19

Biodiversity on Stone Artifacts

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1. Introduction

The biological colonization of stone artifacts exposed under outdoor conditions (e.g. artistic fountains, statues in parks, monuments in urban areas, archeological sites, etc.) is always influenced by the microclimate and the high biodiversity of the opportunistic airflora/airfauna. There is a strength relation between biological development and different factors such as the type of rocks, meteorological phenomena and urban or rural environment. Many organisms, such as phototrophs, may only use the stone as a support, but they can favour more complex colonization, with possible biodeterioration effects. In fact, these organisms are able to growth using the stone mineral components or the superficial deposits of it, exerting damages of stone material by their metabolic activity. Organisms causing biological decay effects are called biodeteriogens. Knowledge of the biodiversity, ecological and physiological aspects of the biological colonization on monumental stones is essential to maintain and preserve the stone cultural heritage for further generations.

2. Bioreceptivity of stone material

Natural and artificial stones have been used from ancient times as building and art materials. Most of the stone artifacts are located outdoor and consequently are frequently subjected to epilithic, chasmolithic and endolithic biological colonization. These biological micro-ecosystems can have different colours and aspects such as pustules, crusts, patinas, biofilms or carpets. Microorganisms may colonize a stone substratum in a variety of ways, especially if they are motile, whereas not motile microorganisms (many cyanobacteria, diatoms) and propagules (spores, seeds, etc.) are presumably deposited on a substratum by gravity from air or water flows. The development of stone biocenosis depends on the combination of environmental location and climatic conditions, in addition to the chemical-physical and petrographic features of stones. A synergistic biodeteriorative action on stone substratum can be started by their concomitant growth of phototrophic and heterotrophic populations during the ecological succession. The whole properties that contribute to biological (flora and/or fauna) colonization has been defined as “bioreceptivity” by Guillitte in 1995, who further defined different types, such as primary, secondary and tertiary bioreceptivity.

2.1 Characteristics of stone substrata

Once extracted from the quarry, the stone material is subjected to irreversible meteoric alteration induced by the synergic action of external and intrinsic factors, with different deteriorating levels, and therefore, with different compositional and geometrical changes. The natural or artificial stones are characterized by their petrographic structure, texture, colour, mineralogical and chemical composition. Among these, the surface roughness, porosity, hygroscopicity, chemical composition, as well as the state of conservation of the stone, are the most important features, which favour the biocolonization and that could, in various ways, lead to deterioration of artworks (Caneva & Ceschin, 2008). Sedimentary (limestones, sandstones, travertine, volcanic tuffs), metamorphic (marble), igneous (basalt) and plutonic rocks (granite, diorite) have been commonly used both as construction materials and artistic compositions. Even artificial stones such as ceramic (bricks, tiles, terracotta), plasters, mortars, stuccos and frescoes, have been used. These have different characteristics and in general a larger porosity with respect to natural stones (Pinna & Salvadori, 2008).

