite), but also the dihydrate (weddelite). Polyfunctional organic acids, such as oxalic, have been shown to enhance the dissolution of siliceous rocks (Bennett *et al.*, 1988), but they may have a protective role in calcareous rock through the formation of calcium oxalate (Di Bonaventura *et al.*, 1999) or malonate (Salinas-Nolasco *et al.*, 2004) films. The brown/yellow oxalate films on stone surfaces are known as ''time patinas'' and are regarded by many as an attractive feature, enhancing the aged appearance of a monument. They may, or may not, be biogenically produced. However, polyfunctional organic acids such as oxalic have been shown to enhance the dissolution of siliceous rocks (Bennett *et al.*, 1988). The ''biomineralization'' caused by this metabolic activity has been documented

using transmission and scanning electron microscopy in association with electron dispersion spectroscopy (Ascaso *et al.*, 2002; De los Rios and Ascaso, 2005; De los Rios *et al.*, 2004).

G. Osmolytes

Osmolytes are a diverse group of polyol substances (which includes glycerol, other sugars and polysaccharides) produced in response to changes in water activity; they are protectants against freezing, excessive heat and drying, salts, acids, alkalis, and factors such as ethanol. They have been reported in all life forms except protozoa, myxomycetes, and some simple animals. Under alkaline conditions (the majority of stone types), polyols degrade siliceous rock by binding to the crystalline layers, causing expansion, and by enhancing the solubility of organosilicon compounds (Gaylarde and Gaylarde, 2004).

It must be pointed out that much of the above is speculative. There is only little experimental evidence, for example, for the production of acids *in situ* and acidic polysaccharides on the stone surface; most investigators that demonstrated the ability of stone-colonizers to produce acids have tested this in artificial media in the laboratory.

IV. MICROORGANISMS DETECTED ON HISTORIC MONUMENTS

A list of microorganisms present on and in the stone of historic monuments, found in the literature, is given in Table 5.1.

A. Phototrophic microorganisms

Cyanobacteria and algae, as phototrophs, do not require organic material for their growth. They can form biofilms and crusts on stone surfaces, which, depending on the environmental conditions and the predominant strains, can be black, grey, brown, green, or red. Under wet conditions, such biofilms tend to be green, while when dry they are grey or black (Ortega-Morales *et al.*, 2004). This does not mean that the organisms within dry biofilms are dead; indeed, cyanobacterial biofilms have been stored for years in dry and dark conditions and remained viable (our unpublished observations and Gaylarde *et al.*, 2006). It has been shown that certain cyanobacteria, such as *Chroococcidiopsis*, the most dessication-resistant cyanobacterium known (Potts and Friedmann, 1981), regain photosynthetic activity within minutes when rehydrated (Hawes *et al.*, 1992).

Green algae are found mainly in damper areas. Their contribution to biodegradation has not been researched thoroughly and is considered to

TABLE 5.1 Microorganisms detected on stone monuments

Microbial group	Family/genus/species	References
Algae	*Apatococcus*, *Asterococcus*, *Cladophora*, *Chlorella*, *Chlorococcum*, *Coccomyxa*, *Chrysocapsa*, *Cyanidium*, *Dimorphococcus*, *Eustigmatos*, *Fragilaria*, *Gongrosira*, *Heterococcus*, *Hormidium*, *Klebsormidium*, *Muriella*, *Nanochlorum*, *Navicula*, *Nitzschia*, *Planktospheria*, *Pleurococcus*, *Protococcus*, *Protoderma*, *Rhizothallus*, *Stichococcus*, *Trentepohlia*, *Ulothrix*.	Crispim *et al.* (2003), Flores *et al.* (1997), Gaylarde *et al.* (2001), Ohba and Tsujimoto (1996), Ortega-Morales *et al.* (2000, 2005), Strzelczyk (1981), Tiano (2002), Tiano *et al.* (1995), Tomaselli *et al.* (2000a,b).
	Apatococcus lobatus, *Botryochloris minima*, *Chlorella vulgaris*, *C. elipsoidea*, *Monodus unipapilla*, *Oocystis parva*, *O. marssoni*, *Protococcus viridis*, *Stichococcus baciliaris*, *T. umbrina*, *Ulothrix punctata*.	Canela *et al.* (2005), Gaylarde *et al.* (2006), Hoppert *et al.* (2004), Ohba and Tsujimoto (1996), Strzelczyk (1981), Tomaselli *et al.* (2000a).
Cyanobacteria	*Arthrospira*, *Calothrix*, *Chlorogloeopsis*, Chroococcales, *Chroococcidiopsis*, *Chroococcus*, *Fischerella*, *Geitlerinema*, *Gloeocapsa*, *Gloethece*, *Hyella*, *Leptolyngbya*, *Lyngbya*, *Mastigocladopsis*, *Microcoleus*, *Myxosarcina*, *Nodularia*, *Nostoc*, Oscillatoriales, *Phormidium*, *Plectonema*, *Pleurocapsa*, Pleurocapsa-group, *Scytonema*, *Stanieria*, Stigonematales, *Synechococcus*, *Synechocystis*, *Tolypothrix*, *Xenococcus*.	Ascaso *et al.* (2002), Crispim *et al.* (2003), Garcia de Miguel *et al.* (1995), Gaylarde and Morton (2002), Gaylarde *et al.* (2001, 2005), Hoppert *et al.* (2004), McNamara *et al.* (2006), Ortega-Morales *et al.* (2000, 2005), Tiano (2002), Tomaselli *et al.* (2000a,b).
	Acaryochloris marina, *Anabaena variabilis*, *Gloeocapsa helvetica*, *G. kuetzingiana*, *G. rupestris*, *Lyngbya matensiana*, *L. aerugineocoerulea*, *Oscillatoria pseudogeminata*, *O. terebriformis*, *O. subtilissima*,	Caneva *et al.* (2005), Gaylarde and Englert (2006), Gaylarde *et al.* (2005), McNamara *et al.* (2006), Strzelczyk (1981).

