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Summary

Biofilm-forming cyanobacteria are widespread inhabitants of exposed stones in archaeological and historical sites and caves. Outdoors, these phototrophic biofilms are adapted to all types of stress imposed by growth at the air-rock interface and have developed the capacity to tolerate excess solar radiation, extreme temperatures and desiccation at different latitudes. Indoors, the typology of the cave or the characteristics of confined environments strongly select the microbial community according to light availability and air humidity. Interactions of cyanobacteria with rocky substrata serving as the source of mineral nutrients are based on the adhesion mechanisms and metabolic processes that allow the development of these biofilms. Both types of subaerial phototrophic community include cyanobacteria that support associated populations of heterotrophic populations of mostly very specialized species. The distribution of particular cyanobacterial taxa on monuments in urban or agricultural areas is related mostly to climatic conditions and the position and orientation of the hard surface with respect to water availability and air circulation.

The chapter provides an overview of the more recent studies on free-living subaerophytic cyanobacteria causing discolouration and erosion of lithic faces. Emphasis is on the biodeterioration of artworks due to physical and chemical processes caused by the growth of epilithic and endolithic organisms. The methods used for studying cyanobacterial communities on rocks and buildings of historic and artistic value are summarized, with the focus on conservation issues. Study techniques which are non-invasive of the underlying substrata are essential and it is important to identify the biodeteriogens responsible for the damage.

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11.1 Cyanobacteria in Subaerial Phototrophic Biofilms

Cyanobacteria successfully colonize almost all illuminated environments, including some of the most hostile for life (Stal 2007). Rocks exposed to the atmosphere are inhabited by extremophilic cyanobacteria that play a basic role as primary producers in a variety of subaerial habitats such as cliffs and pinnacles of dry and humid regions (Pentecost and Whitton 2000; Chap. 10), hot and cold deserts (Wynn-Williams 2000; Vincent 2007) and caves (Hoffmann 2002), and might contribute to the future colonization of other planets (Grilli Caiola and Billi 2007; Billi 2012).

The colonization of rocks is due to lithophytic cyanobacteria growing as epiliths on surfaces and endoliths within the substrata, where they can reach a few millimetres below surface (Golubic et al. 1981). Their typically patchy distribution on solid substrata is due to local inhomogeneities of rock structure, such as differences in porosity, and other environmental differences in their microhabitats. Subaerial lithophytic cyanobacteria form phototrophic biofilms, surface-associated microbial communities with significant environmental and human impact. These multispecies consortia are built by photoautotrophic cyanoprokaryotes (with associated microalgae) and chemoorganotrophs (heterotrophic bacteria and fungi) spatially organised in complex assemblages from different functional groups. Subaerial phototrophic biofilms are embedded in exopolymeric matrices, which mediate adhesion of the microbial communities to the underlying substrata (Karsten et al. 2007; Chap. 18). Because cyanobacteria can both photosynthesize and in many cases fix atmospheric nitrogen, they are primary colonizers of rocks and important lithobiont components of stone biofilms and can also enter into symbiosis with fungi and other phototrophs (Chap. 23).

The morphology, chemistry, physiology and ecology of naturally occurring phototrophic biofilms are as diverse as their constituent microorganisms. While the structure, growth dynamics and physiology of heterotrophic biofilms have been studied extensively, phototrophic biofilms have until recently received less attention, the best described being cyanobacterial mats (Stal 2000; Chap. 4). Similarly to aquatic microbial communities, cyanobacteria in subaerial biofilms generate energy and reduce carbon dioxide and atmospheric nitrogen, providing organic matter and oxygen to heterotrophs. This photosynthetic activity fuels processes and conversions in the biofilm community as a whole, including the degradation of organic compounds and the release of inorganic carbon, nitrogen and phosphorus required by the phototrophic fraction.

Variably coloured, blue-green, grey, brown, violet or black strips on natural rocks are caused by the growth of

cyanobacteria and have long been known as “Tintenstriche” (Jaag 1945; Chap. 8). Epilithic biofilm-forming cyanobacteria discolour natural rocks, buildings and monuments on which they grow not only because of their chlorophyll, phycobiliproteins and carotenoids, but in many cases also UV-screening compounds, such as the yellow-brown scytonemins and the red to blue gloeocapsins (Chap. 19). The latter pigments, which aid survival under extreme solar exposure, can enhance markedly the visibility of cyanobacterial biofilms on rock surfaces. In contrast, endolithic cyanobacteria experience diminished solar radiation, because they occupy cracks and fissures or defined layers within the rock that reduce incident light down to as little as 0.001% surface irradiation (Friedmann and Ocampo-Friedmann 1984; Büdel 1999; Chap. 10).

An epilithic biofilm typically consists of a consortium of microorganisms resistant to variable stresses (poikilotolerant) from diverse taxa, including bacteria, cyanobacteria, algae, mosses, ferns, fungi and lichens. Lithobionts exposed to the atmosphere experience much harsher and more variable conditions than those in soil or water. The poorly buffered subaerial environment results in communities restricted in diversity and biomass (Gorbushina and Broughton 2009).

The metabolic activity of subaerial biofilms is sustained by their ability to retain water through the secretion of compounds that protect the cells from fluctuating light and water conditions and chelate nutrients for the growth of the enclosed microbial community. Generally, biofilms are composed by microbial cells, extracellular polymeric substances or secretions (EPS), biogenic and inorganic particles, and multivalent cations. Microbial strategies for adhesion to stone surfaces are based on the production and secretion at the cell surface of mucilaginous compounds. EPS is secreted by the microorganisms as glycocalyx, sheath or envelope, and acts as an adhesive that allows cells to stick to the substratum, and to form multispecies biofilms. Cyanobacterial capsules and sheaths are structured investments that form an integral component of the cell, whereas EPS loosely attached to the cells or exuded freely in the environment is termed mucilage (or slime) (Stal 2010).

Exposure to high solar radiation and substantial fluctuations in the availability of water restrict survival and growth of subaerial lithobionts. In arid environments, dew, fog, and sparse rain are the only sources of humidity. In extreme hot and cold deserts, dew condensation or melting snow provide organisms with sufficient water, whereas rock-inhabiting biofilms of temperate regions benefit from a temporary or regular supply by rainwater (Büdel et al. 2008, 2009). Even then, extreme temperatures accelerate water loss through evaporation or freezing (Wynn-Williams 2000). The scarcity of water in subaerial environments also imposes salt stress, and the production of osmolites by cyanobacteria is used as a mitigation strategy to protect proteins and pre-

vent desiccation-induced damage in combination with sheath pigments and cytoplasmic mycosporines (Billi and Potts 2000, 2002). High concentrations of sugar alcohols can accumulate inside cells to avoid excess water loss under drought conditions, providing compatible solutes and rapidly available respiratory substrates (Karsten et al. 2007). In the cryptoendolithic desert *Chroococcidiopsis*, the disaccharides trehalose and sucrose-6-phosphate have been shown to accumulate in response to osmotic stress, but data are lacking as to whether or not desiccation has a similar effect (Grilli Caiola and Billi 2007). *Chroococcidiopsis* and *Nostoc* species can dry without dying, a phenomenon known as anhydrobiosis, that allow cyanobacteria to escape the harsh outside climate inside porous rocks where they survive in a dry, metabolic state for prolonged periods. Even though the strategies underlying the ability of anhydrobiotic cyanobacteria to cope with prolonged desiccation are not fully understood, it seems probable that there is an interplay between protection and repair mechanisms (Billi 2009, 2011).

The morphology and diversity of subaerial cyanobacteria adapted to the lithobiotic life includes both unicellular *Synechococcus* only a few microns in diameter and filamentous forms with trichomes more than 30 µm wide. Over a hundred species have been recorded that belong to all subsections and orders of cyanobacteria. Hoffmann (1989) reviewed much of the information available then about lithobiotic algae in subaerial habitats using the terminology for microenvironments proposed by Golubic et al. (1981): epilithic, chasmoendolithic, cryptoendolithic and euendolithic. However, the diversity of lithobiontic cyanobacteria in subaerial biofilms is relatively little understood, and most taxa described so far are still considered to be cosmopolitan, with only a few rare or endemic species (de los Rios et al. 2007; Uzunov et al. 2007).

The identification of cyanobacteria associated with surfaces is usually difficult, because most taxa exhibit few morphological features. However, their taxonomy and systematics is progressing as a result of the sequencing of 16S rRNA and other genes in combination with the analysis of cytomorphological characters (Wilmotte 1994; Wilmotte and Herdmann 2001). The taxonomy of over 150 genera and 1,500 species listed under the International Code of Botanical Nomenclature has been subject to various revisions aimed to define genera as more homogeneous taxonomic groups mainly on the basis of ecological, morphological, and ultrastructural characters (Komárek and Anagnostidis 1998, 2005; Komárek 2010; Komárek and Hauer 2010). Currently the orders of the phylum Cyanobacteria/Cyanophyta correspond to the five subsections accepted by the International Code for Nomenclature of Bacteria mostly on the basis of biochemical and genetic features. Therefore, most researchers are now applying a polyphasic approach that combines phenotypic and genetic studies grouping sequences with more than 95% into the same

genus and 97.5% similarity into operational taxonomic units (OTUs) or phylotypes that may correspond to one (or more) species clearly distinct from others (Rajaniemi et al. 2005; Foster et al. 2008; Zakhis et al. 2008). The use of this approach is providing new insights on the species richness of subaerial communities, where there are many records for new cyanobacterial species and phylogenetic results support the establishment of new genera (Rindi 2007; Bruno et al. 2009).

Marked variations in environmental parameters can lead to time-dependent dynamics of subaerial microbial communities and to the establishment of different micro-habitats at different times. Early studies on the influence of humidity showed the presence of a characteristic epilithic cyanobacterial association on exposed limestone rocks of the Dinaric-Alps (*Scytonema-Gloeocapsetum*) that could be replaced by a different one in shaded areas (*Aphanocapsa-Chroococetum*), while increased humidity supported the growth of various assemblages at high (*Tolypothricetum byssoideae*, *Dichothricetum gypsophila*, *Calothricetum parietinae*) or low light conditions (*Schizothricetum heufleri*, *Schizothricetum lardaceae*, *Hydrocoleetum homoeothrichii*) (Golubic 1967). Phylogeny and biogeography of rock-inhabiting microbes in subaerial communities is showing their similarity on different continents and the cosmopolitan distribution of some rock settlers (Gorbushina and Broughton 2009). This may also apply to epilithic and endolithic cyanobacteria inhabiting similar habitats in other geographical areas (Sigler et al. 2003; Taton et al. 2006).

11.2 The Monument Environment

During the first half of the nineteenth century there were already reports on biological growths on rocks and monuments, such as by A.V. Humboldt, Charles Darwin and J.C. Ehrenberg (Adhikary 2000b). Subsequent accounts of subaerial lithophytic cyanobacteria documented their wide occurrence throughout the world during the past century. Subaerial cyanobacterial biofilms commonly develop on or within man-made surfaces as on any solid mineral substratum exposed to the atmosphere, and are ubiquitous at all latitudes on monuments and buildings, but particularly abundant in warm temperate and tropical regions (Ortega-Calvo et al. 1993; Tripathy et al. 1997, 1999; Gaylarde and Gaylarde 2000; Ortega-Morales et al. 2000; Tomaselli et al. 2000; Pattanaik and Adhikary 2002a; Crispim et al. 2003; Crispim and Gaylarde 2005).

In cultural heritage studies, epilithic cyanobacterial assemblages are usually described as patinas, stone alterations and crusts when they are obvious to the naked eye (Figs. 11.1, 11.2, and 11.3) (Urzi et al. 1992). As mentioned above, the discolouration is due to cyanobacterial and eukaryotic algal pigments. The extracellular pigments may not only protect



Fig. 11.1 Phototrophic biofilms on outdoor monuments in the temperate climate of Rome, Italy: (a) Colonization of organic nutrient enriched rock surfaces and porous mortar layers of a Roman wall on the posterior of the Pantheon temple; (b) Black strips on a marble statue

and (c) spots on one column at the *Terme di Diocleziano* in a heavily air polluted area of the city. Note the growth of cyanobacteria on the wall in the shaded area behind the column, where humidity is high

against high visible and UV irradiance (Roy et al. 1997; Adhikary 2004), but perhaps also help protect against desiccation and temperature stresses (Fleming and Castenholz 2007). The settlement and persistence of multispecies biofilms on exposed rocks relies on their ability to face high solar radiation, repeated cycles of desiccation and rewetting, prolonged desiccation, temperature fluctuations and nutrient limitations (Gorbushina 2007).

The diversity and the deteriogenic activity of epilithic and endolithic cyanobacterial communities depend on the availability of light, water, carbon and other nutrients to sustain microbial metabolism that in turn causes the irreversible transformation and biomineralisation of substrata. Endoliths may be growing in cracks and pores and boring into rocks, although this might be obscured by superficial algal growths, and consequently overlooked (Pentecost 1992). Most of the



Fig. 11.2 Discolouration patterns on surfaces: (a) The Bernini's Elephant statue in the Rome city center (Italy) (b) Façade of St Boniface church in Brussels with black sulphated crusts and phototrophic biofilms in more humid areas. Differently coloured strips are present

on the most exposed and dry upper part of the façade; (c) *Terme di Diocleziano* and (d) Trevi's fountain in Rome (Italy) with subaerial growth of cyanobacteria on marble in sun sheltered areas and constant wetness

relatively few reports on endolithic cyanobacteria on buildings describe the presence of coccoid cyanobacteria (Saiz-Jimenez et al. 1990; Ortega-Morales et al. 2005). Diverse bacterial communities in limestone of Maya archeological sites have shown that the endolithic community is distinctly different from the communities on limestone surfaces due to the influence of the physical and chemical properties of the calcareous stone materials (McNamara et al. 2006). It is, however, not yet clear to what extent taxonomic diversity influences the weathering, nor the quantitative differences between damage caused by endoliths compared to that by epiliths at the same site.

Large seasonal variation of UV radiation and low maximum values are characteristics of high latitudes, while low

latitudes impose stronger exposure that elicits cell responses to survive the damaging effects of UV radiation similar to high altitude exposed sites (Castenholz and Garcia-Pichel 2000). In monuments, stone surfaces at different heights usually harbour different cyanobacterial communities, with the less desiccation-tolerant species developing close to the ground where humidity and nutrients are high, while poikilothermic taxa colonize high and more exposed levels.

The most important limiting factor for the growth of phototrophs is light, the intensity and quality of which depend on the location and specific architectural features of the monument. The average intensity of light reaching a surface on a sunny day ranges from 1,000 to 2,000 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ at mid-day. Light is the driving energy that sustains phototroph



Fig. 11.3 Discolouration of Hindu temples in Bhubaneswar, Orissa, India: (a) The complex of Lingaraj temples and (b) close-up of sandstone with carvings completely covered by dry cyanobacterial biofilms;

(c) Exterior of one temple at the Khandagiri cave colonized by black biofilm strips on rock

development, and appropriate irradiance values or prolonged lighting periods support extensive growth of photosynthetic microorganisms. However, light absorption and scattering at the surface can differ markedly depending on the type of rock. The presence of airborne particles and biofilm of various thickness readily attenuate light within the community and most cyanobacteria, that are usually adapted to low photosynthetic photon flux densities, are often photoinhibited. When available wavelengths vary, cyanobacteria can adjust their light harvesting pigments to optimize light absorption. These photobiological features are at the basis of the acclimation processes of phototrophic biofilms and are reflected by their photosynthetic performance and hence growth.

Subaerial microbial communities are held together and to the substratum by the EPS produced by the phototrophic and heterotrophic component. As a result characteristic microenvironments are originated, which allow the survival of EPS-embedded microorganisms in harsh environments (Stal 2000). EPSs are polyelectrolytes of high molecular weight made by polysaccharides (up to 90%), proteins and nucleic

acids, that contribute to trapping water. These compounds, present as gels in capsules and sheaths or solubilised into the mucilaginous biofilm matrix, are synthesized by one or more of the microbial groups present within the biofilm and contribute to the initial stages of biofilm formation and to the subsequent co-aggregation and stabilisation of a multispecies community (Rickard et al. 2003). EPS also actively participate in the weathering process by contributing to the binding of solubilized minerals (De Philippis and Vincenzini 1998; Pereira et al. 2009) and several studies have reported on the high contents of chelating uronic acids and sulphated groups of the heteropolysaccharides produced by cyanobacteria on lithic faces of archaeological sites (Albertano and Bellezza 2001; Bellezza and Albertano 2003; Bellezza et al. 2005).

Colonization and growth of cyanobacteria and associated microorganisms accelerates weathering and soiling of rocks (Warscheid and Braams 2000; Gaylarde and Morton 2003). Phototrophic and heterotrophic microorganisms cause biodecay and biotransformation of rock substrata while carrying

out normal metabolic activity (Urzi 2004; Gorbushina 2007). Bioerosion of archaeological and artistic lithic surfaces by microbial communities colonizing monument surfaces has been observed in many geographical areas and there has been an increasing concern about the possible damage to the cultural heritage caused by microbial activities. This has stimulated a considerable number of studies in more recent years (Ortega-Calvo et al. 1995; Ciferri 1999; Crispim and Gaylarde 2005; Ortega-Morales 2006). Nevertheless, the deterioration mechanisms of the large range of rock types with different mineralogical characteristics and the varying weathering responses under different climatic and environmental conditions are still far from fully understood. Like all materials, rock is subject to inexorable deterioration, especially if exposed to the weather. Air pollution is one of the major factors responsible for depositing chemical substances and biological agents on outdoor stone surfaces (Zanardini et al. 2000; Nuhoglu et al. 2006).