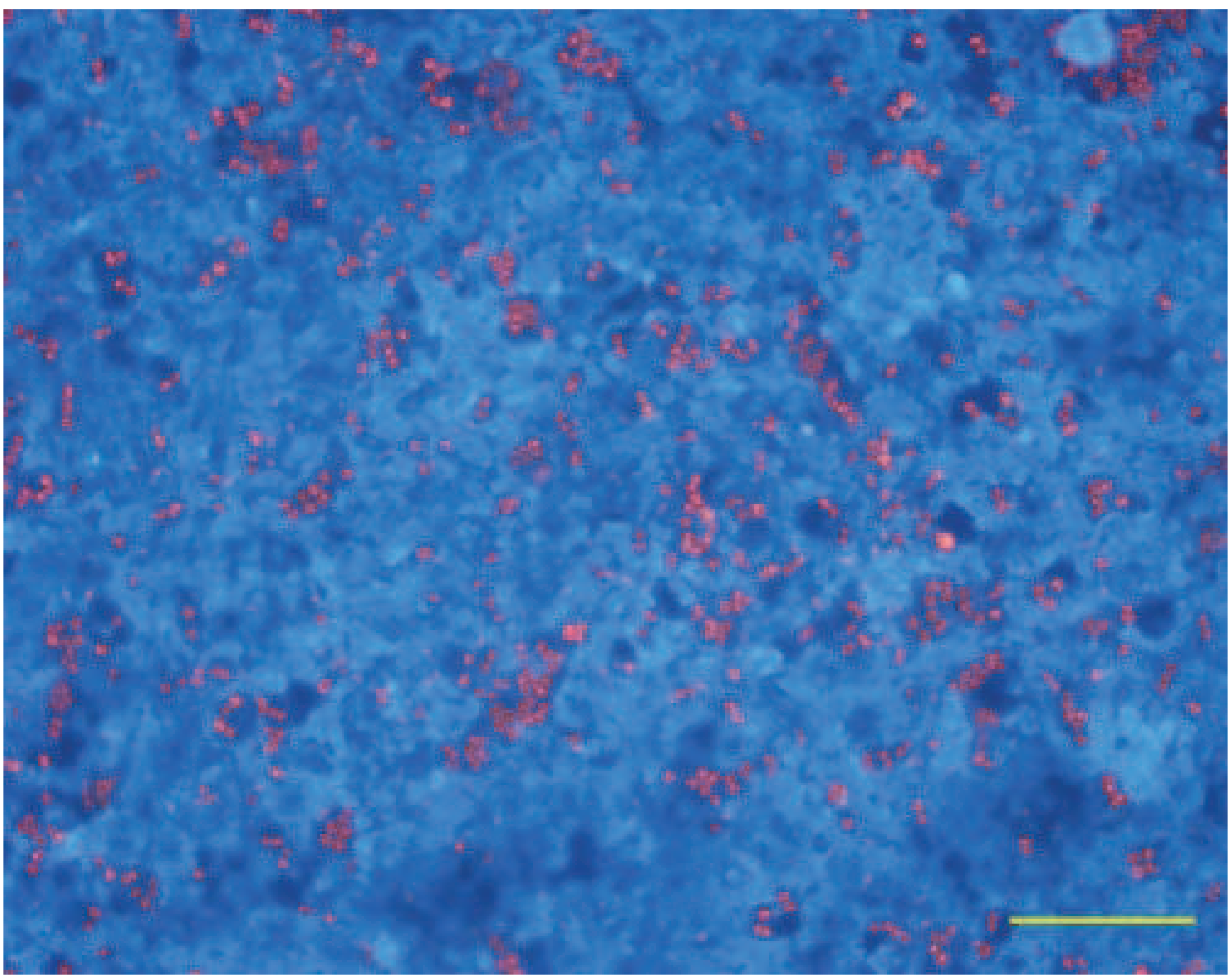

Fig. 1. Phototrophic presence within the micro-irregularities of the surface of a Carrara marble slab under UV light. Scale bar 100 µm

Porosity represents the “empty spaces” in a total volume of a stone material. It affects the permeability and molecular diffusion phenomena. The permeability phenomenon needs usually big pores, while the molecular diffusion can occur even through small interstitial spaces (Amoroso, 2002). Only parts of the void fraction are communicating among them and with the exterior constituting the "open and partial-open pores", while the non-communicating parts are the "closed pores". The distribution, shape and pores size, influence the water absorption and the alterations connected with it (freeze-thaw, salt crystallisation and hydration, biological colonization). Phototrophic micro-organisms, such as coccoid cyanobacteria and algae, start to inhabit in niches formed by its surface porosity, in the primary colonization phase of a stone (Fig. 1). Also, the spores and seeds from air may deposit, by chance, in the pores or crevices and start to develop when microenvironmental conditions are favourable. Some lithotypes are more easily colonized than others, such as highly porous materials (tuffs, limestones, sandstones).