(*continued*)

TABLE 5.1 (*continued*)

Microbial group	Family/genus/species	References
	Phormidium lignicola, *Stigonema ocellatum*, *S. hormoides.*	
Other photosynthetic bacteria	Chloroflexi.	McNamara *et al.* (2006).
	Rhodoplanes elegans.	McNamara *et al.* (2006).
Archaea	Halophilic bacteria: *Halobacillus*, *Halobacterium*, *Halococcus*, *Halomonas*, *Natronobacterium*	Heyrman *et al.* (1999), Piñar *et al.* (2001), Rölleke *et al.* (1998), Rölleke *et al.* (1996).
	Methanogenic bacteria, methanotrophic bacteria	Kussmaul *et al.* (1998).
Chemolithotrophic bacteria	Nitrogen cycle: *Nitrobacter*, *Nitrococcus*, *Nitrosococcus*, *Nitrosoglobus*, *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio*, *Nitrospira*.	Caneva *et al.* (1991), Gorbushina *et al.* (2002), May (2003), Spieck *et al.* (1992).
	Nitrobacter vulgaris, *Nitrosomonas ureae*, *Nitrospira moscoviensis*.	McNamara *et al.* 2006, Pinck and Balzarotti al. (2000).
	Sulfur cycle: *Thiobacillus*.	Caneva *et al.* 1991, Flores *et al.* (1997), Warscheid and Braams, (2000).
	Thiobacillus thiooxidans, *T. thiosporus*, *T. albertis*, *T. neapolitanus*, *T. denitrificans.*	May (2003), Prieto *et al.* (1995).
Chemoorganotrophic bacteria	Acidobacteria, *Bacillus*, *Clostridium*, *Holophaga*, *Melittangium*, *Pseudomonas*, sulfate-reducing bacteria.	Flores *et al.* (1997), Gaylarde *et al.* (2001), Gorbushina *et al.* (2002), Heyrman and Swings (2001), Kussmaul *et al.* (1998), McNamara *et al.* (2006), Ortega-Morales and Hernandez-

		Duque, (1998), Ortega-Morales *et al.* (2005), Rölleke *et al.* (1996), Saarela *et al.* (2004).
	Bacillus circulans, *B. badius*, *B. licheni*, *B. cereus*, *B. licheniformis*, *B. barbaricus*, *B. thuringiensis*, *B. pumilis*, *B. megaterium*, *B. firmus*.	Blazquez *et al*. (2000), Gaylarde *et al.* (2001), Heyrman and Swings (2001), McNamara *et al.* (2006), Prieto *et al.* (1997).
Actinomycetes	*Arthrobacter*, *Aureobacterium*, *Blastococcus*, *Brevibacterium*, *Clavibacter*, *Geodermatophilus*, *Micrococcus*, *Microellobosporium*, *Micromonospora*, *Microphylospora*, *Modestobacter*, *Nocardia*, *Nocardiodes*, *Rhodococcus*, *Rubrobacter*, *Streptomyces*.	Aranyanak (1992), Bassi *et al.* (1986), Caneva and Nugari (2005), Caneva *et al.* (1991), Flores *et al.* (1997), Gorbushina *et al.* (2002), Heyrman and Swings (2001), Hyvert (1966), McNamara *et al.* (2006), May *et al.* (2000), May (2003), Ortega-Morales *et al.* (2004, 2005), Rölleke *et al.*, (1996), Saarela *et al.*, (2004), Tiano, (2002), Warscheid and Braams (2000).
	Arthrobacter (Micrococcus) agilis, Geodermatophilus obscurus, Kocuria rosea, Marmoricola aurantiacus, M. lylae, M. roseus, M. varians, M. halobius, M. agilis, Nocardia restricta, Saccharothrix flava.	Blazquez *et al.* (2000), Eppard *et al.* (1996), McNamara *et al.* (2006), May (2003), Prieto *et al.* (1995).
Fungi	*Acremonium (Cephalosporium)*, *Alternaria*, *Aspergillus*, *Aureobasidium*, *Botrytis*, *Candida*, *Capnobotryella*, *Cladosporium*, *Coniosporium*, *Cryptococcus*, *Dictyodesmium*, *Exophiala*, *Fusarium*, *Hortaea*, *Lichenthelia*, *Mucor*, *Nectria*, *Penicillium*,	Allsopp *et al.* (2003), Blazquez *et al.* (2000), Caneva and Nugari (2005), Gorbushina *et al.* (2002), Hirsch *et al.* (1995b), Monte (2003), Prieto *et al.* (1995), Tiano (2002), Urzi (2004), Urzi

(*continued*)

TABLE 5.1 (*continued*)