Pigment formation can have undesirable masking effects on the undelaying work of art. The chemical interactions of pigments with constituents of the substratum, trace metals and pollutants can further alter the mineral composition of the stone. Various pigmented patinas with patchy distribution due to non-homogeneous growth appear on lithic faces or fissures and cracks or beneath the surface, depending on the conservation state of artefacts. The various microhabitats within a single monument can be colonized by different phototrophs, because of environmental constraints on the phenotypic expression of forms adapted to particular ecological niches.

Another important, but largely neglected, aspect of the ecology of monuments is the impact on humans of active biomolecules produced by cyanobacteria and associated microorganisms. Exposure to toxins is possible by inhalation of the aerosolized cells and molecules. The few reports about this deal with the risks to human health of short- and long-term inhalation effect of toxic volatile compounds produced by biodeteriogenic actinobacteria (Salkinoja-Salonen et al. 2003) and toxigenic airborne cyanobacteria (Kumar et al. 2007).

The development of phototrophs results in physical and chemical damage due to time variable external loadings in conjunction with environmental moisture, heat, freeze-thaw action, shrinkage, chemical and biological dissolution and corrosion. A recent overview of issues related to biodeterioration and conservation of cultural properties around the world provided insights on the interactions between stone and microorganisms, the way they can enhance or retard the overall rate of degradation, and outlined the predominance of bacterial endoliths in calcareous and siliceous stone monuments (Scheerer et al. 2009). Other authors have discussed the threats to a wide range of heritage materials and monuments by biological and chemical agents of decay and

brought together contributions from the field of plant biology related to the biodeterioration, emphasising correlations between deterioration processes, organisms and environment, and describing case studies in various environmental and climatic conditions and diverse geographic settings (May et al. 2008; Caneva et al. 2009).

11.3 Outdoor Monuments

11.3.1 Overview

Microbial colonisation depends on liquid water, that is only periodically available in the form of rain, dew, or condensation of atmospheric humidity. Therefore, the EPS is of great importance for epilithic and endolithic cyanobacteria, as it retains water and acts as osmoprotectant, nutrient reservoir and toxicant chelator. Although atmospheric pollution is generally recognised as a significant physico-chemical factor in the deterioration of cultural properties, biodeterioration caused by cyanobacteria has rarely been analysed in relation to air pollutants. In the presence of atmospheric pollution and a humid environment, calcareous rock is transformed into hydrated calcium sulphate (gypsum, $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). This should be removed from surfaces, as hygrometric fluctuations lead to dissolving and crystalizing processes and subsequent mechanical stress. In areas sheltered from the rain, gypsum embeds mineral and smog particles, leading to the formation of the so-called black crusts that represent chemical alteration and aesthetic disfigurement (McNamara and Mitchell 2005). Ortega-Calvo et al. (1994), studying the effect of black crusts on *Gloeotheca* sp., showed that when gypsum was removed from the rock surfaces of Seville cathedral and added to a mineral salt medium, the surface dissolved slowly, releasing sulphate that was progressively incorporated into the cyanobacterial sheath and used for its growth.

Sedimentary rocks have been extensively used in the construction of monuments and historical buildings. Since the first reports in the 1960s on biodeterioration of stone artworks by phototrophic organisms (Raistrick and Gilbert 1963; Lefèvre et al. 1964), a large number of studies has already assessed the occurrence of cyanobacteria and algae on sedimentary stone materials and cave walls from cultural heritage. However, the effect of weathering by subaerial cyanobacterial communities has only seldom been quantified in nature, though the few calculations made on limestone reported rates between 0.5 and 3 mm/100 years along with exfoliation and pitting of stone surfaces (Pentecost and Whitton 2000).

Cyanobacterial biofilms have been found on limestone and sandstone man-made surfaces of very different types and mineral composition. Floristic records have been reported

for the sandstone of Hindu temples and monuments in various part of India (Pattanaik and Adhikary 2002a, b) and Angkor temples in Cambodia (Lan et al. 2010), marbles in Italy (Tomaselli et al. 2000), granite in Serbia (Grbič et al. 2010); terracotta at Bishnupur temples in West Bengal and brick walls in Tamil Nadu, India (Pattanaik and Adhikary 2002b). Several authors summarized the extant knowledge about taxa recorded for different, mostly limestone, mineral substrata, providing relatively detailed lists (Ortega-Calvo et al. 1991; Adhikary 2000b; Kováčik 2000; Uher et al. 2005; Macedo et al. 2009).

The cyanobacterial component of organisms visible in biofilms over a unit area has been reported to range roughly from 17% to 35% for European versus 25–66% for Latin-American monuments (Gaylarde and Gaylarde 2005). However, quantitative data on biomass for monuments are scarce, making it difficult to compare with natural communities in other subaerial environments; the diversity of methods for expressing the value per unit area adds to the problem.

The development of biofilms on lithic faces is closely related to the environmental humidity necessary for microbial growth. Porosity and hygroscopicity of materials, capillary water absorption and relative humidity strongly influence water availability for the phototrophs, so calcareous substrata are colonized more easily than granite. A survey of the literature on biodeterioration, followed by experimental studies using microscopy and genetic methods (ARDRA), led Tomaselli et al. (2000) to recognize different species associations from different types of environment: *Chroococcus minor*, *Myxosarcina concinna*, *Gloeocapsa biformis*, *Pleurocapsa* and *Scytonema* species with calcareous lithotypes; *Phormidium tenue*, *P. autumnale* and *Microcoleus vaginatus* with siliceous rocks; *Nostoc punctiforme*, *N. muscorum*, *Chroococciopsis* and *Leptolyngbya* species with frescoes and plasters. *Gloeocapsa alpina* isolated from a Portuguese church and inoculated on limestone, granite and white marble to test the bioreceptivity of the different materials, grew on carbonate, but not silicate, substrates (Miller et al. 2006). Sometimes a particular organisms is especially important in a particular region, such as *Gloeocapsa novacekii* on sandstone monuments in Belgrade (Serbia) (Grbič et al. 2010). Other studies have emphasized the importance of factors other than the chemical composition of the substrate. Barberousse et al. (2006a), using multivariate analysis, concluded that precipitation, hygrometry, thermal amplitude, distance from the sea and proximity to vegetation were the major factors influencing the distribution of phototrophic biofilms on building facades in France. An assessment of data for monuments in European Mediterranean countries (45 case studies, 1976–2009) concluded that the occurrence of cyanobacteria on the main lithotypes (marble, limestone, travertine, dolomite, granite, sandstone, terracotta) was only secondarily related to the

lithotype, the main factors being porosity, roughness and permeability of the surface (Macedo et al. 2009). Overall, the majority of literature does not indicate that cyanobacterial populations on exposed surfaces of outdoor monuments are distinctive according to mineral type, but this must be viewed with caution, because so many studies are based on insufficiently critical taxonomic identification. The need for this to be more accurate is made clear by Kaštovský et al. (2010) in their revised checklist of cyanobacteria for the Czech Republic (392 species). In an assessment of common to rare aerophytic epilithic forms on four types of rock environment (wet walls, granite and sandstone, calcareous, serpentinite and ultrabasic), taxon specificity was evident for surfaces with higher water availability versus dry ones, and for acidic versus basic ones, although *Aphanothece caldariorum* proved an exception (Table 11.1). Most of the taxa recorded on outdoor monuments have also been reported from natural environments, so literature about the latter can provide further helpful information e.g. Uzunov et al. (2007).

11.3.2 Temperate and Mediterranean Climates

At latitudes above the tropics, most accounts for cyanobacteria on outdoor monuments have come from Europe. These include biofilms on marble statues and fountains in Tuscany (Tomaselli et al. 2000; Cuzman et al. 2010), archaeological sites, churches and historic buildings in France, Greece, Italy, Serbia, Spain, Ukraine, Turkey, Belgium, Germany, Ireland and Sweden (Ortega-Calvo et al. 1993; Adhikary 2000b; Darienko and Hoffmann 2003; Kováčik 2000; Macedo et al. 2009; Grbič et al. 2010). Tomaselli et al. (2000) made a literature survey to quantify the relative importance of taxa. *Phormidium* was the most widespread genus (seven species, *P. autumnale* and *P. tenue* the most frequent), with the next most important being *Nostoc* (six species, mostly *N. punctiforme*), *Microcoleus* (four species, mostly *M. vaginatus*) and *Plectonema* (four species, mostly *P. boryanum*). Values for building facades in France (Barberousse et al. 2006b) gave *Cyanosarcina parthenonensis* (28%), *Chroococcus lithophilus* (21%), *Gloeocapsa sanguinea* (15%) for coccoid species, and *Calothrix pulvinata* (21%), *Leptolyngbya foveolarum* (18%) and *Phormidium corium* (13%) for filamentous species. The unsightly effect of dark-coloured stains in Renaissance and Baroque cloisters of the Santo Domingo College (Orihuela, Alicante, S-E Spain), was due to 14 epilithic species with Chroococcales and Oscillatoriales the most important (Sánchez-Antón and Asencio-Martínez 2007). *Chroococcus* and *Tolypothrix* showed the highest species diversity, while *Pseudocapsa* was the most frequent genus. Of the 37 genera and 96 species listed by Macedo et al. (2009), the most widespread taxa on all substrata

Table 11.1 Valid names of common and rare aerophytic cyanobacterial species reported as epiliths (or chasmo-endoliths*) on rocky substrata in the Czech Republic (Data adapted from Kaštovsky et al. 2010)

Wet walls	Granite and sandstone	Calcareous rocks	Serpentine and ultrabasic rocks
<i>Aphanocapsa muscicola</i>	<i>Aphanothece caldariorum</i>	<i>Aphanocapsa parietina</i>	<i>Aphanothece caldariorum</i>
<i>Aphanothece bullosa</i>	<i>A. saxicola</i>	<i>Aphanothece castagnei</i>	<i>Chroococcus spelaeus</i>
<i>Chroococcus cohaerens</i>	<i>Chr. turgidus</i>	<i>Calothrix parietina</i>	<i>Entophysalis atroviolacea</i>
<i>Chr. helveticus</i>	<i>Chr. various</i>	<i>Chlorogloea novacekii</i>	<i>Gloeocapsa alpina</i>
<i>Cyanosarcina huebeliorum</i>	<i>Cyanothece aeruginosa</i>	<i>Chl. microcystoides</i>	<i>G. compacta</i>
<i>Gloeocapsa punctata</i>	<i>Dichothrix orsiniana</i>	<i>Chondrocystis dermochroa</i>	<i>G. novacekii</i>
<i>G. rupicola</i>	<i>Gloeocapsa bituminosa</i>	<i>Chroococcus ercegovicii</i>	<i>Gloeocapsopsis chroococcoides</i>
<i>Gloeothece palea</i>	<i>G. fuscolutea</i>	<i>Chr. pallidus</i>	<i>Gl. dvorakii</i>
<i>Glo. tepidariorum</i>	<i>G. haematodes</i>	<i>Chr. spelaeus</i>	<i>Gl. pleurocapsoides</i>
<i>Leptolyngbya angustissima</i>	<i>G. kuetzingiana</i>	<i>Chr. turicensis</i>	<i>Hassallia byssoydea</i>
<i>L. carnea</i>	<i>G. reicheltii</i>	<i>Chr. various</i>	<i>Scytonema crustaceum</i>
<i>L. cataractarum</i>	<i>G. sanguinea</i>	<i>Dichothrix gypsophila</i>	<i>Stigonema panniforme</i>
<i>L. cebennensis</i>	<i>Homoeothrix janthina</i>	<i>Entophysalis atroviolacea</i>	<i>St. tomentosum</i>
<i>L. compacta</i>	<i>Microchaete brunescens</i>	<i>Gloeocapsa aeruginosa</i>	
<i>L. edaphica</i>	<i>Scytonema mirabile</i>	<i>G. alpina</i>	
<i>L. foveolarum</i>	<i>Stigonema hormoides</i>	<i>G. biformis</i>	
<i>L. hennigsii</i>	<i>St. informe</i>	<i>G. compacta</i>	
<i>L. subtilissima</i>	<i>St. minutum</i>	<i>G. nigrescens</i>	
<i>Merismopedia minima</i>		<i>G. novacekii</i>	
<i>Microcoleus subtorulosus</i>		<i>G. rupestris</i>	
<i>M. vaginatus</i>		<i>G. violacea</i>	
<i>Nostoc calcicola</i>		<i>Gloeocapsopsis chroococcoides</i>	
<i>N. microscopicum</i>		<i>Gl. dvorakii</i>	
<i>N. punctiforme</i>		<i>Gl. pleurocapsoides</i>	
<i>Oscillatoria rupicola</i>		<i>Gloeothece confluens</i>	
<i>Phormidium kolkwitzii</i>		<i>Glo. rupestris</i>	
<i>P. papyraceum</i>		<i>Hassallia byssoydea</i>	
<i>P. rimosum*</i>		<i>Leptolyngbya gracillima</i>	
<i>P. schroeteri</i>		<i>Phormidium rimosum*</i>	
<i>P. violaceum</i>		<i>Rivularia haematites</i>	
<i>Pseudophormidium tenue</i>		<i>Scytonema crustaceum</i>	
<i>Scytonema hofmanni</i>		<i>S. drilosiphon</i>	
<i>S. myochrous</i>		<i>Tolypothrix bouteillei</i>	
<i>Symploca muralis</i>		<i>T. elenkini</i>	
<i>Sy. muscorum</i>			

were *Chroococcus*, *Gloeocapsa* and *Phormidium*, with *Chroococcus minor*, *Gloeocapsa biformis* and *Phormidium foveolarum* the most frequent species. *Pleurocapsa minor* on marble associated with fountains was also one of the most frequently recorded taxa, whereas other forms of *Pleurocapsa* and also *Scytonema* on Mediterranean monuments remain unidentified to the species level.

Fountains and nymphaea are characterized by having some areas continuously or sporadically wetted by running water, while others are always dry. The presence or absence of water, its physical and chemical features and the exposure to direct or shaded sunlight all influence phototroph distribution (Pietrini and Ricci 2009). A recent overview on the biological patinas that developed in fountains in Italy and

Spain compared the biodiversity of the different biotopes in relationships to water availability, light exposure, and lithotypes (Cuzman et al. 2010). Using microscopy combined with automated ribosomal RNA intergenic spacer analysis and the principal component analysis of obtained profiles, the authors identified 32 cyanobacteria showing a precise match of molecular phylogeny data and morphological identification of cyanobacterial isolates, that separated in four clusters according to the microenvironmental conditions of each fountain. Species of *Chlorogloea*, *Chroococcus*, *Pleurocapsa* and *Phormidium* were the most common colonizers of wet spots along with *Calothrix* and *Nostoc*, whilst *Chroococcidiopsis* occurred in dry areas (Cuzman et al. 2010).

11.3.3 Tropical Climates

Kumar and Kumar (1999) provided an overview of general biodeterioration problems in tropical climates and mentioned cyanobacteria as one of the main biodeteriogenic groups of organisms. In the case of Latin America, most surveys of cyanobacteria in biofilms on historical buildings have been made at archaeological sites on Mexico (Gaylarde et al. 2001; Ortega-Morales et al. 2000; McNamara et al. 2006) and Brazil, though some have also been investigated in Argentina, Bolivia and Peru (Gaylarde and Gaylarde 2000). Ortega-Morales (2006) concluded from the literature that Pleurocapsales were the main colonizers at the Mayan site of Uxmal in Mexico, but *Synechocystis*, *Gloeocapsa* and *Xenococcus* were generally the dominants elsewhere on monuments in Latin America, accompanied by *Lyngbya*, *Plectonema* and *Nostoc*. Ramírez et al. (2010), who studied buildings at Palenque, another archaeological site in Mexico, described the three-dimensional structure and distribution on rock, stucco and concrete of photosynthetic microorganisms in the biofilms dominated by the desiccation-tolerant *Scytonema guyanense* and *Asterocapsa divina*, that occupy respectively dry and more shaded-humid habitats. Cyanobacterial diversity was higher at biofilm surfaces under low sunlight and prolonged wet conditions than those exposed to full solar radiation. This led them to discuss the implications for the development and persistence of phototrophic species able to withstand temporal heterogeneity resulting mainly from the alternating wet and dry seasons. The more vulnerable species seemed to grow in the bottom layer of the biofilm, their persistence depending on the ability to tolerate the annual desiccation period, whereas species on top probably took advantage of fast growth rates under favourable rainy conditions.