Microbial group	Family/genus/species	References
	Phaeococcomyces, *Phaeosclera*, *Phaeotheca*, *Phoma*, *Phialostele*, *Pseudotaeniolina*, *Rhinocladiella*, *Rhizopus*, *Rhodotorula*, *Sarcinomyces*, *Sporobolomyces*, *Sporotrichum*, *Trichoderma*, *Trimmatostroma*, *Ulocladuim*.	and De Leo (2001), Urzi *et al.* (2000), Warscheid and Braams (2000).
	Acremonium murorum, *A. niger*, *A. versicolor*, *A. wentii*, *Aurobasidium pullulans*, *Capnobotryella renispora*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Coniosporium apollinis*, *C. perforans*, *C. uncinatus*, *Exophiala jeanselmei*, *E. monileae*, *Hortaea werneckii*, *Phialophora melinii*, *Sarcinomyces petricola*, *Trichoderma viride*, *Trimmatostroma abietis*, *Verticillium nigrescens*.	Blazquez *et al.* (2000), Caneva and Nugari (2005), Gorbushina *et al.* (2002), Hoppert *et al.* (2004), Urzi (2004).
Lichens	*Aspicilia*, *Caloplaca*, *Lecanora*, *Protoplastenia*, *Thyrea*, *Verrucaria*, *Xanthoria*.	Ascaso *et al.* (1998, 2002), Tiano (2002).
	Caloplaca aurantiaca, *C. ceria*, *C. citrina*, *C. holocarpa*, *C. trachyphylla*, *C. concolor*, *C. vitellina*, *Collera crispum*, *Diploicia canescens*, *Dirina massiliensis*, *Lecania rabenhorstii*, *Lecanora hageni*, *Ochrolechia parella*, *Phaeophysica hirsute*, *Tephronella atra*	Ascaso *et al.* (1998), Frey *et al.* (1993), Hoppert *et al.* (2004), Prieto *et al.* (1997, 1999), Seaward (2003).

be mainly to promote the growth of other organisms. This is not so for cyanobacteria, whose role in the deterioration of surfaces of historic buildings has been the subject of several recent studies and reviews (Crispim and Gaylarde, 2004; Ortega-Morales *et al.*, 2001; Tomaselli *et al.*, 2000b). These organisms are generally adapted to resist adverse conditions because of their thick outer envelopes and the presence of protective pigments (Chazal and Smith, 1994; Garcia-Pichel *et al.*, 1992). Cyanobacteria are probably the most resistant of the microflora on monument surfaces (with lichens in second place), if we relate this environment to desert crusts (West, 1990). The Atacama and Namib deserts, the most extreme on earth, have crusts in which cyanobacteria are the only phototrophs (Evenari *et al.*, 1985). The ability of cyanobacteria to survive repeated cycles of dehydration and high levels of UV radiation (Chazal and Smith, 1994; Garcia-Pichel *et al.*, 1992; Potts, 1994) makes them particularly important organisms on outdoor stone surfaces. In spite of their resistance to UV, superficial growth of cyanobacteria and algae is stronger on sheltered indoor surfaces of historic limestone buildings, which have reduced illumination, higher humidity, and more organic nutrients (Gaylarde *et al.*, 2001; Ortega-Morales *et al.*, 2000).

Cyanobacteria have been suggested to be of higher ecological importance as pioneer organisms on exposed stone surfaces of buildings than any other organism (Grant, 1982) and may have the most important influence on weathering of exposed stone (Gaylarde and Morton, 2002). Cyanobacteria have been shown to constitute the major biomass on external surfaces of ancient stone structures in Latin America (Gaylarde *et al.*, 2001; Ortega-Morales *et al.*, 2000), Greece (Anagnostidis *et al.*, 1983), and India (Tripathy *et al.*, 1999). In fact, cyanobacteria and eukaryotic algae had been found, until the recent molecular biology study of McNamara *et al.* (2006), to be the most widespread microorganisms in the endolithic habitat (Sigler *et al.*, 2003). The ability to fix carbon dioxide, and in some species atmospheric dinitrogen (N_2), gives the cyanobacteria an obvious advantage over heterotrophic bacteria. Light quality and intensity are the main factors that control the minimum and maximum depth at which endolithic phototrophic communities grow (Nienow *et al.*, 1988).

Apart from their evident aesthetic deterioration of the stone monument, these phototrophs may cause chemical and physical deterioration by the excretion of chelating agents and stone-dissolving acids (Albertano, 2003; May, 2003; Urzi and Krumbein, 1994), as well as by yet undefined boring activity (Carcia-Pichel, 2006), documented for the Pleurocapsa-group (Mao-Che *et al.*, 1996), *Synechocystis*, *Gloeocapsa*, *Stigonema*, *Schizothrix* (Hoffman, 1989), *Scytonema* (Golubic *et al.*, 2000), and *Mastigocladus* (*Fischerella*) (Boone *et al.*, 2001). Ortega-Morales *et al.* (2000) and Gaylarde and Englert (2006) showed scanning electron micrographs that demonstrate coccoid cyanobacteria sitting in cell-sized depressions in the stone

surface, while Gaylarde *et al.* (2006) report the presence of pure colonies of the alga *T. umbrina* within colony-sized pits on limestone. All these images indicate that the cells themselves are causing the degradation.

Photosynthetic organisms deposit $CaCO_3$ in the presence of light and solubilize it at night. The precipitation of calcium salts on cyanobacterial cells growing on limestone suggests the migration of calcium from neighboring sites (Ascaso *et al.*, 1998; de los Rios, 2005; Ortega-Morales *et al.*, 2000; Schultze-Lam and Beveridge, 1994). The external S-layer of *Synechococcus* GL24 binds calcium ions (Schultze-Lam and Beveridge, 1994), which complex with carbonate ions at the pH values (>8.3) produced around the cells (Miller *et al.*, 1990). *Synechococcus* cells can become encrusted with calcite within 8 h in an aqueous environment and must continually shed patches of the mineralized S-layer to remain viable (Douglas and Beveridge, 1998). This mobilization of calcium ions and the trapping of released particles of calcite in the gelatinous sheaths or capsules of cyanobacterial cells (Pentecost, 1987, 1988) are important mechanisms of degradation of calcareous stone. Nitrogen and phosphorus may also be mobilized from the stone and metabolized or stored within the organisms (Albertano, 2003). Warscheid and Braams (2000) also mentioned the possibility that phototrophic organisms take up and accumulate sulfur and calcium into their cells.

Lichens are symbiotic associations between fungi and one or two photobionts, which can be algae or cyanobacteria. They are frequent colonizers of stone monuments and have been mistaken for the remains of ancient rendering when they cover substantial areas of the surface (Seaward, 2003). Lichens are particularly sensitive to air pollution and, indeed, are used as bioindicators of such. It has been suggested that improved air quality has already, or may in the future, lead to an increase in colonization of stone by lichens (Ardron, 2002; Young, 1997). Although many people find lichen growth on stone pleasing, it can be a problem in obscuring fine details of carvings and it is certainly inherently damaging to the structure. Nimis and Monte (1988) reported an interesting effect of lichen growth on the Orvieto duomo (Italy). The alternating dark basalt and light limestone bands have been colonized, respectively, by light and dark, or orange, lichens, completely eliminating the effect desired by the artist on the northern facade.

Lichens cause mechanical damage due to penetration of their rhizines, composed of fungal filaments, and the expansion/contraction of the thallus on wetting/drying, which can lift grains of stone off the surface (De los Rios *et al.*, 2004; Gaylarde and Morton, 2002) (Fig. 5.6). Accumulation of small stone fragments (as small as 5 μm) within the lower thallus have been reported (Gadd, 2007). The depth of penetration depends on the stone substrate and the type of lichen; lichen structures can sometimes be found at least 3 cm below the stone surface (Lee *et al.*, 2003). They also

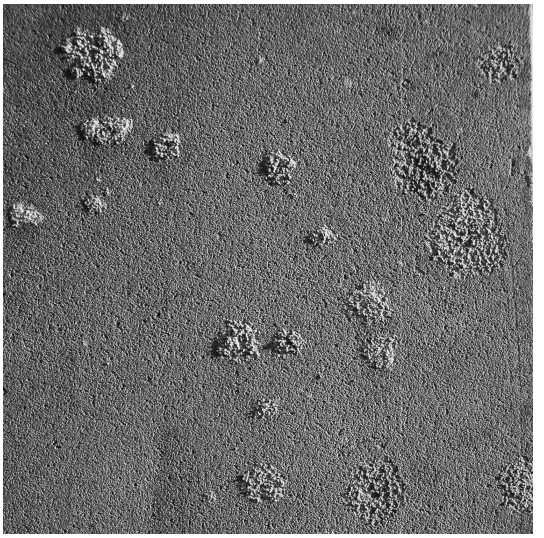

FIGURE 5.6 Damage caused by lichens on a sandstone tomb in Cardiff, Wales.

cause direct chemical attack by the production of significant amounts of acids. ''Lichen acids'' have been shown to cause damage at the stone/lichen interface (Cameron *et al.*, 1997; Seaward, 2003). The principal acid produced is oxalic, which leads mainly to the formation of calcium oxalate and its different hydrate forms whewellite and wedellite (Gaylarde and Morton, 2002; Tiano, 2002). The lichen thallus has been shown to accumulate from 1% to 50% calcium oxalate, depending on the substrate. Even on siliceous stone, some lichens can accumulate this compound, using calcium from the air, or leachates (Seaward, 2003). Lichens on historic stone buildings have been reviewed recently by Lisci *et al.* (2003).

The CO_2 produced by lichens is transformed within the thallus to carbonic acid (Tiano, 2002), which, although a weak acid, seems to be able to solubilize calcium and magnesium carbonates in calcareous stone. Lichens have been demonstrated to biomobilize certain elements from the stone matrix (De los Rios *et al.*, 2004; Tiano, 2002). The former workers demonstrated magnesium-depleted areas of the stone substrate around the lichen thallus. Saxicolous lichens mobilize magnesium and silicon in rock, causing biochemical weathering (Aghamiri and Schwartzman, 2002). Gordon and Dorn (2005) calculated that a saxicolous lichen increased the weathering rate of basalt by a factor of at least 1.7. The weathering was greatest directly under the lichen colony. About 0.5 mm below the colony, weathering rates fell to those of uncolonized surfaces. Banfield *et al.* (1999) proposed a model, based on high-resolution transmission electron microscopy, of the weathering of silicate rocks by lichen

activity. Clear boundaries are shown in the vertical profile, with a direct ''biochemical'' effect first produced, followed by predominantly biophysical action in the deeper layer of material.