Reports on cyanobacteria on Asian monuments include a few for Cambodia (Lan et al. 2010), Korea (Fusey and Hyvert 1964; Tripathy et al. 2007), Iran (Mohammadi and Krumbein 2008), and most extensively in India (Roy et al. 1997; Tripathy et al. 1997; Adhikary 2000a, b; Pattanaik and Adhikary 2002b). A total of 30 species belonging to 13 different genera were reported by Tripathy et al. (1999); their detailed account described seven species of *Tolypothrix* along with *Gloeocapsopsis dvorakii*, *Lyngbya corticola*, *Phormidium truncicola* and *Plectonema puteale* as the major components of crusts and tufts on Indian stones. Species of *Gloeotheca*, *Chroococidiopsis*, *Myxosarcina*, *Plectonema*, *Calothrix*, *Nostoc*, *Chroogloeopsis*, *Fischerella* and *Hapalosiphon* appeared in the enrichment culture as minor components of the biofilms. A study by Pattanaik and Adhikary (2002) identified 46 cyanobacterial taxa at Indian archaeological sites and caves in Orissa, Maharashtra, Karnakata, Uttar Pradesh, Tamil Nadu, Delhi and West Bengal. These phototrophic communities showed a marked capacity to tolerate the extreme environmental conditions

thanks to the stability of their chlorophyll *a*, ability to grow in the dark, production of heat-shock proteins, scytonemin and mycosporin-like aminoacids (Roy et al. 1997; Adhikary 2000a, b). In a study by Pattanaik et al. (2004) the scytonemin was sufficient to cause a dark pigmentation of the stone, while heat-shock proteins supported resistance to temperatures >60°C.

More recently, some of the thousand sandstone temples in the Golden triangle of Orissa, N-E. India, which date back to the eighth to twelfth centuries (AD), have been investigated to understand the processes which have led to changes in stone microhabitats since the 1990s. These have been caused largely by a combination of unsuitable conservation strategies and the effects of climate change, which together have led to the modification of the microbial communities described above and increased corrosion of the sandstone. The following are some of the studies relating to cyanobacteria.

These architectural complexes have an extensive cover formed by two main types of cyanobacterial biofilm. This is a stable black or brownish crust during the dry season (Fig. 11.3) and a thick blue-green biofilm during the monsoon period. The latter biofilm survives the extended period of drought, temperature and high radiation stress in a vegetative state and metabolic activity revives soon after rewetting (Adhikary 2004). Among the 57 taxa reported for building facades in India only *Scytonema pseudoguyanense* and *Gloeocapsa kuetzingiana* thrive on sandstone during the period of extreme temperature and desiccation (Lakshmi and Adhikary 2008). However, variations were detected in the type of coccoid and filamentous species during a 3-year study, with an increase of the total number of phototrophic taxa particularly during the monsoon season. The most extensive alterations of the lithic faces were still due to biofilms of *Tolypothrix* and *Lyngbya* during the dry season (Fig. 11.4), while samples collected during the rainy season showed an almost stable number of cyanobacterial taxa, but also a few eukaryotic algae (S.P. Adhikary, personal communication).

Tolypothrix byssoidea (Adhikary and Satapathy 1996) is one of the most common cyanobacteria on exposed surfaces of Hindu temples thanks to its scytonemin and mycosporin-like amino-acids absorbing UV radiation, together with a range of heat-shock proteins (Adhikary 2000a, b; Pattanaik et al. 2007). The analysis of biofilm matrices, cyanobacterial and bacterial capsules revealed the abundance of compounds acting in the protection from water limitation and dangerous radiation. The heteropolysaccharides extracted from some isolates were shown to have a high affinity for bivalent metal cations, i.e. calcium, magnesium and iron, suggesting their ability to actively contribute to weakening the mineral substrata. Eleven to 12 neutral and acidic sugars were detected in the slime secreted by these strains, two of which showed 98% sequence similarity of the 16S rRNA gene to a strain of *Chroogloeocystis siderophila* (Rossi et al. 2012).



Fig. 11.4 Cyanobacterial biofilms on Hindu temples: (a, b) Abundant development of biofilms on the walls during the monsoon period leads to the formation of black crusts during the dry season;

(c) Severe exfoliation and erosion of sandstone caused by the detachment of biofilms; (d) Dried cyanobacterial crusts in a sheltered area

Büdel et al. (2004) reported a different weathering mechanism in South African sandstone formations, where the cryptoendolithic *Choococidiopsis* sp., *Nostochopsis lobata* and *Trichocoleus* cf. *sociatus* induced weathering by substrate alkalization due to positive net photosynthesis coupled to a pH increase to 9.5–10.5, values high enough to solubilize silica. As a result of deprotonation of Si-O-H bonds, the upper rock part was loosened and then eroded away by wind and water flow. Intermittent swelling of the EPS during water uptake also played a role in the final loosening of rock flakes. This special type of ‘exfoliation’ seems to be widely distributed in Africa and other continents and probably affects sandstone monuments around the world.

11.4 Caves and Other Subterranean Sites

11.4.1 The Cave Environment

Caves with rock art are widespread in several countries in all continents. Many cavities occur at various depths in a cave system due to the continual seepage and flow of water through

the deposits, while underground rivers may eventually carve their way through a mountainside, creating openings and entrances to the outside. Caves are usually in connection with the outdoor environment, and thus subjected to climatic and microclimatic shifts. The most common caves are solutional caves, called limestone caves for the common type of soluble rock in which they form. Limestone caves are widespread all over the world and can range from a few metres to many kilometres in length and depth. Weak carbonic acid reacting with the chemicals in the rock, dissolves and erodes away the limestone as the water filtered into the underlying depths of sediments. Sandstone, also known as arenite, is a sedimentary rock of clastic origin composed mainly of sand-sized minerals or rock grains, mainly quartz and/or feldspar. Depending on the types of mineral component, some sandstones are resistant to weathering, while others are more friable. Sandstone caves are shallow caves that form at the base of cliffs, carved out by water and wind. The water loosens the natural cement holding the sand particles together, then the moving water, while wind carries away the sand grains.

Large hollow limestone cavities and smaller sandstone caves formed over thousands of years have become colonized by a



Fig. 11.5 Indoor development of phototrophic biofilms: (a) Roman frescoes at the Domus aurea and (b) wall paintings at the St. Domitilla catacombs of Rome (Italy) disfigured by the presence of thick biofilms;

(c) Patchy distribution of cyanobacterial colonies on top of the wall and ceiling of a corridor and (d) on the vault of an “arcosolium” all occurring on plaster in close proximity to lamps

range of specialised organisms, including cyanobacteria. The natural cave environment is usually characterised by high relative humidity, although dry caves are also known, stable temperature throughout the year and light gradients provided by solar radiation at the entrances or penetrating through holes in the ceilings (Pentecost and Whitton 2000; Hoffmann 2002).

Caves have always been natural attractions for man and the remarkable mural paintings on the walls of Lascaux caves, in southern France (Lefèvre 1974), and the Altamira caves, in northern Spain, witness the presence of prehistoric men and their artistic ability. Numerous prehistoric caves, which have been naturally closed for thousands of years, have been discovered in the last two centuries, and then opened to the public with dramatic consequences on the cave microclimate (Gonzalez et al. 1999). Stones and mural paintings in caves and other hypogean environments (crypts, tombs, etc.) frequently suffer from biodeterioration (Figs. 11.5 and 11.6), and the scarce literature on this topic is at last starting to increase, though slowly (Urzi et al. 2010). This includes not only cyanobacteria, but new chemoorganotrophic bacterial species.

In general, high values of humidity along with light conditions, and probably also nutrient input, appear to be the

most important factors allowing growth of phototrophs here (Albertano et al. 2009; Pietrini et al. 2009). Caves are usually oligotrophic environments, where primary production depends on well-established autotrophic communities, both chemoautotrophic and photoautotrophic. However, cyanobacterial exudates and cell debris along with inputs of organic matter from above ground may support the growth of several chemoorganotrophic microbes.

The transport of viable cells to underground sites is due to air currents, water flow and contamination by animals and humans. Colonizing microorganisms are usually distributed on the mineral surface layer, but a few develop beneath it or actively bore into the mineral substrata. Chasmoendolithic cyanobacteria were observed in a cave in S-E. Spain (Asencio and Aboal 2000a), while monospecific assemblages formed by *Chlorogloea* sp. or mixed populations of *Chroococcus spelaeus* associated with *Aphanocapsa muscicola*, *Chroococcus turgidus*, *Gloeocapsa bififormis* and *Leptolyngbya gracillima* were found inside a limestone cave in Greece (Lamprinou et al. 2009). Pleurocapsalean endoliths occur on the salted-ceiling of a limestone tunnel at the Edzna pyramid in Yucatan, Mexico (Ortega-Morales et al. 2005).

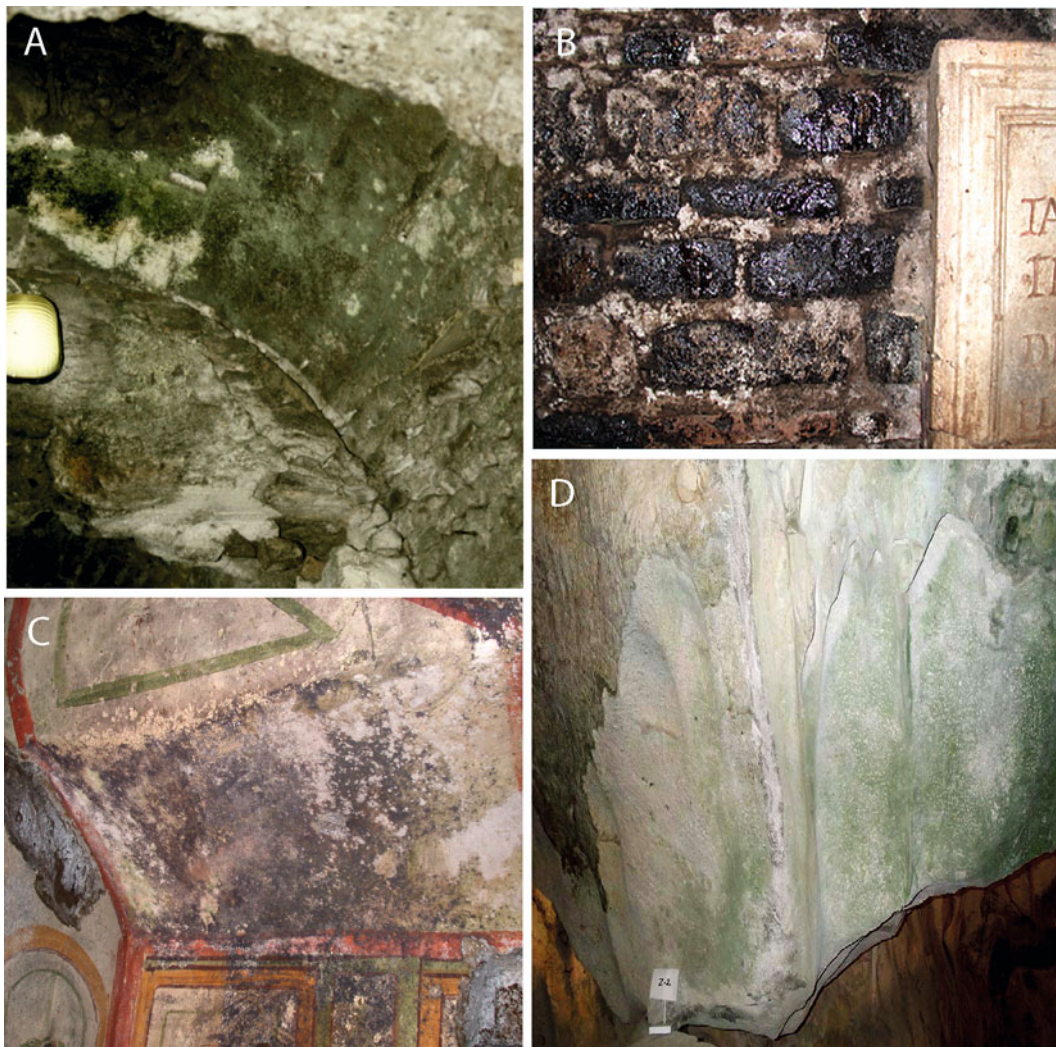


Fig. 11.6 Cyanobacterial biofilms on different lithotypes indoors: (a) Tufa rock at the Catacomb of Priscilla, Rome; (b) Brick wall in St. Domitilla catacombs, Rome; (c) Wall paintings inside the Ocean's

cubiculum at St. Callistus catacombs, Rome; (d) Limestone at the Cave of Bats Zuheros, Cordoba, Spain

In general, phototrophic communities colonize surfaces at the entrances, but most can be found inside tourist caves, where light allows the growth of cyanobacteria, green algae, diatoms and lichens (Roldán et al. 2004, 2006). These associations known as 'lampenflora', develop in natural and artificial caves around lighting sources (Dobat 1998). Since humidity is usually high and temperature stable, the light energy drives microbial communities towards autotrophy that in turn supports associated heterotrophs. The photosynthetic communities that inhabit the caves are mainly epilithic on the surface of rocks speleothems, stalactites and stalagmites, that provide them with a variety of ecological niches (Roldán and Hernández-Maríné 2009).

Similarly to caves, phototrophic biofilms develop inside hypogeal archaeological sites on lithic faces near artificial light sources and in areas adjacent to entrances or openings to the surface, such as wells and air vents. These regions show a

range of microclimatic conditions, reflecting the transition from the outdoor environment to the influence of the constant humidity, temperature and poor air circulation of the inner regions. Much also depends on the size and architecture of the hypogea. In the outdoor-indoor transitional areas, phototrophic communities include saxicolous lichens, which are absent in the deepest parts (Roldán and Hernández-Maríné 2009). Several studies have reported or reviewed the presence of abundant populations of cyanobacteria in caves of Australia, Belgium, China, Croatia, France, Israel, Italy Hungary, Slovenia, Spain, UK and USA (Abdelahad and Bazzichelli 1988; Abdelahad 1989; Hoffmann 1989; Garbacki et al. 1999; Pentecost and Whitton 2000; Smith and Olson 2007; Mulec et al. 2008). Although few studies have attempted to quantify biomas, Mulec et al. (2008) showed that the maximum concentration of chlorophyll a per unit area of lampenflora biofilms in Slovenian karst caves was slightly

Table 11.2 Some recorded ranges (min and max values) of the environmental conditions that supported the development of lithobiontic subaerial populations of cyanobacteria in caves and other archaeological underground sites of the Mediterranean area with different stone substrata

Location and substratum	PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Temperature ($^{\circ}\text{C}$)	Air humidity (RH%)	References
Leontari cave, Attica, Greece – L	0.0001–0.64	11–17.5	77–91.5	Lamprinou et al. (2009)
Koutouki cave, Attica, Greece – L	0.02–0.035	15.7–16.5	92.3–95.1	Lamprinou et al. (2011)
Gelada cave, SE Spain – L	0.001–0.06	5.4–18	55–95	Martinez and Asencio (2010)
L'Aigua cave, SE Spain – L	0.3–(1,254)	15–29.4	24.7–81.5	Beltrán and Asencio (2009)
Cave of Bats, Zuheros, SW Spain – L	0.05–3.8 (18)	8.2–14.4	54–93.8	Urzi et al. (2010)
Catacomb of St. Callistus, Rome, Italy – P	0.01–0.15	18–20	94–95	Albertano and Bellezza (2001)
Catacomb of St. Domitilla, Ipogeo dei Flavi, Rome, Italy – T	0.05–2	17–22	87.5–97.6	Cadel and Albertano (unpublished)
Catacomb of Priscilla, Rome, Italy – T	0.2–0.6	16.5–17	99.0–99.9	Albertano and Urzi (1999)

L limestone, P wall paintings, T tufa rock. Values in brackets refer to the entrance areas

higher ($2.44 \mu\text{g cm}^{-2}$) than that of the epilithic assemblages at the cave entrance (up to $1.71 \mu\text{g cm}^{-2}$). Comparisons of values for the cyanobacterial contribution to phototrophic diversity in caves gave remarkably similar values in Belgium (54%) and Germany (55%) to those in Slovenia (51%) (Mulec et al. 2008). The extensive studies of Slovenian karst caves reported a total of 197 cyanobacterial species.

In a study of the lampenflora of caves in Moravia, Czech Republic, about 20 cyanobacteria species contributed to 31% of the phototrophic diversity at one site (Pouličková and Hašler 2007). Beltrán and Asencio (2009) showed that among the thirteen epilithic and chasmoendolithic cyanobacteria colonizing the walls of L'Aigua cave, S-E Spain, the proportion were 54% Chroococcales, 23% Oscillatoriales and 23% Nostocales. *Calothrix elenkinii*, *Gloeothece confluens* and *Hormoethece cylindrocellulare* were observed for the first time in caves, along with chasmoendolithic growths of *Aphanothece saxicola*, *Gloeothece confluens* and *Pleurocapsa minor*. The values for the Gelada Cave were 77% Chroococcales, 14% Oscillatoriales, 4.5% Nostocales and 4.5% Stigonematales (Martinez and Asencio 2010). Of the 22 species identified, the most common were *Asterocapsa divina*, *Leptolyngbya leptotrichiformis* and *Scytonema julianum*. A decrease of Chroococcales and increase of Oscillatoriales from the entrance to the end of the cave has also been observed in caves of Israel (Vinogradova et al. 1998, 2009).