Certain lichens can grow endolithically (Gaylarde and Gaylarde, 2005; Gerrath *et al.*, 1995). They are slow-growing, stress-tolerant organisms, which have been stated to have a similar physiology to epilithic crustose lichens (Tretiach and Pecchiari, 1995), and lead to similar destructive effects.

Under conditions of high abiotic weathering, lichens have been suggested to provide protection for the stone surface from wind and rain through the insoluble oxalate layer (Bungartz *et al.*, 2004; Di Bonaventura *et al.*, 1999; Warscheid and Braams, 2000), or to limit erosion by reducing the level of water within the rock (Garcia-Vallès *et al.*, 2003); their retention of moisture within the thallus reduces thermal stress on a limestone surface (Carter and Viles, 2003). However, they are generally defacing and intrinsically damaging. Even when a protective effect can be shown, subsequent decay of the lichen thallus (which occurs in the centre of the colony of some species) can open this area to further weathering, resulting in cratered mounds on the rock surface (Mottershead and Lucas, 2000). The mechanical removal of crustose lichens is particularly difficult because the thallus forms an intimate association with the substrate. Hence, its removal leads to severe structural damage (Allsopp and Gaylarde, 2004; Gaylarde and Morton, 2002).

Recent work based on molecular approaches has shown that, in addition to algae, lichens, and cyanobacteria, other previously unrecognized phototrophic microorganisms may occur in stone monuments. Ortega-Morales *et al.* (2004) found bacteria related to the Ectothiorhodospiraceae in certain samples at the Mayan site of Uxmal, while McNamara *et al.* (2006) detected *Chloroflexi*-related organisms. This new data, added to the already known complex nature of lithic biofilms on historic monuments, indicates that these organisms may contribute to the carbon pool in autotrophic biofilms. It is likely that their role in stone deterioration, as for algae, is supporting the growth of associated heterotrophs, although the production of osmolytes cannot be ruled out. Interestingly, the halophily of these organisms is congruent with the measured levels of salts in some monuments, where significant amounts of sulfate, chloride, and nitrate have been found (Ortega-Morales, 1999; Ortega-Morales *et al.*, 2004, 2005).

B. Chemoorganotrophic microorganisms

The contribution of heterotrophic microorganisms to stone deterioration, particularly as pioneering colonizers, had long been neglected; however, their degradative role by acid/alkali production and by chelation is now well accepted (Gaylarde and Morton, 2002).

1. Fungi

The effects of fungi are due to physical and chemical actions, which are often synergistic in the degradation of stone. They were recently reviewed by Gadd (2007), and will therefore only be mentioned briefly here. The fungal stone flora consists of filamentous fungi (ubiquitous hyphomycetes and coleomycetes) and microcolonial fungi (black yeasts and yeast-like meristematic fungi) (Gorbushina *et al.*, 2002, 2003; May, 2003; Sterflinger, 2000; Urzi *et al.*, 2000). Meristematic fungi produce swollen, isodiameteric cells with thick, melanin containing cell walls. They remain metabolically active even in low nutrient conditions and have high resistance to desiccation, UV radiation, and osmotic stress (Urzi *et al.*, 2000), thus being well adapted to growth on external walls. Wollenzien *et al.* (1995) suggested that these are the resident fungi in Mediterranean climates; the fast growing, filamentous hyphomycetes being present only in the colder and more humid winter months and therefore considered contamination in this climatic area. Hyphomycetes tend to be the major fungal population in more northerly parts of Europe (Sterflinger, 2002). However, the ubiquitous hyphomycetes can also be found in (sub)tropical climates. Resende *et al.* (1996) identified a wide range of filamentous fungi in soapstone and quartzite in churches in the Brazilian state of Minas Gerais. The most common genera were *Cladosporium* and *Penicillium*. However, it must be emphasized that the detection technique affects the results of such investigations.

Gorbushina *et al.* (2002) detected mainly deuteromycetes, such as *Alternaria*, *Cladosporium*, and *Trichoderma*, on historic marble monuments in St. Petersburg and Moscow. Many of the organisms were obviously derived from the surrounding plants. They applied Koch's postulates to two of the isolates and showed that they could grow on and discolor sterile marble blocks.

Sterflinger (2000) indicated *Aspergillus niger*, *Penicillium simplissimum*, and *Scopulariopsis brevicaulis* as important fungi that attack siliceous stone. These dark pigmented mitosporic fungi (''black fungi'') can actively penetrate limestone and marble and produce pits of up to 2 cm diameter on rock surfaces. (Sterflinger and Krumbein, 1997). They are especially important in arid and semiarid environments (hot and cold deserts) because of their ability to resist high temperatures, desiccation, and osmotic stress (Sterflinger, 1998).

In fact, several cryptoendolithic fungi may actively bore into the stone and hence physically disrupt its integrity (Gadd, 2007; Hoffland *et al.*, 2004). Fungi, unlike the phototrophs, do not require light for growth, and so their boring activity can penetrate to greater depths. Golubic *et al.* (2005) discussed such a tunneling activity in carbonate substrates (particularly mollusk shells) in marine environments. Hyphal penetration of materials involves swelling/deflation effects and channeling of water

into the substrate. It can form cracks, fissures, and crevices, extend existing ones and lead to the detachment of crystals (Sterflinger, 2000; Urzi *et al.*, 2000). Weaker areas of the stone will be preferably penetrated by thigmotropism (contact guidance on solid surfaces to explore new substrates; Gadd, 2007; Watts *et al.*, 1998).