11.4.2 Light

The characteristically extremely low values for photosynthetic photon flux density (PPFD) of caves and other underground archaeological and historical sites (Table 11.2) can be changed when energy is introduced in the environment as occurs when lighting systems are installed. Variable regimes of natural and artificial light, temperatures and air humidity have been recorded in caves in France (Leclerc et al. 1983), Italy (Albertano and Urzi 1999; Hernández-Mariné et al. 2003), Slovenia and Spain (Asencio and Aboal 1996, 2000b). At the L'Aigua cave mentioned above, cyanobacterial growth

occurred within specific ranges of fluctuation of PPFD and relative humidity (Beltrán and Asencio 2009). In the Gelada Cave the availability of light was the primary stress factor, followed by humidity, lack of nutrients and temperature (Martinez and Asencio 2010). Changes in local air currents caused by warming in the proximity of lamps can also favour microbial growth. Mulec and Kosi (2009) showed the influence of lighting on relative humidity in Slovenian caves with falls from 95% to 73% at 20 cm from a lamp, while temperature could increase by 8°C at 50 cm from a light source.

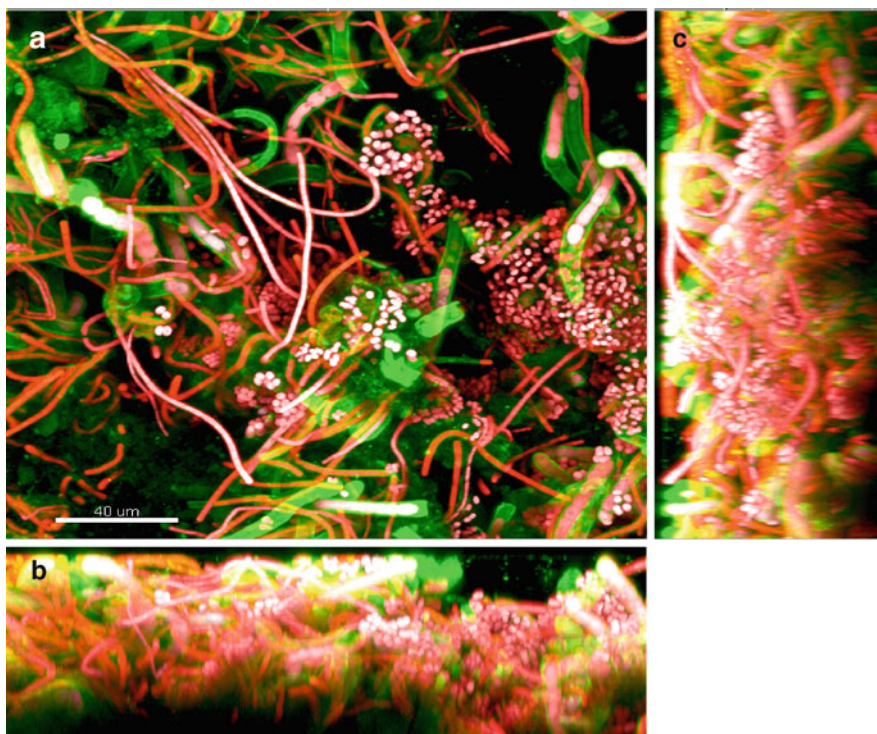
The depth to which phototrophic biofilms extend into the cave interior depends on the light gradients and, more precisely, on the amount of light at various wavelengths available for primary production (Albertano and Bruno 2003). In spite of the restricted emission in the visible part of the spectrum of the lamps used for lighting, numerous troglophilic cyanobacteria have adapted to the particular light conditions and contribute most of the phototrophic biomass (Table 11.3 and Fig. 11.7). The light available for photosynthesis in the inner part of caves and hypogea is usually restricted to periods when visitors are present and the cyanobacterial species composition is mostly determined by the available light (Albertano 1993). In the catacombs of Rome, the illuminated areas supporting phototrophic growth usually extend to around 1.0 m from the lamp. Although the floristic composition was not related clearly to decreasing irradiance, there was a trend for phototroph diversity to decrease under lower irradiances, with the biofilms at the lowest PPFD being uniquely built by erect filaments of *Leptolyngbya* (Hernández-Mariné et al. 2003).

Caves and other types of confined environment open to the public increasingly suffer due to the increases in air temperature, relative humidity and carbon dioxide concentration. The visitor influx also favours the condensation of water, mostly on vaults and the upper part of walls and in turn this all favours photosynthetic carbon fixation (Pulido-Bosch et al. 1997; Sanchez-Moral et al. 2005). The presence of atmospheric pollutants from urban and industrial emissions, namely CO_2 and NO_x , might enhance the growth of biofilms. Microbial diversity in caves can also be greatly

Table 11.3 Some of the cyanobacterial species recently reported for subterranean sites of the Mediterranean area

Species	References
<i>Aphanocapsa muscicola</i>	Lamprinou et al. (2009)
<i>Aphanocapsa parietina</i>	Roldán and Hernández-Mariné (2009), Urzì et al. (2010)
<i>Aphanothece saxicola</i>	Beltrán and Asencio (2009), Martínez and Asencio (2010)
<i>Asterocapsa divina</i>	Aboal et al. (2003), Martínez and Asencio (2010)
<i>Calothrix elenkinii</i>	Beltrán and Asencio (2009)
<i>Chlorogloea microcystoides</i>	Imperi et al. (2007)
<i>Chroococciopsis doonensis</i>	Asencio and Aboal (2000a), Lamprinou et al. (2009)
<i>Chroococcus lithophilus</i>	Imperi et al. (2007)
<i>Chroococcus minor</i>	Tomaselli et al. (2000), Macedo et al. (2009)
<i>Chroococcus spelaeus</i>	Lamprinou et al. (2009), Martínez and Asencio (2010)
<i>Chroococcus turgidus</i>	Lamprinou et al. (2009)
<i>Chroococcus westii</i>	Martínez and Asencio (2010)
<i>Cyanobacterium cedrorum</i>	Martínez and Asencio (2010)
<i>Cyanosaccus aegeus</i>	Martínez and Asencio (2010)
<i>Cyanosaccus atticus</i>	Martínez and Asencio (2010)
<i>Cyanostylon gelatinosus</i>	Albertano and Bellezza (2001)
<i>Cyanostylon microcystoides</i>	Martínez and Asencio (2010)
<i>Eucapsis terrestris</i>	Albertano and Bellezza (2001)
<i>Fischerella maior</i>	Albertano and Urzì (1999)
<i>Geitleria calcarea</i>	Abdelahad and Bazzichelli (1988), Ariño et al. (1997)
<i>Gloeocapsa alpina</i>	Urzì et al. (2010)
<i>Gloeocapsa biformis</i>	Asencio and Aboal (2000a), Imperi et al. (2007), Lamprinou et al. (2009), Martínez and Asencio (2010)
<i>Gloeocapsa kuetzingiana</i>	Imperi et al. (2007)
<i>Gloeocapsa nigrescens</i>	Martínez and Asencio (2010)
<i>Gloeocapsa novacekii</i>	Martínez and Asencio (2010)
<i>Gloeocapsa rupestris</i>	Imperi et al. (2007)
<i>Gloeocapsopsis magma</i>	Roldán and Hernández-Mariné (2009)
<i>Gloeocasa rupicola</i>	Martínez and Asencio (2010)
<i>Gloeothece confluens</i>	Beltrán and Asencio (2009)
<i>Gloeothece membranacea</i>	Albertano and Bellezza (2001), Bellezza and Albertano (2003)
<i>Gloeothece rupestris</i>	Imperi et al. (2007)
<i>Herpyzonema pulverulentum</i>	Hernández-Mariné and Canals (1994), Albertano et al. (2003)
<i>Hormothece cylindrocellulare</i>	Beltrán and Asencio (2009)
<i>Iphinoe spelaebios</i>	Lamprinou et al. (2011)
<i>Leptolyngbya carnea</i>	Martínez and Asencio (2010)
<i>Leptolyngbya gracillima</i>	Asencio and Aboal (2000a), Albertano and Bellezza (2001), Lamprinou et al. (2009)
<i>Leptolyngbya leptotrichiformis</i>	Martínez and Asencio (2010)
<i>Leptolyngbya</i> sp. Green	Bruno et al. (2009)
<i>Leptolyngbya</i> sp. Red	Bruno et al. (2009), Martínez and Asencio (2010)
<i>Loriella osteophila</i>	Albertano et al. (2003), Bellezza et al. (2005)
<i>Loriellopsis cavernicola</i>	Lamprinou et al. (2011)
<i>Myxosarcina</i>	Abdelahad (1989)
<i>Phormidium molle</i>	Asencio and Aboal (2000a), Lamprinou et al. 2009,
<i>Pleurocapsa minor</i>	Beltrán and Asencio (2009), Martínez and Asencio (2010)
<i>Pseudocapsa dubia</i>	Imperi et al. (2007), Lamprinou et al. (2009), Martínez and Asencio (2010)
<i>Scytonema julianum</i>	Pietrini and Ricci (1993), Aboal et al.(1994), Ariño et al. (1997), Albertano and Urzì (1999), Lamprinou et al. (2009), Roldán and Hernández-Mariné (2009), Martínez and Asencio (2010)
<i>Scytonema ocellatum</i>	Albertano and Urzì (1999)
<i>Symphonema cavernicolum</i>	Asencio et al. (1996), Martínez and Asencio (2010)

Fig. 11.7 Epilithic biofilm structure: (a) Coccoid and filamentous cyanobacteria with different morphologies and various autofluorescence due to the photosynthetic pigments provide a picture of the phenotypic diversity within biofilms as shown in confocal laser scanning microscopy by bi-channel extended-focus projection images (*section mode*) that allow lateral view in XZ (b) and YZ (c) of the community layering. Bar = 40 μm



affected by biocidal treatments in combination with other factors resulting from human activity.

11.4.3 The Organisms and Their Response to the Environment

In addition to the floristic information included in the reports mentioned above, there are a number of other detailed accounts of cyanobacteria adapted to extremely low PPFD values. These include ones about wall paintings in Italy (Nugari et al. 2009) and Malta (Zammit et al. 2011a), sinkholes and caves in Spain (Hernández-Marín et al. 2001; Asencio and Aboal 2001, 2004; Roldán et al. 2004; Uher et al. 2005; Urzì et al. 2010), Greece (Lamprinou et al. 2009), Israel (Cor and Dor 1999) and Kentucky, USA (Smith and Olson 2007). Subterranean sites are revealing previously undescribed forms, such as *Leptolyngbya* species (Bruno et al. 2009) and several Stigonematales (Asencio et al. 1996; Lamprinou et al. 2011). The latter include *Iphinoe spelaeobios* gen. nov., sp. nov. in two Greek caves, *Loriellopsis cavernicola* gen. nov., sp. nov. in a cave in Catalonia, and *Symphyonema caverniculum* sp. nov. in Alicante, Spain. Different OTUs have also been described from the palaeo-Christian catacombs of St Agatha and St Paul at Rabat in Malta and trees have been constructed using 16S rRNA gene sequences as a step towards clarifying their taxonomic position (Zammit et al. 2010).

Observations on the ultrastructure of chasmoendolithic *Chroococcidiopsis*, *Cyanosarcina*, *Leptolyngbya*, *Phormidium*

and *Pseudocapsa* in cave cyanobacteria in the Murcia region of S-E. Spain (Asencio and Aboal 2004) showed wide sheaths, a well developed thylakoid system and examples of the all the most widely known cell inclusions (carboxysomes, glycogen and cyanophycin granules, lipid globules, polyphosphate and poly- β -hydroxybutyric bodies). During a study on the morphological and ultrastructural variability of a red *Leptolyngbya* species from a site in Rome and *Rhabdogloea brasiliica* from Brazilian caves using a temperature – light cross-gradient system, the values for both light and temperature optima were found to differ than those recorded *in situ* (Albertano and Kovacik 1996; Azevedo and Kováčik 1996). One remarkable feature of the red *Leptolyngbya* strain VRUC 135, very recently described as *Oculatella subterranea* gen. nov., sp. nov. (Zammit et al. 2012), is its phototactic activity and the presence of an eye-spot like structure made of carotenoid globules and rhodopsin-like pigment at the tip of the apical cell (Albertano et al. 2000a). This complex photoreceptive structure may have an evolutionary importance since it resembles that observed in chloroplast of green flagellates.

Physiological adaptations of cyanobacteria to light limitation in caves and hypogea have seldom been studied. However, Mulec et al. (2008) reported a general increase of chlorophyll *a* and phycobiliprotein contents with decreasing light, features which are widely applicable to cyanobacteria in general. The light saturation values for cyanobacteria in culture range between 50 and 100 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$, with the typical compensation point at about 5–6 $\mu\text{mol photon}$

$\text{m}^{-2} \text{s}^{-1}$. Nevertheless the latter values are higher than PPFD values measured at most sites, as has been shown for a *Phormidium* sp. living in the Frasassi caves, Italy, where the irradiance needed to compensate for respiration, $13 \mu\text{mol photon m}^{-2} \text{s}^{-1}$, exceeded the available light, $6\text{--}10 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ (Giordano et al. 2000). Nevertheless, phototrophic biofilms have been found on the frescoes of the Domus aurea in Rome at PPFD $<0.05 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ with a phycobiliproteins: chlorophyll *a* ratio >4 (Albertano and Grilli Caiola 1989). Most phototrophic biofilms in Roman catacombs develop at $<2 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ (Albertano and Urzì 1999; Albertano and Bellezza 2001). Amperometric measurement with oxygen microelectrodes generally showed high photosynthetic efficiency, photoinhibition at $>200 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ and confirmed low photosynthetic maxima and high respiration rates, possibly due to the heterotrophic associated bacteria (Compagnone et al. 1999). These biofilms, in culture at PPFD ten times those *in situ*, showed sheath thickening and a reduced amount of glycogen in dominant green *Leptolyngbya* species; filamentous bacteria were closely associated with the cell wall of the cyanobacterium (Albertano et al. 1991). Photosynthetic light saturation values $<60 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ have been measured in most of the strains isolated from the Domus aurea and catacombs of Rome including the red ex *Leptolyngbya* (*Oculatella subterranea*) species after acclimation of cultures to PPFD $<10 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ (Bruno and Albertano 1999). These strains possessed high number of phycobilisomes and thylakoids, and high phycobilin to chlorophyll ratios (between 10 and 17) (Bruno and Albertano 1999). Adaptation to cave habitats also includes the ability to use organic sources and grow heterotrophically (Adhikary 2002). *Scytonema coactile*, isolated from the twilight zone of an Indian cave and studied under different light – dark regimes, could use several exogenous sugars for heterotrophic and photoheterotrophic growth (Lakshmi et al. 2008); there was no requirement for protein induction before growth could occur in the dark.

11.4.4 Calcification in Subterranean Sites

Deposition of calcium carbonate on cyanobacterial filaments has been frequently observed in subterranean sites. *Scytonema julianum* is one of the calcifying species able to mobilize calcium ions from mineral substrata, as are *Geitleria calcarea*, *Herpyzonema pulverulentum* and *Loriella* sp. (Hernández-Mariné and Canals 1994; Ariño et al. 1997; Hernández-Mariné et al. 1999) and some *Leptolyngbya* and *Fischerella* spp. (Albertano 1997) (Fig. 11.8).