Biochemical actions of fungi can lead to microtopological alterations through pitting and etching, mineral dislocation and dissolution (Gadd, 2007). They are associated with extracellular mucilaginous substances, which contain, amongst many other metabolites, acidic, and metal-chelating compounds (Burford *et al.*, 2003). Acidic metabolites (oxalic, acetic, citric, and other carbonic acids) deteriorate the stone minerals by a solubilizing and chelating effect (Sterflinger, 2000; Urzi and Krumbein, 1994). Ortega-Morales *et al.* showed that fungi isolated from deteriorated limestone at the Mayan site of Uxmal, Mexico, produced oxalic acid, which reacted with solubilized calcium from the stone to produce crystals of whewellite and weddelite (unpublished results). Fungal oxalic acid had previously been reported to solubilize metals (e.g., iron, aluminum, lithium, manganese) from various other substrates to form oxalates (Devevre *et al.*, 1996; Strasser *et al.*, 1994). Acidolysis and complexolysis, which have been reported to be the primary deteriorative mechanisms of fungi (Gadd, 2007), act on the stone mineral by proton efflux (plasma membrane H^+-ATPase, maintenance of charge balance during nutrient uptake) and siderophores, which mobilize iron (III), or CO_2 production (Gadd, 2007).

Oxidation and reduction of mineral cations are also triggered by fungal activity (Gadd, 2007). Iron and manganese particularly are removed from the stone lattice by redox processes (Warscheid and Braams, 2000) and may be reoxidized at the stone surface, forming ''patinas'' or ''crusts.'' This biotransfer of metal ions and subsequent formation of patina [called ''rock varnish'' by Krumbein and Jens (1981) and Krumbein and Giele (1979)], can lead to hardening of the surface layer and exfoliation (Tiano, 2002). However, Urzi *et al.* (2000) emphasized that there is no evidence that meristematic fungi produce acids, oxidize manganese, or are directly responsible for the formation of ''rock'' or ''desert varnish.''

Various metabolic substances excreted by fungi are colored, leading to staining of the substrate (Tiano, 2002). The production of melanins by dematiaceous (dark pigmented mitosporic) fungi darkens the stone surface, leading to significant aesthetic alterations, and physical stress.

The literature suggests that fungi are present in low numbers on the surfaces of historic stone buildings. Populations of 10^2–10^5cfu$\cdot g^{-1}$are common (Gaylarde *et al.*, 2001; Hirsch *et al.*, 1995b; Ortega-Morales *et al.*, 2000; Resende *et al.*, 1992; Urzi, 1993). However, this does not mean that they are unimportant; their activity may be high and erosive! In addition,

fungi may be the most important endoliths in built stone, according to De los Rios and Ascaso (2005). They have higher tolerance of low water activity than algae and bacteria and require low nutrient concentrations, as well as having no need for light.

2. Actinomycetes

These filamentous bacteria penetrate their substrate by mechanisms similar to those employed by fungi; they also excrete a wide range of enzymes. They can form a whitish veil on stone or produce various water-soluble dark pigments. Laboratory experiments have demonstrated their ability to utilize nitrites and nitrates and to reduce sulfates (Caneva *et al.*, 1991), and, of course, they are well recognized as degraders of a wide range of different carbon and nitrogen sources. Probably for these reasons, the gram-positive actinomycetes tend to predominate over gram-negative bacteria on exposed stone surfaces (Dornieden *et al.*, 2000; Saarela *et al.*, 2004; Warscheid and Braams, 2000). Their acidic metabolic products can attack calcareous stone, hydrolyze some silicate minerals, and chelate metal ions (Kumar and Kumar, 1999). However, they have been reported to rarely, if ever, produce noteworthy amounts of organic acids and chelates in a rock decay environment (Urzi and Krumbein, 1994). In spite of this, they may cause structural damage by their extensive biofilm formation and penetration of their filaments into the stone substrate.

Actinomycetes have been found as important endoliths in various types of built stone (McNamara *et al.*, 2006; Ortega-Morales *et al.*, 2005), emphasizing their degradative ability in this situation. Ortega-Morales *et al.* (2004), for example, found almost exclusively *Rubrobacter xylanophilus*-related bacteria on external biofilms in Uxmal. Although the genus *Geodermatophilus* has been suggested to be common on and in limestone (Eppard *et al.*, 1996), Urzi *et al.*, 2001 using amplified 16S rDNA analysis (ARDRA) and partial sequencing, found that many of the Geodermatophilaceae family on stone in the Mediterranean belonged to other genera (closest to *Modestobacter multiseptatus*).

There have been a number of publications on the presence of actinomycetes in caves (Laiz *et al.*, 2000; Schabereiter-Gurtner *et al.*, 2004), but, in spite of their obvious importance, there is little in the built cultural heritage literature on this group of microorganisms, apart from mainly superficial comments about their presence. This is an area that demands further attention.

3. Nonfilamentous bacteria

The contribution of heterotrophic bacteria to stone deterioration had long been neglected, as insufficient organic nutrients were assumed to be present on stone surfaces. However, these organisms have been isolated frequently from such surfaces; and it has been found that organic

contaminants, such as soil, dust, and dirt, are sufficient to support heterotrophic growth. Furthermore, several of these heterotrophic bacteria are oligotrophic (May, 2003). Chemoorganotrophic bacteria utilize a wide range of nutrients and may serve other microorganisms by the breakdown of poorly degradable compounds (e.g., from atmospheric pollution), which could otherwise not be utilized.