Scytonema julianum can form an extensive cover on lithic faces with a grey-greenish crusty or powdery surface alterations; it prefers rocks that are rich in calcium carbonate, but

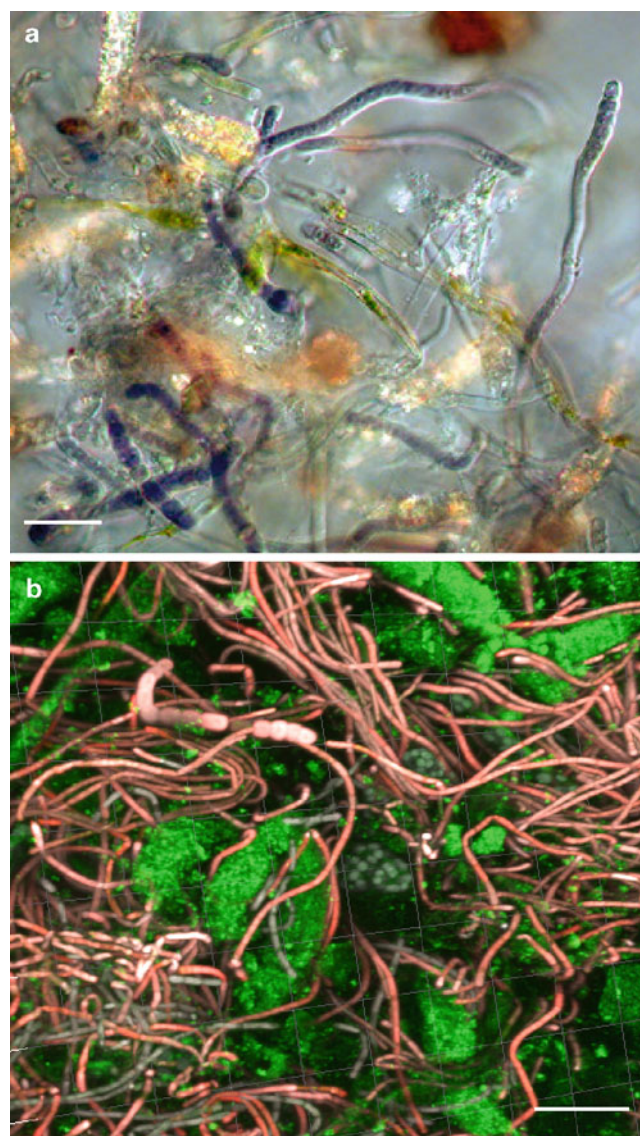
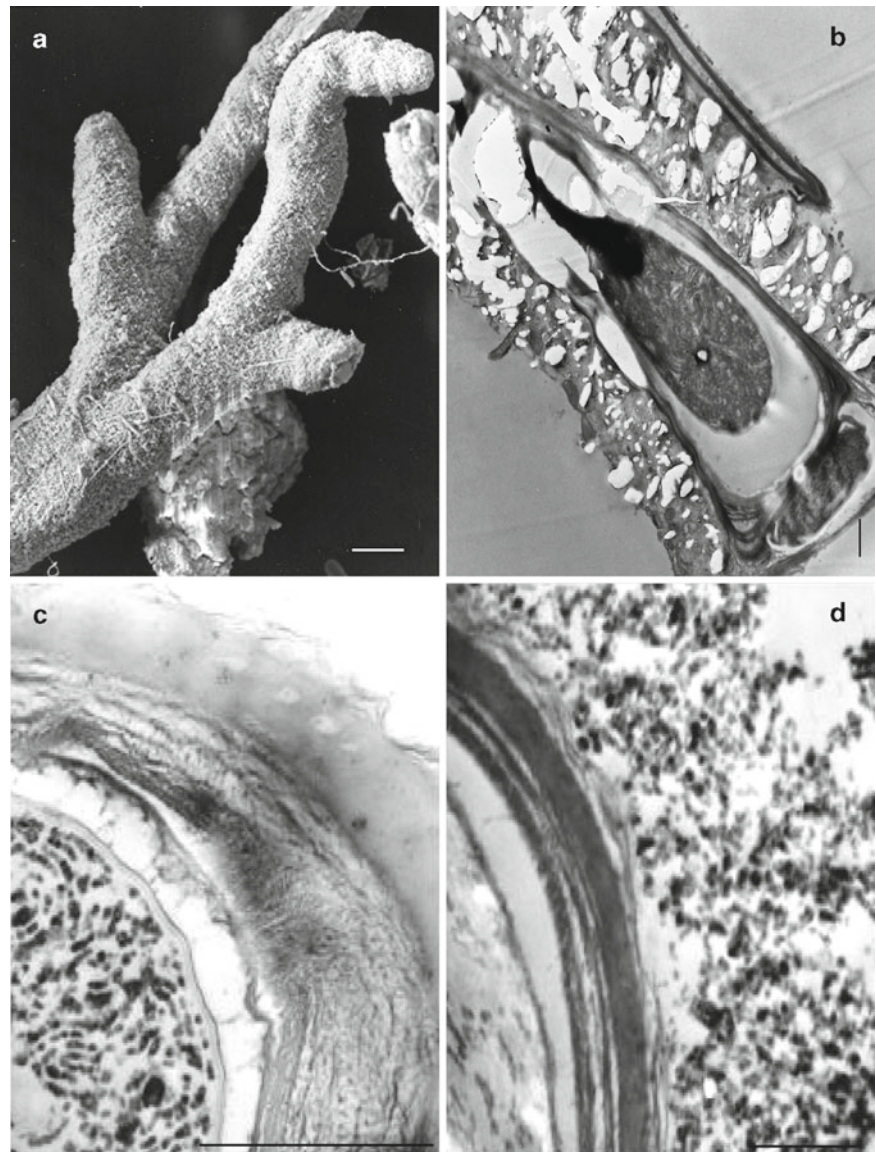


Fig. 11.8 Cyanobacterial communities in low light habitat: (a) Light microscopy of a biofilm fragment), with phycoerythryn- and calcifying filamentous species; (b) Confocal laser scanning microscopy of a *S. julianum* (calcite in green) and thin *Leptolyngbya* (pink) dominated community from St. Domitilla catacombs in Rome (Italy). Bars = 20 μm

never wet. The areas colonized are usually well protected from air currents and characterized by high relative humidity (72–100%) and PPFD up to $8 \mu\text{mol photon m}^{-2} \text{s}^{-1}$. Since it was first reported in the Roman Catacombs of Rome in 1992 (Ortega et al. 1993), it has been found on the Roman mortars of the Carmona necropolis, Spain (Ariño et al. 1997), in the rock church of Matera, Italy (Pietrini and Ricci 1993), and in several natural caves and archaeological hypogea (Albertano and Urzì 1999; Garbacki et al. 1999; Tomaselli et al. 2000; Cañaveras et al. 2001; Sanchez-Moral et al. 2005). In the sheath of *S. julianum* both acidic (glucuronic and galacturonic acids) and sulphated polysaccharides have been shown

Fig. 11.9 Calcifying cyanobacteria: (a) Scanning electron microscopy view of the true-branching filaments of *Loriella* with mineralised sheath; (b) Transmission electron microscopy view of a longitudinal section of *Scytonema julianum* showing calcite crystals deposited within the outermost sheath layer; (c, d) Details of the by-layered sheath in cross-section after cytochemical stain to evidence the presence of polysaccharides throughout the sheath thickness (c) and glycoproteins (d) in the innermost part. Bars = 5 μm (a), 1 μm (b–d)



by cytochemistry, and further confirmed by chromatography and circular dichroism (Albertano and Bellezza 2001; Bellezza et al. 2005), whilst *S. ocellatum* and *Fischerella maior* isolates lacked glucuronic acid (Bellezza et al. 2006). The sheath of *S. julianum* in samples from Roman catacombs appeared to be organised in a thick diffuent outer layer rich in acidic polysaccharides and impregnated by calcite crystals, and an inner dense layer with a complex substructure (Fig. 11.9) (S. Cadel and the author, unpublished data). Negatively charged carboxylic and sulphated groups and positively charged glycoproteins made up the outer mucilage in which triradial calcite crystals (Hoffmann 2002; Ariño et al. 1997) and anastomosing crystals (Aboal et al. 1994) could be observed. The crystals at the sheath surface probably undergo a repeated calcification/decalcification process depending on changes in pH, CO_2 species and calcium con-

centration (Riding 2006). Similarly, incrustation of calcite on *Geitleria calcarea* may be controlled by the organism itself (Pentecost and Whitton 2000). Indeed, photosynthesis linked alkalization has been measured using potentiometric microsensors within cyanobacterial biofilms during illumination with pH shifts above neutrality, sufficient to induce precipitation of carbonates (Albertano et al. 2000b). Precipitation of mineral particles on cells can lead epilithic strains to become endolithic (Asencio and Aboal 2001).

The preference for calcareous substrata is well documented also for *Loriellopsis cavernicola* (previously reported as *Loriella* sp. by Hernández-Mariné et al. 1999) in Spanish caves (Lamprinou et al. 2011). However, PPF values supporting the development of *Loriella osteophila* in Roman catacombs (Fig. 11.9a) were lower, 0.05–2 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$, than for *S. julianum*, which inhabits more illuminated

areas in the catacombs of St Domitilla in Rome (Table 11.2). *Loriella* also secretes a bilayered sheath characterized by complex polysaccharides, carboxylic and sulphated groups and glycoproteins, which are positively charged (S. Cadel and author, unpublished data), and similarly to *Loriellopsis*, both the inner and outer sheath layers are densely covered by crystals in shape of acinose granules or little sticks (Hernández-Mariné et al. 1999).

The presence of carboxylic and sulphated groups and glycoproteins positively charged in the capsular polysaccharides of the red *Leptolyngbya* species and *Fischerella maior* (Bellezza et al. 2003, 2005) adds further support to the suggested role of EPS in calcium accumulation in the sheath, which can favour the mineralisation processes in combination with the photosynthetic accumulation of OH⁻ (Riding 2006).

11.5 Colonization of Stone

Monuments can be considered as pristine environments that soon after their creation (as well as after each cleaning intervention) are exposed to several biophysical and biochemical processes that start with the adhesion of epilithic and endolithic organisms. Because of the autotrophic nature and ecological role of photosynthetic microorganisms, cyanobacteria, microalgae, mosses and lichens play a particular role in the deterioration of stone surfaces.

Passive dispersal of viable cyanobacteria and algae by means of water, air and other organisms is therefore a prerequisite to establish an active population at a particular site. In subaerial environments most microorganisms are transported by air and settle on surfaces where they can grow into biological patinas, the biofilms, where suitable conditions are suitable. Airborne cells and spores of microorganisms together with pollens and other biological particles attach to the exposed surfaces. Since Round (1981, as quoted in Kumar et al. 2007), remarked that there was no information on transport mechanism of algae, their dispersal mechanisms has occasionally received attention. The review by Kumar et al. (2007) about airborne algae reported 32 cyanobacterial species and gave an outline of the environmental factors that control aerosolization and transport, along with the biogeographical implications. The authors indicated that repeated cycles of drying/wetting favour the exfoliation of phototrophic biofilms during the dry period and the transport of airborne cyanobacterial cells spores from the soil by ascending currents to the atmosphere at distances up to 10 km. In confined environments the limited air circulation favours an increase of particle concentration and the chances of settlement on surfaces.

Biofilm development starts when microorganisms adhere to a surface, though adhesion mechanisms vary depending

on the organism and substrate. The successful development of a species is determined by the nature and properties of mineral constituents of the substratum, its pH, salinity, water content, texture and porosity, and by environmental factors as temperature, relative humidity, light conditions, atmospheric pollution levels, wind, and rainfall. Prieto and Silva (2005) assessed potential bioreceptivity of granite varieties before exposure to specific environmental conditions, by measuring intrinsic rock properties such as abrasion pH, bulk density, open porosity and capillary water. All these properties were used to evaluate differences in the rock/water interactions, and hence in the bioreceptivity of granite to cyanobacteria during a 2-months experiment in growth chambers. Restoration treatments can favour microbial colonization when carried out without the appropriate methods and microbiological knowledge, since they may involve the use of inorganic (even water) or organic compounds that can support the growth of autotrophs or heterotrophs (Bastian and Alabouvette 2009).

As soon as microorganisms grow and divide, EPS secretion provides them with a protective highly hydrated matrix, which contributes to an increase in the bulk volume of the biofilm. This dynamic community can then spread across surfaces and incorporate other microorganisms. The production and composition of EPS seem to be remarkably variable depending on the microorganisms and nutritional conditions.

Gliding hormogonia and photoreception, as in red *Leptolyngbya* (*Oculatella subterranea*) species, allow effective colonization of rock substrata. Motility and phototaxis have important roles in success under the low light conditions of the catacombs (Albertano et al. 2000a, b). Furthermore, the ability to secrete sheath-forming EPS rich in polysaccharides allowing adhesion can provide the strength sufficient to adhere to surfaces, as shown for subaerophytic green algae (Karsten et al. 2007). Alternatively, mucilage pads resulting from the lysis of necrotic cells can remain attached to *Scytonema* hormocytes and act as extracellular spots for adhesion (Hernández-Mariné et al. 2001). Six years after restoration of the marbles of the statues in the Boboli Gardens, Florence, Italy, Lamenti et al. (2000) calculated a biofilm cover of 3×10^4 cells cm⁻², these consisting of a green alga and associated cyanobacteria. Laboratory studies on colonisation of stone by cyanobacteria have shown that in some cases appropriate culture conditions may allow reconstruction of natural communities from monuments to compare their capacity to colonize different lithotypes (Miller et al. 2006). Multidimensional and multispecies mathematical models, based on the theory of mixtures, are also developing to describe the cyanobacterial biofilm formation and the time evolution of live and dead cells and EPS on monument surfaces (Clarelli et al. 2009).

11.6 Interactions with Chemoorganotrophs

Although phototrophs are usually the first colonizers of bare rocks, the establishment of heterotrophic communities is possible without the pioneering participation of phototrophs, and may in turn sustain the subsequent growth of photosynthetic populations. In this case, organic matter naturally present in sedimentary rock (perhaps 0.2–2%), airborne particles, organic vapours, excreted metabolites and decaying biomass are used by the heterotrophs along with synthetic or natural organic substances from previous restoration treatments (Warscheid and Braams 2000).

Biodeterioration processes are rarely caused by a single group of microorganisms, and most often a synergistic effect on stone surfaces is achieved by the concomitant growth of phototrophic and heterotrophic populations. Bacteria and fungi can use the organic matter produced by phototrophs to release organic acids that dissolve the minerals of the substratum. Actinobacteria, microcolonial ascomycetous fungi and microscopic green algae are usually present in outdoor habitats, while spore-forming bacteria, troglotic actinobacteria, diatoms and mosses form the subaerial biofilm adapted to low irradiance and high carbon dioxide (and other gases) concentrations. However, apart from the information on biofilms on stone monuments, little is still known about these associations. Scheerer et al. (2009) summarized data on the occurrence of previously unrecognized phototrophic bacteria related to *Chloroflexus* and Ectothiorhodospiraceae in samples at the Mayan site of Uxmal, and gave a short account of the contribution of these and other microorganisms to stone deterioration, their degradative role by acid/alkali production and by chelation along with the presence of chemoautotrophs (sulphur oxidizers, nitrifying bacteria, ferrous and manganese oxidizers).

In caves and confined environments, almost nothing appears to be known about fungi and their interactions with free-living phototrophs (Jurado et al. 2009), but different level of cyanobacterial association with bacteria have been observed *in situ* and in laboratory cultures. The adhesion of bacteria and penetration of actinobacteria into the mucilaginous sheath surrounding the trichomes of *Leptolyngbya* and *Scytonema* has been frequently observed, suggesting the establishment of a distinct syntrophism (Albertano and Urzì 1999). Strains of *Pseudomonas* and *Stenotrophomonas* have been recorded as the dominant airborne bacteria in the catacombs of St. Domitilla and St. Callistus in Rome (Saarela et al. 2004), and subsequently recognized in culture associations. Genome highly iterated octameric palindrome (HIP) sequences were used for PCR-fingerprinting of non-axenic *Leptolyngbya* strains maintained in culture for 11–20 years. These revealed the identity at genus and species levels of Gram-negative and Gram-positive bacteria from

biofilms of the Roman catacombs from which the cyanobacteria had been isolated (Bruno et al. 2006). Other studies have shown non-culturable Acidobacteria as a relevant microbial component associated with cyanobacteria in many cave habitats and hypogea, including catacombs (Zimmerman et al. 2005, 2006). These cyanobacteria/bacteria relationships are regarded as a mutual-beneficial associations, where bacteria benefit from organic substances released from cyanobacteria and in return remineralized inorganic nutrients and carbon dioxide that can be used by the phototrophs. The previously unrecognized diversity of these subterranean sites was also revealed by studies that allowed the detection of new species and the identification of several other taxa of bacteria associated with cyanobacteria (Urzì et al. 2008, 2010 and references therein).

Comparison of fresh and old collected biofilms from sandstone of the Bayon Temple in Angkor, Cambodia, showed that the bacterial community of old biofilm was very similar to the newly formed biofilm in terms of bacterial composition, but the eukaryotic communities were distinctly different between the two (Lan et al. 2010). Microscopy of biofilms from hindu temples in India has shown that Pseudomonadaceae and filamentous and rod-shaped actinobacteria are the most common heterotrophic prokaryotes associated with stress-tolerant cyanobacteria. Together with the fungi *Aspergillus*, *Cladosporium*, *Rhodotorula*, *Trichoderma* and *Ulocladium* they may represent a constant component of those tropical communities (C. Urzì, personal communication).

11.7 Methods for Studying Biodeterioration of Cyanobacterial Biofilms

The measurement of physical, chemical and climatic conditions in the microarea to be sampled, the recording of georeferenced coordinates and visual images of the position in the monument are the basic information needed to map subaerial biofilms on rocks and to understand the role they play in biodeterioration. In the field of cultural heritage the need for a multidisciplinary scientific approach is particularly evident. Archaeologists, restorators and conservators continuously interact with researchers of scientific disciplines to set up dedicated methods, and discover and adapt new prevention and control measures for a specific monument.

To cope with the need of preserving man-made surface during the sampling of biofilms, techniques which do not increase the damage to the underlying substratum are much preferred (Zammit et al. 2008). Non-invasive techniques (NIT) for sampling are those which do not require a sample to be removed from the object and which essentially leave the object in the same state as before the analysis. Non-invasive sampling is undertaken by the application of adhesive tape strips (Gaylarde and Gaylarde 2000; Urzì and De

Leo 2001) and sterile humid paper filters to the surface to ensure the removal of biofilm and cells only. Non-destructive techniques (NDT) are used for analyses, while respecting the physical integrity of the sample. In addition, the removal of low-invasive micro-samples (1×1 mm) including a portion of the underlying substratum, can sometimes be permitted to investigate the interaction of the biofilm with the underlying substratum. This type of sample can subsequently be processed for structural and chemical analysis by non-destructive techniques using confocal laser scanning microscopy and environmental scanning electron microscopy coupled to Energy Dispersive X-Ray Spectroscopy (SEM-EDS), X-ray micro-fluorescence (μ XRF) and X-ray micro-diffraction (μ XRD) (Cuezva et al. 2009; Zammit et al. 2011b).