Organisms of the genus *Bacillus* have been very frequently identified on stone buildings (Blazquez *et al.*, 2000; Gaylarde *et al.*, 2001; Heyrman and Swings, 2001; Kiel and Gaylarde, 2006; Laiz *et al.*, 2003; Ortega-Morales *et al.*, 2004; Prieto *et al.*, 1995; Rölleke *et al.*, 1996). This is not unexpected, as they are very common in soil and are able to withstand extreme environments because of their spore-forming ability and ease of culture. Laiz *et al.* (2003), comparing culture and molecular biology techniques, suggested that their proportion of the biofilm on external surfaces of historic buildings is overestimated. However, McNamara *et al.* (2006), using only molecular biology, found that many of the clones were closely related to the low GC Firmicutes and considered that culture techniques may not, in fact, be entirely misleading.

Rather more important than the simple presence of these bacteria in the biofilm, is their potential degradative activity. Kiel and Gaylarde (2006) found that some of their *Bacillus* isolates produced acids and surfactants with autoemulsifying activity in the laboratory, indicating that they had the capacity to accelerate stone degradation. Once again, however, beware extrapolation from laboratory experiments to the real world!

One surprising component of the stone microflora is the group of bacteria producing or utilizing methane. These were isolated from 44 of 225 stone samples from 19 historic buildings in Germany and Italy (Kussmaul *et al.*, 1998). All were Type II methanotrophs, that is, those found at oligotrophic sites under nitrogen limiting conditions. It was suggested that the methane necessary for methanotrophic growth could originate from anthropogenic sources and from endolithic methanogens, which were detected in four of the samples, presumably in anaerobic niches. ''Mini-methane producers'', such as *Clostridium*, were found in almost half of the 47 samples tested for this activity.

C. Chemolithotrophic microorganisms

The presence of chemolithoautotrophic microorganisms, such as sulfur oxidizers, nitrifying bacteria, and iron and manganese oxidizers, depends on the availability of the specific nutrients supporting their growth (Warscheid and Braams, 2000). Although they were the first group of microorganisms to be implicated in stone decay, their assumed importance has been superceded by later research that suggests the greater role

of phototrophs and chemoorganotrophs. Gaylarde and Morton (2002) emphasized that there is little doubt that chemolithotrophic microorganisms have the potential to cause damage to stone; however, their significance to biodeterioration of outdoor stone monuments is still in question.

It appears that sulfur oxidizers and nitrifying bacteria play a more significant role in biodeterioration in humid areas, because of their sensitivity to desiccation (Warscheid and Braams, 2000). In fact, nitrifying bacteria have been suggested to be the most important microbial factors in the decay of sandstone in northern Europe (Bock *et al.*, 1988; Meincke *et al.*, 1988)

1. Sulfur compound oxidizers and reducers

Sulfur-oxidizing bacteria obtain energy by the oxidation of reduced or elemental sulfur to sulfuric acid. Sulfuric acid may react with calcium carbonate to form calcium sulfate (gypsum), which is more soluble in water than the calcium carbonate of the parental rock (Urzi and Krumbein, 1994; Warscheid and Braams, 2000), and thus more readily leached. However, sulfuric acid and calcium sulfate are not always of biogenic origin; they may also derive from atmospheric pollution and acid rain (May, 2003). In fact, Tiano (2002) emphasized that there is as yet no experimental evidence that confirms the direct action of sulfur-oxidizing bacteria in the development of gypsum layers on stone surfaces.

Sulfate-reducing bacteria (which are not chemolithotrophic, but chemoorganotrophs) have been detected in biofilms on limestone (Gaylarde *et al.*, 2001; Ortega-Morales and Hernández-Duque, 1998), but this is apparently rare and no role has been suggested for them in stone decay.

2. Nitrifying bacteria

Ammonia and nitrites on the stone surface are oxidized by chemolithotrophic and, partly, by heterotrophic ammonia and nitrite oxidizers to nitrous and nitric acid, respectively. Ammonia tends to derive from airborne ammonium salts, whereas nitrites may originate from automobiles, industry, and soil (May, 2003). The acids that are produced attack calcium carbonate and other minerals (Urzi and Krumbein, 1994; Warscheid and Braams, 2000). The CO_2 produced can be utilized by the cells to form organic compounds, while calcium cations from the stone matrix form nitrates and nitrites, which are more soluble again than the original mineral phases and thus are leached out of the stone by rain. The characteristic symptom of the activity of nitrifying bacteria is a change in stone properties with no obvious biofilm. It becomes more porous, exfoliation occurs, and fine powder may fall off (Urzi and Krumbein, 1994).

3. Iron- and manganese-oxidizing microorganisms

Iron oxidation is usually rapid and sensitive to pH and oxygen concentrations. Iron-oxidizing bacteria obtain energy by oxidizing ferrous iron in iron-containing minerals to ferric iron, which reacts with oxygen to form iron oxide. The latter process determines the characteristic discoloration and patina formation on stones. Many bacteria and fungi, even algae, are capable of these oxidation steps, causing damaging lesions (Barrionuevo and Gaylarde, 2005; Caneva *et al.*, 1991; Urzi and Krumbein, 1994). It is difficult to distinguish such biooxidation from chemical processes, although evidence for the involvement of living organisms in the formation of a red patina on a dolomite cathedral in Spain has been presented by Valls del Barrio *et al.* (2002).

V. CONTROL OF BIODETERIORATING MICROORGANISMS

This topic deserves a review of its own and will only be mentioned briefly here.

The removal of the microbial community from any given surface is an intervention that must be carefully evaluated. Biocidal treatments may have negative effects on the artifacts (Webster *et al.*, 1992). The removal of the microbial community may give rise to a new succession of microorganisms, which may be more damaging than the old microbial surface populations; and the inhibition of specific groups of microorganisms may favour the growth of others (May, 2003; Warscheid and Braams, 2000). The approach to control biodeterioration must be a polyphasic, interdisciplinary one that considers the history and condition of the artifact as well as physical and chemical damaging factors.

Actions against microbial growth can be divided into four major categories: (1) indirect control by altering environmental conditions; (2) mechanical removal of biodeteriogens; (3) chemicals (biocides); (4) physical eradication methods. Biocides often exhibit detrimental effects on the stone, for example, discoloration, oxidation/reduction of stone compounds, and salt formation, with subsequent crystallization upon drying, leading to exfoliation (Cameron *et al.*, 1997; Caneva *et al.*, 1991; Kumar and Kumar, 1999; Warscheid and Braams, 2000). The ecotoxicity of commercial biocides may make them poor candidates for use in outdoor environments and many countries have prohibited the use of some of the previously most common (and effective) biocides. Furthermore, nitrogen-containing biocides may serve as nutrients for surviving or newly attaching microorganisms (Warscheid and Braams, 2000). Where possible, microbial growth should be prevented by altering growth-supporting conditions (e.g., introduction of a drainage system).

Physical methods such as UV light have long been overlooked in their application on cultural heritage objects, owing to reported long treatment times and low penetration depth (Van der Molen *et al.*, 1980). However, a more recent preliminary study on the removal of lichens by means of a high-intensity pulsed xenon flash lamp gave encouraging results (Leavengood *et al.*, 2000). More research is needed into its effectiveness in controlling a broader range of microorganisms that cause biodeterioration of stone.

There have been a number of publications on the protective effects of microorganisms against biodeterioration of stone. Krumbein (1969) pointed out that after initial destructive processes the microorganisms and the substrate may establish an equilibrium leading to a protective ''patina.'' The influence of lichens has already been discussed and Mottershead *et al.* (2003) suggested that abundant microbial growth on sandstone could protect against salt weathering. They pointed out, however, that this was not the case where colonization is patchy, when degradation was increased. Although a biological control option for stone decay is attractive, it is still some distance in the future.

The human influence of restoration/conservation and its integrated antimicrobial approaches on stone monuments can itself be detrimental, unwittingly improving conditions for microbial colonization and growth. The changes following the discovery of the siliceous stone hieroglyphic stairway at Copan, Honduras, cited by Caneva *et al.* (2005), is a good example. Early removal of the tree cover in the surroundings led to increased exposure to sunlight and better air circulation and resulted in lichen growth. In 1985, a tarpaulin cover was installed to protect the stairway from rain, after various treatments with biocides to remove the growth. By 2005, lichens were much reduced, but there was heavy algal and cyanobacterial growth, interpreted by the authors as the beginning of a new colonization sequence, and doubtless aided by the increased humidity beneath the tarpaulin. Although earlier treatments resulted in a decrease of the more obvious type of biofilms (lichens), the old endolithic population was still present (mainly filamentous fungi and moss protonema were detected), and its removal would be extremely difficult.

VI. CONCLUSIONS

Although it is well established that microorganisms can cause serious damage to stone monuments, knowledge of the precise mechanisms of decay is still fragmentary. This is a field that demands more attention. The development of new identification methods provides us with a broader understanding of the diversity of organisms present on outdoor monuments, and may expand our knowledge of new types of microbial

metabolism occurring in these habitats. Most likely, the list of organisms will expand dramatically as further analytical methods for detection and taxonomy are developed. However, very little work has been carried out in studying the general physiology and potential deteriorative activity of the newly identified organisms, using, for example, proteomics. A proteomic and genomic approach would not only shed light on the potential activity of microorganisms, but would also help to design new strategies for isolating and successfully culturing new organisms. Even microorganisms that have long been known to occupy the surface of stone monuments have only rarely been appropriately examined for their actual contribution to stone decay *in vivo*. In order to compare results of different research groups, a standardization of methods for the detection, assessment, and quantification of biodeterioration is necessary.

The possibility of biologically induced stabilization of stone needs to be more thoroughly investigated. Understanding the interactions between microorganisms and with their environment is crucial to determine whether the organism is damaging or protective to the art object. A description of criteria for determining that the decay of a monument is due to microbial action is rare in the literature. Similarly, very few studies aim to quantify biodeterioration processes. In fact, there have been no attempts to define the degree of biodeterioration of an artifact and at what stage antimicrobial actions should be initiated. In order to assess the contribution of microorganisms to the deterioration of cultural heritage objects, as well as the possibilities for their control, interdisciplinary research projects between conservators and scientists, such as microbiologists, geologists, and chemists, are needed.

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