The application of light and electron microscopy to the study of biofilm-forming microbial communities is commonly used in the visualization of cyanobacterial biofilms, providing the means to characterize structural interactions between species, and resolving ultrastructural details at a micro- and nanometer scale. A variety of microscopy techniques (Fig. 11.7), are applied for the examination of microorganisms in the hypogean environment to assess their cytomorphological features and nutritional status (Albertano et al. 1991, 2003). Light microscopy epifluorescence, confocal laser microscopy scanning, and transmission electron microscopy allow to understand the organism relationships within the polymicrobial population and the interactions between mineral substrata and microorganisms by using micro-samples (Urzi and Albertano 2001).

These studies include the assessment of microbial diversity on surfaces. To improve conservation strategies relevant to the particular biofilm, with its structure and species, culture-dependent and culture-independent techniques are applied to study the genetic diversity of the biofilm. Because of the limitations of culture-based methods, molecular approaches such as those based on fluorescence in situ hybridization (FISH) techniques (La Cono and Urzi 2003) and those involving amplification of the 16S rRNA gene as universal phylogenetic markers are widely used to retrieve essential information on the structure of the communities. Thanks to the small amount of sample required by these approaches, denaturing gradient gel electrophoresis (DGGE) and single strand conformation polymorphism (SSCP) can be used to unravel the high diversity that cultural methods could resolve only poorly, and have become essential to investigate the microbial aetiology of the biodeterioration of monuments and art objects (Gonzalez and Saiz-Jimenez 2005; Miller et al. 2008, 2009; Macedo et al. 2009).

The convenience of combining 16S rRNA-based methods, i.e. ARDRA amplified rDNA restriction analysis and DGGE, with micro-Raman spectroscopy analysis for the identification of the aetiological agent(s) of a specific microbial-induced deterioration process on cave paintings has

been reported by Hernanz et al. (2006) and Imperi et al. (2007). In many conservation laboratories, Raman spectroscopy has gained a leading position among standard techniques in the investigation of pigments from art and antiquities by virtue of its non-destructive nature and its ability to provide molecular information on a micrometer scale along with its applicability to both inorganic and organic substances.

Various methodological approaches have been developed to standardize rules and protocols for cultural heritage (Fassina 2008), along with specific methods to investigate phototrophic biofilms on stone (Albertano 2003; Albertano et al. 2003; Urzi and Albertano 2001).

Particularly important are the monitoring light detection and ranging (LIDAR) technique for the remote sensing of photoautotrophic biodeteriogens (Raimondi et al. 2009) and techniques based on the recording of visible light emission by portable spectroradiometry (Bruno et al. 2001; Albertano and Bruno 2003; Albertano et al. 2005), and spectral fingerprints (Roldán et al. 2004; Polerecky et al. 2009). The latter are usually applied to study the role of the spatial organization of microorganisms in the ecological functioning of complex microbial communities and for non-invasive monitoring of changes in the spatial organization and/or composition of a microbial community in response to environmental factors. Other spectral imaging systems have been developed for minimally invasive identification, localization, and relative quantification of pigments in cells and microbial communities. For pigment identification *in vivo* absorption and/or autofluorescence spectra are used as the analytical signals in CLSM.

The increasing interest in non-invasive approaches of monitoring and assessing damage and biodecay processes has boosted the use of microsensors to study changes in composition of chemical species mobilized upon stone surfaces during microbial metabolism. The growth of cyanobacterial films can induce more or less pronounced variation of the chemical parameters that characterize a microhabitat, and might possibly cause deleterious deteriorogenic effects on the substrata. Amperometric oxygen microsensors were applied to measure photosynthesis and respiration in phototrophic biofilms that develop in Roman catacombs in order to record simultaneously curves of photosynthesis at increasing irradiance (P/I) (Compagnone et al. 1999), to measure phosphate concentration within biofilms (Calvo Quintana et al. 2004) and to apply potentiometric microelectrodes for the measurements of pH, K^+ and Ca^{2+} (Calvo Quintana et al. 2002). A decrease of potassium concentration occurred during light exposure in strains of *Scytonema julianum*, perhaps due to active uptake sustained by the photosynthetic activity. No appreciable decrease of soluble calcium due to the metabolic activity was observed in natural and artificial biofilms of the same species unless accumulation occurred in low active biofilms.

A final comment has to be made on the general approaches adopted in avoiding cyanobacterial growth on lithic faces. Among physical methods, monochromatic lamps resulted the most effective in reducing biofilm development in Roman catacombs (Albertano et al. 2005, 2007) and in Spanish cave (Roldán and Hernández-Mariné 2008). The established use of biocides, although with low toxicity, is still debated because of the unwanted effects on environment and human health, and in selecting microorganisms (Adhikary 2000a; Urzì and De Leo 2007; Bastian and Alabouvette 2009; Cappitelli et al. 2009; Nugari et al. 2003, 2009). In subterranean sites, a suitable combination of physical and chemical approaches can represent the best solution, while alternative methods may be based on the development and application of nanocomposites (Rodea-Palomares et al. 2010).

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References

- Abdelahad N (1989) On four *Myxosarcina*-like species (Cyanophyta) living in the Infernglio cave (Italy). *Arch Hydrobiol Suppl Algal Stud* 54:3–13
- Abdelahad N, Bazzichelli G (1988) *Geitleria calcarea* Friedmann, Cyanophycée cavernicole nouvelle pour l'Italie. *Nova Hedwig* 46:265–270
- Aboal M, Ad A, Prefasi M (1994) Studies on cave cyanophytes from Southeastern Spain: *Scytonema julianum* Meneghini. *Arch Hydrobiol Suppl Algal Stud* 75:31–36
- Aboal M, Ad A, Lopez-Jiménez E (2003) Morphological, ultrastructural and ecological study of *Asterocapsa divina* Komárek (Chroococaceae, Cyanobacteria) from a cave of SouthEastern Spain. *Arch Hydrobiol Suppl Algal Stud* 109:57–65
- Adhikary SP (2000a) Chlorophyll stability of epilithic cyanobacteria from temples of India. *Arch Hydrobiol Suppl Algal Stud* 98:119–131
- Adhikary SP (2000b) Epilithic cyanobacteria on the exposed rocks and walls of temples and monuments of India. *Indian J Microbiol* 40:67–81
- Adhikary SP (2002) Survival in darkness and heterotrophic growth of epilithic cyanobacteria from temples of India. *Arch Hydrobiol Suppl Algal Stud* 105:141–155
- Adhikary SP (2004) Survival strategies of lithophytic cyanobacteria on the temples and monuments. In: Jain PC (ed) *Microbiology and biotechnology for sustainable development*. CBS Publishers and Distributors, New Delhi, pp 187–194
- Adhikary SP, Satapathy DP (1996) *Tolypothrix byssoidea* (Cyanophyceae/Cyanobacteria) from temple rock surfaces of coastal Orissa, India. *Nova Hedwig* 62:419–423
- Albertano P (1993) Epilithic algal communities in hypogean environments. *G Bot Ital* 127:386–392
- Albertano P (1997) Elemental mapping as a tool in the understanding of microorganisms-substrate interactions. *J Comp Assist Microsc* 9: 81–84
- Albertano P (2003) Methodological approaches to the study of stone alteration caused by cyanobacterial biofilms in hypogean environments. In: Koestler RJ, Koestler VR, Charola AE, Nieto-Fernandez FE (eds) *Art, biology, and conservation: biodeterioration of works of art*. The Metropolitan Museum of Art, New York, pp 302–315
- Albertano P, Bellezza S (2001) Cytochemistry of cyanobacterial exopolymers in biofilms from Roman hypogea. *Nova Hedwig* 123: 501–518
- Albertano P, Bruno L (2003) The importance of light in the conservation of hypogean monuments. In: Saiz-Jimenez C (ed) *Molecular biology and cultural heritage*. Swets and Zeitlinger BV, Lisse, pp 171–177, 287 pp
- Albertano P, Grilli Caiola M (1989) A hypogean algal association. *Braun Blanquetia* 3:386–392
- Albertano P, Kovacic L (1996) Light and temperature responses of terrestrial sciaphilous strains of *Leptolyngbya* sp. in cross-gradient cultures. *Arch Hydrobiol Suppl Algal Stud* 83:17–28
- Albertano P, Urzì C (1999) Structural interactions among epilithic cyanobacteria and heterotrophic microorganisms in Roman hypogea. *Microb Ecol* 38:244–252
- Albertano P, Luongo L, Grilli Caiola M (1991) Observations on cell structure of micro-organisms o fan epilithic phototrophic community competing for light. *Nova Hedwig* 53:369–381
- Albertano P, Barsanti L, Passarelli V, Gualtieri P (2000a) A complex photoreceptive structure in the cyanobacterium *Leptolyngbya* sp. *Micron* 31:27–34
- Albertano P, Bruno L, D'Ottavi D, Moscone D, Palleschi G (2000b) Effect of photosynthesis on pH variation in cyanobacterial biofilms from Roman catacombs. *J Appl Phycol* 12:279–384
- Albertano P, Moscone D, Palleschi G, Hermosín B, Saiz-Jimenez C, Sánchez-Moral S, Hernández-Mariné M, Urzì C, Groth I, Schroeckh V, Saarela M, Mattila-Sandholm T, Gallon JR, Graziottin F, Bisconti F, Giuliani R (2003) Cyanobacteria attack rocks (CATS): control and preventive strategies to avoid damage caused by cyanobacteria and associated microorganisms in Roman hypogean monuments. In: Saiz-Jimenez C (ed) *Molecular biology and cultural heritage*. Swets and Zeitlinger BV, Lisse, pp 151–162, 287 pp
- Albertano P, Bruno L, Bellezza S (2005) New strategy for the monitoring and control of cyanobacterial films on valuable lithic faces. *Plant Biosyst* 139:311–322
- Albertano P, Bruno L, Piermarini S, Bellezza S (2007) Monochromatic light and portable spectroradiometry for the conservation of stone monuments affected by phototrophic micro-organisms. In: Drdacky M, Chapuis M (eds) *Safeguarded cultural heritage – understanding and viability of the enlarged Europe*, vol 2. Glos Semily, Praha, pp 814–817
- Albertano P, Urzì C, Caneva G (2009) Problems of biodeterioration in relation to particular types of environments. – Tombs, Catacombs and other Hypogea. In: Caneva G, Nugari MP, Salvadori O (eds) *Plant biology for cultural heritage*. Getty Conservation Institute, Los Angeles, pp 184–189
- Ariño X, Hernández-Mariné M, Saiz-Jiménez C (1997) Colonization of Roman tombs by calcifying cyanobacteria. *Phycologia* 36:366–373
- Asencio AD, Aboal M (1996) Cyanophytes from Andragulla cave (Murcia, SE Spain) and their environmental conditions. *Arch Hydrobiol Suppl Algal Stud* 83:55–72
- Asencio AD, Aboal M (2000a) A contribution to knowledge of chasmoendolithic algae in cave-like environments. *Arch Hydrobiol Suppl Algal Stud* 98:133–151
- Asencio AD, Aboal M (2000b) Algae from La Serreta cave (Murcia, SE Spain) and their environmental conditions. *Arch Hydrobiol Suppl Algal Stud* 96:59–78

- Asencio AD, Aboal M (2001) Biodeterioration of wall paintings in caves of Murcia (SE Spain) by epilithic and chasmoendolithic algae. *Arch Hydrobiol Suppl Algal Stud* 103:131–142
- Asencio AD, Aboal M (2004) Cell inclusions in the chasmoendolithic Cyanophytes from cave-like environments in Murcia (SE Spain). *Arch Hydrobiol Suppl Algal Stud* 113:117–127
- Asencio AD, Aboal M, Hoffmann L (1996) A new cave-inhabiting blue-green alga: *Symphonema cavernicolum* sp. nov. (Mastigocladaceae, Stigonematales). *Arch Hydrobiol Suppl Algal Stud* 83:73–82
- Azevedo MT, Kováčik L (1996) *Rhabdogloea brasiliica* sp. nov. (Chroococcales, Synechococcaceae): morphological and morphometric variability under cross-gradient cultures. *Arch Hydrobiol Suppl Algal Stud* 83:83–92
- Barberousse H, Lombardo RJ, Tell G, Couté A (2006a) Factors involved in the colonisation of building façades by algae and cyanobacteria in France. *Biofouling* 22:69–77
- Barberousse H, Tell G, Yéprémian C, Couté A (2006b) Diversity of algae and cyanobacteria growing on building façades in France. *Arch Hydrobiol Suppl Algal Stud* 120:81–105
- Bastian F, Alabouvette C (2009) Lights and shadows on the conservation of a rock art cave: the case of Lascaux Cave. *Int J Speleol Spec Issue Cave Microbiol* 38:55–60
- Bellezza S, Albertano P (2003) A Chroococcalean species from Roman hypogean sites: characterisation of *Gloeothece membranacea* (cyanobacteria, Synechococcaceae). *Arch Hydrobiol Suppl Algal Stud* 109:103–112
- Bellezza S, Paradossi G, De Philippis R, Albertano P (2003) *Leptolyngbya* strains from Roman hypogea: cytochemical and physico-chemical characterisation of exopolysaccharides. *J Appl Phycol* 15:193–200
- Bellezza S, Albertano P, De Philippis R, Paradossi G (2005) Exopolysaccharides in cyanobacterial biofilms from Roman catacombs. *Arch Hydrobiol Suppl Algal Stud* 117:117–132
- Bellezza S, De Philippis R, Paradossi G, Albertano P (2006) Exopolysaccharides of two cyanobacterial strains from Roman hypogea. *Geomicrobiol J* 23:301–310
- Beltrán JA, Asencio AD (2009) Cyanophytes from the L'Aigua cave (Alicante, SE Spain) and their environmental conditions. *Algal Stud* 132:21–34
- Billi D (2009) Subcellular integrities in *Chroococcidiopsis* sp. CCME029 survivors after prolonged desiccation revealed by molecular probes and genome stability assays. *Extremophiles* 13:49–57
- Billi D (2012) Anhydrobiotic rock-inhabiting cyanobacteria: potential for astrobiology and biotechnology. In: Stan-Lotter H, Fendrihan F (eds) *Adaptation of microbial life organisms in extreme environments: research and application*. Springer, Wien New York, pp 119–132
- Billi D, Potts M (2000) Life without water: responses of prokaryotes to desiccation. In: Storey KB, Storey JM (eds) *Cell and molecular response to stress: environmental stressors and gene responses*. Elsevier, Amsterdam, pp 181–192
- Billi D, Potts M (2002) Life and death of dried prokaryotes. *Res Microbiol* 153:7–12
- Billi D, Viaggiu E, Cockell CS, Rabbow E, Horneck G, Onofri S (2011) Damage escape and repair in dried *Chroococcidiopsis* spp. from hot and cold deserts exposed to simulated space and Martian conditions. *Astrobiology* 11:65–73
- Bruno L, Albertano P (1999) Photoacclimation of sciaphilous epilithic cyanobacteria isolated from Roman hypogea. *Arch Hydrobiol Suppl Algal Stud* 94:89–103
- Bruno L, Piermarini S, Albertano P (2001) Characterisation of spectral emission by cyanobacterial biofilms in the Roman Catacombs of Priscilla in Rome (Italy). *Nova Hedwig* 123:229–236
- Bruno L, Billi D, Urzì C, Albertano P (2006) Genetic characterisation of epilithic cyanobacteria and their associated bacteria. *Geomicrobiol J* 23:293–299
- Bruno L, Billi D, Bellezza S, Albertano P (2009) Cytomorphological and genetic characterization of troglophilic *Leptolyngbya* strains isolated from Roman hypogea. *Appl Environ Microbiol* 75:608–617
- Büdel B (1999) Ecology and diversity of rock-inhabiting cyanobacteria in tropical regions. *Eur J Phycol* 34:361–370
- Büdel B, Weber B, Kühl M, Pfanz H, Sültemeyer D, Wessels D (2004) Reshaping of sandstone surfaces by cryptoendolithic cyanobacteria: bioalkalization causes chemical weathering in arid landscapes. *Geobiology* 2:261–268
- Büdel B, Bendix J, Bicker FR, Allan Green TG (2008) Dewfall as a water source frequently activates the endolithic cyanobacterial communities in the granites of Taylor Valley, Antarctica. *J Phycol* 44:1415–1424
- Büdel B, Darienko T, Deutschewitz K, Stephanie Dojani S, Friedl T, Mohr KI, Salisch M, Reisser W, Weber B (2009) Southern African biological soil crusts are ubiquitous and highly diverse in drylands, being restricted by rainfall frequency. *Microb Ecol* 57:229–247
- Calvo Quintana J, Piermarini S, Albertano P, Palleschi G, Moscone D (2002) Potentiometric microsensors for cyanobacterial biofilms monitoring in hypogean environments. In: Di Natale C, D'Amico A, Dori L, Cardinali GC, Nicoletti S (eds) *Sensors and microsystems*. World Scientific, River Edge, pp 270–274
- Calvo Quintana J, Idrissi L, Palleschi G, Albertano P, Amine A, El Rhazi M, Moscone D (2004) Investigation of amperometric detection of phosphate. Application in seawater and cyanobacterial biofilm samples. *Talanta* 63:567–574
- Cañaveras JC, Sanchez-Moral S, Soler V, Saiz-Jimenez C (2001) Microorganisms and microbially induced fabrics in cave walls. *Geomicrobiol J* 18:1521–1529
- Caneva G, Nugari MP, Salvadori O (2009) *Plant biology for cultural heritage*. Getty Conservation Institute, Los Angeles, 408 pp
- Cappitelli F, Abbruscato P, Foladori P, Zanardini E, Ranalli G, Principi P, Villa F, Polo A, Sorlini C (2009) Detection and elimination of cyanobacteria from frescoes: the case of the St. Brizio Chapel (Orvieto Cathedral, Italy). *Microb Ecol* 57:633–639
- Castenholz RW, Garcia-Pichel F (2000) Responses to UV-radiation. In: Whitton BA, Potts M (eds) *The ecology of cyanobacteria: their diversity in time and space*. Kluwer Academic Publishers, Dordrecht, pp 591–611, 669 pp
- Ciferri O (1999) Microbial degradation of paintings. *Appl Environ Microbiol* 65:879–885
- Clarelli F, Di Russo C, Natalini R, Ribot M (2009) Mathematical models for biofilms on the surface of monuments. In: De Bernardis E, Spigler R, Valente V (eds) *Advances in mathematics for applied science* 82. World Scientific Publication, Singapore, pp 220–231
- Compagnone D, Di Carlo V, Bruno L, Albertano P, Palleschi G (1999) Development of oxygen microsensor for monitoring cyanobacterial photosynthesis in Roman hypogea. *Anal Lett* 32:213–222
- Crispim CA, Gaylarde CC (2005) Cyanobacteria and biodeterioration of cultural heritage: a review. *Microb Ecol* 49:1–9
- Crispim CA, Gaylarde PM, Gaylarde CC (2003) Algal and cyanobacterial biofilms on calcareous historic buildings. *Curr Microbiol* 46:79–82
- Cuezva S, Sanchez-Moral S, Saiz-Jimenez C, Canaveras JC (2009) Microbial communities and associated mineral fabrics in Altamira caves. *Int J Speleol* 38:83–92
- Cuzman OA, Ventura S, Sili C, Mascalchi C, Turchetti T, D'Acqui LP, Tiano P (2010) Biodiversity of phototrophic biofilms dwelling on monumental fountains. *Microb Ecol* 60:81–95
- Darienko T, Hoffmann L (2003) Algal growth on cultural monuments in Ukraine. *Biologia* 56:575–587
- de los Rios A, Grube M, Sancho LG, Ascaso C (2007) Ultrastructural and genetic characteristics of endolithic cyanobacterial biofilms colonizing Antarctic granite rocks. *FEMS Microbiol Ecol* 59:386–395
- De Philippis R, Vincenzini M (1998) Exocellular polysaccharides from cyanobacteria and their possible applications. *FEMS Microbiol Rev* 22:151–175

- Dobat K (1998) Flore de la lumière artificielle (lampenflora-maladie verte). In: Juberthie C, Deçu V (eds) *Encyclopaedia Biospeologica*, Tome 2. Société de Biospéologie, Moulis-Bucarest, pp 1325–1335
- Dor I, Dor Y (1999) Cyanobacterial flora of the Soreq stalactite Cave (Israel) and way of its control. *Arch Hydrobiol Suppl Algal Stud* 94:115–120
- Fassina V (2008) European Technical Committee 346 – Conservation of Cultural Property – Updating of the activity after a three-year period. In: Notea A, Shoef Y (eds) *Art 2008: non-destructive testing, microanalysis and preservation in the conservation of cultural and environmental heritage*. ISAS International Seminars Ltd., Jerusalem, pp 1–9
- Fleming ED, Castenholz RW (2007) Effects of periodic desiccation on the synthesis of the UV-screening compound, scytonemin, in cyanobacteria. *Environ Microbiol* 9:1448–1455
- Foster JS, Green SJ, Ahrendt SR, Golubic S, Reid RP, Hetherington KL, Bebout L (2008) Molecular and morphological characterization of cyanobacterial diversity in the stromatolites of Highborne Cay, Bahamas. *ISME J* 3:573–587
- Friedmann EI, Ocampo-Friedmann R (1984) Endolithic microorganisms in extreme dry environments: analysis of alithobiontic microbial habitat. In: Klug MJ, Reddy CA (eds) *Microbial ecology*. American Society for Microbiology, Washington, DC, pp 177–185
- Fusey P, Hyvert G (1964) Les altérations physico-chimiques et biologiques des gres des monuments Khmers. *Compt Rend Hebd Séances Groupes* 13, 258(26):6573–6575 (in French)
- Garbacki N, Ector L, Kostikov I, Hoffmann L (1999) Contribution à l'étude de la flore des grottes de Belgique. *Belg J Bot* 132:43–76
- Gaylarde PM, Gaylarde CC (2000) Algae and cyanobacteria on painted buildings in Latin America. *Int Biodeter Biodegr* 46:93–97
- Gaylarde PM, Gaylarde CC (2005) A comparative study of the major microbial biomass of biofilms on exteriors of buildings in Europe and Latin America. *Int Biodeter Biodegr* 55:131–139
- Gaylarde CC, Morton LHG (2003) Biodeterioration of mineral materials. In: Bitton G (ed) *Encyclopedia of environmental microbiology*. Wiley, New York, pp 516–527
- Gaylarde PM, Gaylarde CC, Guimet P, Gomez de Saravia S, Videla H (2001) Biodeterioration of Mayan buildings at Uxmal and Tulum, Mexico. *Biofouling* 17:41–45
- Giordano M, Mobili F, Pezzoni V, Hein MK, Davis JS (2000) Photosynthesis in the caves of Frasassi (Italy). *Phycologia* 39: 384–389
- Golubic S (1967) Algenvegetation der Felsen, eine ökologische Algenstudie im dinarischen Karstgebiet. In: Elster HJ, Ohle W (eds) *Die Binnengewässer*, vol 23. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, p 183
- Golubic S, Friedmann I, Schneider J (1981) The lithobiontic ecological niche, with special reference to microorganisms. *J Sediment Petrol* 5:475–478
- Gonzalez JM, Saiz-Jimenez C (2005) Application of molecular nucleic acid-based techniques for the study of microbial communities in monuments and artworks. *Int Microbiol* 8:189–194
- Gonzalez I, Laiz L, Hermosin B, Caballero B, Incerti C, Saiz-Jimenez C (1999) Bacteria isolated from rock art paintings: the case of Atlanterra shelter (South Spain). *J Microbiol Methods* 36:123–127
- Gorbushina AA (2007) Life on the rocks. *Environ Microbiol* 9:1613–1631
- Gorbushina AA, Broughton WJ (2009) Microbiology of the atmosphere-rock interface: how biological interactions and physical stresses modulate a sophisticated microbial ecosystem. *Annu Rev Microbiol* 63:431–450
- Grbić ML, Vukojević J, Subakov Simić G, Krizmanić J, Stupar M (2010) Biofilm-forming cyanobacteria, algae and fungi on two historic buildings in Belgrade, Serbia. *Arch Biol Sci Belgrad* 62:625–631
- Grilli Caiola M, Billi D (2007) *Chroococciopsis* from desert to Mars. In: Seckbach J (ed) *Algae and cyanobacteria in extreme environments*. Springer, Dordrecht, pp 555–568
- Hernández-Mariné M, Canals T (1994) *Herpyzonema pulverulentum* (Mastigocladaceae), a new cavernicolous atmophytic and lime-encrusted cyanophyte. *Arch Hydrobiol Algal Stud Suppl* 75:123–136
- Hernández-Mariné M, Asencio AD, Canals A, Ariño X, Aboal M, Hoffmann L (1999) Discovery of populations of the lime-encrusting genus *Loriella* (Stigonematales) in Spanish caves. *Arch Hydrobiol Algal Stud Suppl* 94:121–138
- Hernández-Mariné M, Roldán M, Clavero E, Canals A, Ariño X (2001) Phototrophic biofilm morphology in dim light. The case of the Puigmoltó sinkhole. *Nova Hedwig* 123:237–253
- Hernández-Mariné M, Clavero E, Roldán M (2003) Why there is such luxuriant growth in the hypogean environments. *Arch Hydrobiol Suppl Algal Stud* 109:229–240
- Hernanz A, Mas M, Gavilán B, Hernández B (2006) Raman microscopy and IR spectroscopy of prehistoric paintings from Los Murciélagos cave (Zuheros, Córdoba, Spain). *J Raman Spectrosc* 37:492–497
- Hoffmann L (1989) Algae of terrestrial habitats. *Bot Rev* 55:77–105
- Hoffmann L (2002) Caves and other low-light environments: aerophytic photoautotrophic microorganisms. In: Bitton G (ed) *Encyclopedia of environmental microbiology*. Wiley, New York, pp 835–843
- Imperi F, Caneva G, Laura Cancellieri L, Ricci MA, Sodo A, Visca P (2007) The bacterial aetiology of rosy discoloration of ancient wall paintings. *Environ Microbiol* 8:2894–2902
- Jaag O (1945) Untersuchungen über die Vegetation und Biologie der Algen des nackten Gesteins in den Alpen, im Jura und im schweizerischen Mittelland. *Beitr Kryptogamenflora Schweiz* 9:1–560
- Jurado V, Fernandez-Cortes A, Cuezva S, Laiz L, Canaveras JC, Sanchez-Moral S, Saiz-Jimenez C (2009) The fungal colonisation of rock-art caves: experimental evidence. *Naturwissenschaften* 96:1027–1034
- Karsten U, Schumann R, Mostaert AS (2007) Aeroterrestrial algae growing on man-made surfaces. In: Seckbach J (ed) *Algae and cyanobacteria in extreme environments*. Springer, Dordrecht, pp 585–597, 811 pp
- Kaštovský J, Hauer T, Komárek J, Skácelová O (2010) The list of cyanobacterial species of the Czech Republic at the end of 2009. *Fottea* 10:235–249
- Komárek J (2010) Recent changes (2008) in cyanobacteria taxonomy based on a combination of molecular background with phenotype and ecological consequences (genus and species concept). *Hydrobiologia* 639:245–259
- Komárek J, Anagnostidis K (1998) Cyanoprokaryota I. Teil: Chroococcales. In: Ettl H, Gärtner G, Heynig H, Mollenhauer D (eds) *Süßwasserflora von Mitteleuropa* 19/1. Gustav Fischer, Jena/Stuttgart/Lübeck/Ulm, 548 pp
- Komárek J, Anagnostidis K (2005) Cyanoprokaryota 2. Teil: Oscillatoriales. In: Büdel B, Krienitz L, Gärtner G, Schagerl M (eds) *Süßwasserflora von Mitteleuropa* 19/2. Elsevier GmbH, Spektrum Akademischer Verlag, Heidelberg, 759 pp
- Komárek J, Hauer T (2010) *CyanoDB.cz* – On-line database of cyanobacterial genera. –World-wide electronic publication, University of South Bohemia and Institute of Botany ASCR. <http://www.cyanodb.cz>
- Kováčik L (2000) Cyanobacteria and algae as agents of biodeterioration of stone substrata of historical buildings and other cultural monuments. In: Choi S, Suh M (eds) *New millenium international forum on conservation of cultural property*. Institute of Conservation Science for Cultural Heritage, Kongju, pp 44–58
- Kumar R, Kumar AV (1999) Biodeterioration of stone in tropical environments – an overview. *J. Paul Getty Trust, Los Angeles*, 85 pp
- Kumar NS, Kumar R, Singh S, Brown RM Jr (2007) Airborne algae: their present status and relevance. *J Phycol* 43:615–627

- La Cono V, Urzi C (2003) Fluorescent *in situ* hybridization (FISH) applied on samples taken with adhesive tape strips. *J Microbiol Methods* 55:65–71
- Lakshmi KS, Adhikary SP (2008) Diversity of microalgae and cyanobacteria on building facades and monuments of India. *Algae* 23:91–114
- Lakshmi KS, Jnanendra R, Adhikary SP (2008) Growth response and protein profile of two different *Scytonema* species from cave walls and soil crusts in light and dark. *Algal Stud* 127:49–60
- Lamenti G, Tiano P, Tomaselli L (2000) Biodeterioration of ornamental marble statues in the Boboli Garden (Florence, Italy). *J Appl Phycol* 12:427–433
- Lamprinou V, Pantazidou A, Papadogiannaki G, Radea C, Economou-Amilli A (2009) Cyanobacteria and associated invertebrates in Leontari Cave, Attica (Greece). *Fottea* 9:155–164
- Lamprinou V, Hernández-Mariné M, Canals T, Kormas K, Economou-Amilli A, Pantazidou A (2011) Two new Stigonematalean cyanobacteria: *Iphinoe spelaeobios* gen. nov., sp. nov. and *Loriellopsis cavernicola* gen. nov., sp. nov. from Greek and Spanish caves. Morphology and molecular evaluation. *Int J Syst Evol Microbiol*. doi:10.1099/ijs.0.029223-0
- Lan W, Hui Li H, Wang W-D, Katayama Y, Gu J-D (2010) Microbial community analysis of fresh and old microbial biofilms on Bayon Temple Sandstone of Angkor Thom, Cambodia. *Microb Ecol* 60:105–115
- Leclerc JC, Couté A, Dupuy P (1983) Le climat annuel de deux grottes et d'une église du Poitou, ou vivent des colonies pures d'algues sciaphiles. *Cryptogam Algol* 4:1–19
- Lefèvre M (1974) La maladie verte de Lascaux. *Stud Conserv* 19:126–156
- Lefèvre M, Laporte G, Bauer J (1964) Sur les microorganismes envahissant les peintures rupestres de la grotte préhistorique de Lascaux. *C R Acad Sci* 258:5116–5118
- Macedo MF, Miller AZ, Dioniso A, Saiz-Jimenez C (2009) Biodiversity of cyanobacteria and green algae on monuments in the Mediterranean Basin: an overview. *Microbiology* 155:3476–3490
- Martinez A, Asencio AD (2010) Distribution of cyanobacteria at the Gelada Cave (Spain) by physical parameters. *J Cave Karst Stud* 72:11–20
- May E, Jones M, Mitchell J (2008) Heritage microbiology and science: microbes, monuments and maritime materials. Cambridge RCS Publishing, Cambridge, 305 pp
- McNamara CJ, Mitchell R (2005) Microbial deterioration of historic stone. *Front Ecol Environ* 3:445–451
- McNamara CJ, Perry TD, Bearce KA, Hernandez-Duque G, Mitchell R (2006) Epilithic and endolithic bacterial communities in limestone from a Maya archaeological site. *Microb Ecol* 51:51–64
- Miller A, Dioniso A, Macedo MF (2006) Primary bioreceptivity: a comparative study of different Portuguese lithotypes. *Int Biodeter Biodegr* 57:136–142
- Miller AZ, Laiz L, Gonzalez JM, Dionísio A, Macedo MF, Saiz-Jimenez C (2008) Reproducing stone monument photosynthetic-based colonization under laboratory conditions. *Sci Total Environ* 405:278–285
- Miller AZ, Laiz L, Dionísio A, Macedo MF, Saiz-Jimenez C (2009) Growth of phototrophic biofilms from limestone monuments under laboratory conditions. *Int Biodeter Biodegr* 63:860–867
- Mohammadi P, Krumbein WE (2008) Biodeterioration of ancient stone materials from the Persepolis monuments (Iran). *Aerobiologia* 24:27–33
- Mulec J, Kosi G (2009) Lampenflora algae and methods of growth control. *J Cave Karst Stud* 71:109–115
- Mulec J, Kosi G, Vrhovšek D (2008) Characterization of cave aerophytic algal communities and effects of irradiance levels on production of pigments. *J Cave Karst Stud* 70:3–12
- Nugari MP, Ricci S, Roccardi A, Monte M (2003) Churches and hypogea. In: Mandrioli P, Caneva G, Sabbioni C (eds) Cultural heritage and aerobiology, methods and measuring techniques for biodeterioration monitoring. Kluwer Academic Publishers, Dordrecht, pp 207–244
- Nugari MP, Pietrini AM, Caneva G, Imperi F, Visca P (2009) Biodeterioration of mural paintings in a rocky habitat: the Crypt of the Original Sin (Matera, Italy). *Int Biodeter Biodegr* 63:705–711
- Nuhoglu Y, Oguz E, Uslu H, Ozbek A, Ipekoglu B, Ocak I, Hasenekoglu I (2006) The accelerating effects of the microorganisms on biodeterioration of stone monuments under air pollution and continental-cold climatic conditions in Erzurum, Turkey. *Sci Total Environ* 364:272–283
- Ortega-Calvo JJ, Hernández-Mariné M, Saiz-Jiménez C (1991) Biodeterioration of building materials by cyanobacteria and algae. *Int Biodeter* 28:165–185
- Ortega-Calvo JJ, Hernandez-Mariné M, Saiz-Jimenez C (1993) Cyanobacteria and algae on historic building and monuments. In: Garg KL, Garg N, Mukerji KG (eds) Recent advances in biodeterioration and biodegradation, vol 1. Naya Prokash, Calcutta, pp 173–203
- Ortega-Calvo JJ, Arifto X, Stal LJ, Saiz-Jimenez C (1994) Cyanobacterial sulfate accumulation from black crust of a historic building. *Geomicrobiol J* 12:15–22
- Ortega-Calvo JJ, Ariño X, Hernandez-Mariné M, Saiz-Jimenez C (1995) Factors affecting the weathering and colonization of monuments by phototrophic microorganisms. *Sci Total Environ* 167:329–341
- Ortega-Morales O (2006) Cyanobacterial diversity and ecology on historic monuments in Latin America. *Rev Latinoam Microbiol* 48:188–195
- Ortega-Morales O, Guezennec J, Hernandez-Duque G, Gaylarde CC, Gaylarde PM (2000) Phototrophic biofilms on ancient Mayan buildings in Yucatan, Mexico. *Curr Microbiol* 40:81–85
- Ortega-Morales O, Gaylarde CC, Englert GE, Gaylarde PM (2005) Analysis of salt-containing biofilms on limestone buildings of the Mayan culture at Edzna, Mexico. *Geomicrobiol J* 22:261–268
- Pattanaik B, Adhikary SP (2002a) Blue green algal flora at some archaeological sites and monuments in India. *Feddes Rept* 113:289–300
- Pattanaik B, Adhikary SP (2002b) Blue green algal flora on archaeological monuments of India. *Bull Bot Surv India* 44:61–74
- Pattanaik B, Sahu JK, Adhikary SP (2004) Changes in the protein profile of cyanobacteria from terrestrial habitats in response to heat and UV-B radiation. *Archiv Hydrobiol Algal Stud* 113:175–182
- Pattanaik B, Schumann R, Karsten U (2007) Effects of ultraviolet radiation on cyanobacteria and their protective mechanisms. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Dordrecht, pp 31–45, 811 pp
- Pentecost A (1992) Growth and distribution of endolithic algae in some North Yorkshire streams (UK). *Br Phycol J* 27:145–151
- Pentecost A, Whitton BA (2000) Limestones. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publisher, Dordrecht, pp 257–279, 669 pp
- Pereira S, Zille A, Micheletti E, Modaras-Ferreira P, De Philippis R (2009) Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. *FEMS Microbiol Rev* 33:917–941
- Pietrini AM, Ricci S (1993) Occurrence of a calcareous blue-green alga, *Scytonema julianum* (Kütz) Meneghini, on the frescoes of a church carved from the rock in Matera, Italy. *Cryptogamic Bot* 3:290–295
- Pietrini AM, Ricci S (2009) Problems of biodeterioration in relation to particular types of environments. – Fountains and Nynphaea. In: Caneva G, Nugari MP, Salvadori O (eds) Plant biology for cultural heritage. Getty Conservation Institute, Los Angeles, pp 200–205
- Pietrini AM, Ricci S, Nugari MP (2009) Churches and crypts. In: Caneva G, Nugari MP, Salvadori O (eds) Plant biology for cultural

- heritage. Biodeterioration and conservation. Getty Conservation Institute, New York, pp 179–183
- Polerecky L, Bissett A, Al-Najjar M, Faerber P, Osmers H, Suci PA, Stoodley P, de Beer D (2009) Modular spectral imaging system for discrimination of pigments in cells and microbial communities. *Appl Environ Microbiol* 75:758–771
- Pouličková A, Hašler P (2007) Aerophytic diatoms from caves in central Moravia (Czech Republic). *Preslia* 79:185–204
- Prieto B, Silva B (2005) Estimation of the potential bioreceptivity of granitic rocks from their intrinsic properties. *Int Biodeter Biodegr* 56:206–215
- Pulido-Bosch A, Martín-rosales W, López-Chicano M, Rodríguez-Navarro CM, Vellejos A (1997) Human impact in a tourist karstic cave (Aracena, Spain). *Environ Geol* 31:142–149
- Raimondi V, Cecchi G, Lognoli D, Palombi L, Grönlund A, Johansen A, Svanberg S, BarupK HJ (2009) The fluorescence LIDAR technique for the remote sensing of photoautotrophic biodeteriogens in the outdoor cultural heritage: a decade of *in situ* experiments. *Int Biodeter Biodegr* 63:823–835
- Raistrick A, Gilbert OL (1963) Malham Tarn House: its building materials, their weathering and colonization by plants. *Field Stud* 1:89–115
- Rajaniemi P, Hrouzek P, Kaštovská K, Willame R, Rantala A, Hoffmann L, Komárek J, Sivonen K (2005) Phylogenetic and morphological evaluation of the genera *Anabaena*, *Aphanizomenon*, *Trichormus* and *Nostoc* (Nostocales, Cyanobacteria). *Int J Syst Evol Microbiol* 55:11–26
- Ramírez M, Hernández-Mariné M, Novelo E, Roldán M (2010) Cyanobacteria-containing biofilms from a Mayan monument in Palenque, Mexico. *Biofouling* 26:399–409
- Rickard AH, Gilbert P, High NJ, Kolenbrander PE, Handley PS (2003) Bacterial co-aggregation: an integral process in the development of multi-species biofilms. *Trends Microbiol* 2:94–100
- Riding R (2006) Cyanobacterial calcification, carbon dioxide concentrating mechanisms, and Proterozoic–Cambrian changes in atmospheric composition. *Geobiology* 4:299–316
- Rindi F (2007) Diversity, distribution and ecology of green algae and cyanobacteria in urban habitats. In: Seckbach J (ed) *Algae and cyanobacteria in extreme environments*. Springer, Dordrecht, pp 621–638, 811 pp
- Rodea-Palomares I, Boltes K, Fernández-Piñas F, Leganés F, García-Calvo E, Santiago J, Rosal R (2010) Physicochemical characterization and ecotoxicological assessment of CeO₂ nanoparticles using two aquatic microorganisms. *Toxicol Sci* 119:135–145
- Roldán M, Hernández-Mariné M (2008) The influence of green light on cyanobacterial fine structure: applicability for dim environments. *Algal Stud* 126:159–171
- Roldán M, Hernández-Mariné M (2009) Exploring the secrets of the three-dimensional architecture of phototrophic biofilms in caves. *Int J Speleol* 38:41–53
- Roldán M, Clavero E, Canals A, Gómez-Bolea A, Ariño X, Hernández-Mariné M (2004) Distribution of phototrophic biofilms in cavities (Garraf, Spain). *Nova Hedwig* 78:329–351
- Roldán M, Oliva F, Gonzalez del Valle MA, Saiz-Jimenez C, Hernandez-Mariné M (2006) Does green light influence the fluorescence properties and structure of phototrophic biofilms? *Appl Environ Microbiol* 72:3026–3031
- Rossi F, Micheletti E, Bruno L, Adhikary SP, Albertano P, De Philippis R (2012) Characteristics and role of the exocellular polysaccharides produced by five cyanobacteria isolated from phototrophic biofilms growing on stone monuments. *Biofouling* 28:215–224
- Roy A, Tripathy P, Adhikary SP (1997) Epilithic blue-green algae/cyanobacteria from temples of India and Nepal. II. Presence of UV sunscreen pigments. *Arch Hydrobiol Algal Stud* 86:147–161
- Saarela M, Halakomi HL, Suihko ML, Maunuksela L, Raaska L, Mattila-Sandholm T (2004) Heterotrophic microorganisms in air and biofilm samples from Roman catacombs, with special emphasis on actinobacteria and fungi. *Int Biodeter Biodegr* 54:27–37
- Saiz-Jimenez C, Garcia-Rowe J, Garcia del Cura MA, Ortega-Calvo JJ, Roekens E, van Grieken R (1990) Endolithic cyanobacteria in Maastricht limestone. *Sci Total Environ* 94:209–220
- Salkinoja-Salonen MS, Peltola J, Andersson MA (2003) Microbial toxins in moisture damaged indoor environment and cultural assets. In: Saiz-Jimenez C (ed) *Molecular biology and cultural heritage*. Swets and Zeitlinger BV, Lisse, pp 93–105
- Sánchez-Antón F, Asencio-Martínez AD (2007) Participation of Cyanophyceae in the biodeterioration of the stones of the Santo Domingo College in Orihuela, Alicante (SE Spain). *Algal Stud* 124:95–108
- Sanchez-Moral S, Luque L, Cuezva S, Soler V, Benavente D, Laiz L, Gonzalez JM, Saiz-Jimenez C (2005) Deterioration of building materials in Roman catacombs: the influence of visitors. *Sci Total Environ* 349:260–276
- Scheerer S, Ortega-Morales O, Gaylarde C (2009) Microbial deterioration of stone monuments -an updated overview. *Appl Microbiol* 66:97–139
- Sigler WV, Bachofen R, Zeyer J (2003) Molecular characterization of endolithic cyanobacteria inhabiting exposed dolomite in central Switzerland. *Environ Microbiol* 5:618–627
- Smith T, Olson R (2007) A taxonomic survey of Lamp Flora (Algae and Cyanobacteria) in electrically lit passages within Mammoth Cave National Park, Kentucky. *Int J Speleol* 36:105–114
- Stal LJ (2000) Cyanobacterial mats and stromatolites. In: Whitton BA, Potts M (eds) *The ecology of cyanobacteria: their diversity in time and space*. Kluwer Academic Publishers, Dordrecht, pp 61–120, 669 pp
- Stal LJ (2007) Cyanobacteria: diversity and versatility, clues to life in extreme environments. In: Seckbach J (ed) *Algae and cyanobacteria in extreme environments*. Springer, Dordrecht, pp 661–680, 811 pp
- Stal LJ (2010) Microphytobenthos as a biogeomorphological force in intertidal sediment stabilization. *Ecol Eng* 36:236–245
- Taton A, Grubisic S, Balthasart P, Hodgson DA, Laybourn-Parry J, Wilimotte A (2006) Biogeographical distribution and ecological ranges of benthic cyanobacteria in East Antarctic lakes. *FEMS Microbiol Ecol* 57:272–289
- Tomaselli L, Lamenti G, Bosco M, Tiano P (2000) Biodiversity of photosynthetic microorganisms dwelling on stone monuments. *Int Biodeter Biodegr* 46:251–258
- Tripathi SN, Chung IK, Lee JA (2007) Diversity and characteristics of terrestrial cyanobacteria near Gimhae City, Korea. *J Plant Biol* 50:50–59
- Tripathy P, Roy A, Adhikary SP (1997) Survey of epilithic blue-green algae (Cyanobacteria) from temples of India and Nepal. *Arch Hydrobiol Suppl Algal Stud* 87:43–57
- Tripathy P, Roy A, Anand N, Adhikary SP (1999) Blue green algal flora on the rock surface of temples and monuments of India. *Feddes Reper* 110:133–144
- Uher B, Aboal M, Kovacik L (2005) Epilithic and chasmoendolithic phycoflora of monuments and buildings in South-Eastern Spain. *Crypt Algal* 26:275–358
- Urzi C (2004) Microbial deterioration of rocks and marble monuments of the Mediterranean basin: a review. *Corros Rev* 22:441–459
- Urzi C, Albertano P (2001) Studying phototrophic and heterotrophic microbial communities on stone monuments. *Methods Enzymol* 336:340–355. Academic Press, San Diego
- Urzi C, De Leo F (2001) Sampling with adhesive tape strips: an easy and rapid method to monitor microbial colonization on monument surfaces. *J Microbiol Methods* 44:1–11
- Urzi C, De Leo F (2007) Evaluation of the efficiency of water-repellent and biocide compounds against microbial colonization of mortars. *Int Biodeter Biodegr* 60:25–34
- Urzi C, Krumbein WE, Warscheid T (1992) On the question of biogenic colour changes of Mediterranean monuments (coating, crust, micro-

- stromatolite, patina, scialbatura, skin, rock varnish). In: Decrouez D, Chamay J, Zezza F (eds) Proceedings of the 3rd international symposium on the conservation of stone. Musée d'Art et d'Histoire, Genève, pp 397–420
- Urzi C, De Leo F, Schumann P (2008) *Kribbella catacumbae* sp. nov. and *Kribbella sancticallisti* sp. nov., isolated from whitish-grey patinas in the Catacombs of St Callistus in Rome, Italy. *Int J Syst Evol Microbiol* 58:2090–2097
- Urzi C, De Leo F, Bruno L, Albertano P (2010) Microbial diversity in Paleolithic caves: a study case on the phototrophic biofilms of the Cave of Bats (Zuheros, Spain). *Microb Ecol* 60:116–129
- Uzunov BA, Stoyneva MP, Gärtner G (2007) Review of the studies on aero-terrestrial cyanoprokaryotes and algae in Bulgaria with a checklist of the recorded species. I. *Phytol Balc* 13:65–73
- Vincent W (2007) Cold tolerance in cyanobacteria and life in the cryosphere. In: Seckbach J (ed) *Algae and cyanobacteria in extreme environments*. Springer, Dordrecht, pp 289–301, 811 pp
- Vinogradova ON, Kovalenko OV, Wasser SP, Nevo E, Weinstein-Eviron M (1998) Species diversity gradient to darkness stress in blue-green algae/cyanobacteria: a microscale test in a prehistoric cave, Mount Carmel, Israel. *Isr J Sci* 46:229–238
- Vinogradova ON, Nevo E, Wasser SP (2009) Algae of the Sefunim Cave (Israel): species diversity affected by light, humidity and rock stresses. *Int J Algae* 11:99–116
- Warscheid T, Braams J (2000) Biodeterioration of stone: a review. *Int Biodeter Biodegr* 46:343–368
- Wilmotte A (1994) Molecular evolution and taxonomy of the cyanobacteria. In: Bryant DA (ed) *The molecular biology of cyanobacteria*. Kluwer Academic Publishers, Boston, pp 1–25
- Wilmotte A, Herdmann M (2001) Phylogenetic relationships among the cyanobacteria based on 16S rRNA sequencing. In: Boone DR, Castenholz RW (eds) *Bergey's manual of systematic bacteriology*, vol I. Springer, New York, pp 487–493
- Wynn-Williams DD (2000) Cyanobacteria in deserts – life at the limits? In: Whitton BA, Potts M (eds) *The ecology of cyanobacteria: their diversity in time and space*. Kluwer Academic Publishers, Dordrecht, pp 341–366, 669 pp
- Zakhis F, Jungblut A-D, Taton A, Vincent WF, Wilmotte A (2008) Cyanobacteria in cold ecosystems. In: Margesin R, Schinner F, Marx J-C, Gerday C (eds) *Psychrophiles: from biodiversity to biotechnology*. Springer, Dordrecht, pp 121–128
- Zammit G, Psaila P, Albertano P (2008) An investigation into biodeterioration caused by microbial communities colonising Maltese Palaeo-Christian Catacombs. In: Notea A, Shoef Y (eds) *Art 2008: non-destructive testing, microanalysis and preservation in the conservation of cultural and environmental heritage*. ISAS International Seminars Ltd., Jerusalem, pp 1–10
- Zammit G, Kastovsky J, Albertano P (2010) A first cytomorphological and molecular characterization of a new Stigonematalean cyanobacterial morphotype isolated from Maltese catacombs. *Algol Stud* 135:1–14
- Zammit G, Billi D, Shubert ET, Kastovsky J, Albertano P (2011a) The biodiversity of subaerophytic phototrophic biofilms from Maltese hypogea. *Fottea* 11:187–201
- Zammit G, Sanchez-Moral S, Albertano P (2011b) Bacterially mediated mineralisation processes lead to biodeterioration of artworks in Maltese catacombs. *Sci Total Environ* 409:2773–2782
- Zammit G, Billi D, Albertano P (2012) The subaerophytic cyanobacterium *Oculatella subterranea* (Oscillatoriales, Cyanophyceae) gen. et sp. nov: a cytomorphological and molecular description. *Euro J Phycol* 47 (in press)
- Zanardini E, Abbruscato P, Ghedini N, Realini M SC (2000) Influence of atmospheric pollutants on biodeterioration of stone. *Int Biodeter Biodegr* 45:35–42
- Zimmerman J, Gonzales JM, Ludwig W, Saiz-Jimenez C (2005) Detection and phylogenetic relationships of highly diverse uncultured acidobacterial communities in Altamira cave using 23S rRNA sequence. *Geomicrobiol J* 22:379–388
- Zimmerman J, Gonzales JM, Saiz-Jimenez C (2006) Epilithic biofilms in Saint Callixtus Catacombs (Rome) harbour a broad spectrum of Acidobacteria. *Antonie Van Leeuwenhoek* 89:203–208