Fig. 2. Different phototrophic colonization in accordance with moisture availability in a stone microhabitat (wall from historical center of Gavorrano, Italy)

Beside the external factors involved in a biological dwelling and growth, the stone intrinsic properties are very important. These are related to the porosity and water transport, retention and diffusion. Therefore, *hygroscopicity* is a significant characteristic to be considered. It is related to the porous structure and capillary pressures of the water present in the stone. The water, in vapour or liquid phase, may follow different penetration mechanisms, by imbibition or condensation processes. The water vapours are strictly correlated with the surrounding environment tending to reach an equilibrium i.e. higher is the relative humidity, higher is the moisture absorption. The liquid water is absorbed by capillarity and moves by diffusion and/or osmosis inside the stone. The water availability is essential for the life development, but it is crucial how long the moisture is retained on/into the stone. Therefore, another parameter to be considered is the *water evaporation process* – a low evaporation usually leads to a higher biological colonization. In Fig. 2 can be seen how different stones show different colonization levels due to their different properties, even if they are located in the same micro-environment. A long lasting living microbial establishment can occur in fine-grained stones that hold moisture for long time, while coarse-grained stones, with high water permeability, can favour only temporary biological colonisations (Warscheid & Braams, 2000; Tomaselli et al., 2002; Prieto & Silva, 2005; Miller et al., 2009).

Surface roughness represents the complex vertical deviations in time of the topography of a stone surface. In fact, this is changing due to the climatic action, soiling processes, eutrophication and biological succession. Many authors (Tiano et al., 1995; Tomaselli et al., 2000; Miller et al., 2009) confirm that higher microbial colonisation occurs when the roughness increases, because this enhances the total surface area, diminishing the shear forces and increasing water absorption.

Chemical components such as macro and microelements, can influence the types of biological growth allowing the availability of inorganic micronutrients. For example, Miller et al. (2006) observed that *Gloeocapsa alpina* and *Sticochoccus bacillaris* were able to grow on carbonate substrates, but showed very limited growth on silicate substrate. Also, many lichen species are growing on a specific type of substratum, such as on siliceous (*Acarospora fuscata*, *Caloplaca flavovirescens*, *Lecanora rupicola*) or calcareous stone (*Acrocordia conoidea*, *Lecanora spadicea*, *Protoblastenia incrustans*). When the stone surface is eutrophicated, and therefore the chemical composition of the stone become less important, other microorganisms can develop, such as microfungi, heterotrophic bacteria and nitrophilus lichens (*Xanthoria calcicola*, *X. parietina*, *Diplocacia canescens*) (Nimis et al., 1992).

2.2 Environment, stone material and biodiversity

Each stone material is part of an ecosystem, being interconnected with surrounding abiotic and biotic factors. At a global level, an ecosystem is part of a biome, and the latter one is part of the ecosphere - the planetary system consisting of atmosphere, geosphere, hydrosphere and biosphere, which are continuously exchanging matter and energy in order to reach a dynamic equilibrium. The climate is a term used to describe the above mentioned interlinked systems powered by the sun, in time and space scale. Macro-climate includes a large area, such as a region or a country, while micro-climate includes only a small area (still part of the macro-climate) such as a single building or a statue (Fig. 3). The artwork ecosystem is a complex self-regulating system, composed by the biotope (stone material), the biocenosis (organisms) and the surrounding environment (micro-climate). The climate is the principal factor controlling the distribution and dynamics of ecosystems (Levéquê, 2003). Pollution is a consequence of industrialization and urbanization of the society and is the main cause of climate changes. This has an impact not only on human health and natural environment, but also on the built environment where, chemical, physical and biological weathering are influenced by climatic factors. The effect of climatic and weather changes on all living organisms (bioclimate) is evidenced especially by loss of biodiversity and increasing of uniformity, both on macro- and micro-ecosystem scale (Caneva, 2010). Studies on biodeterioration of monuments in relation to climatic changes (Caneva et al., 1995; Ariño et al., 2010) stressed the floristic diversity reduction and disappearance of the most sensitive species, such as many lichens. In the same

time, other species among algae, cyanobacteria, fungi and lichens developed resistance to air pollutants, becoming invasive species. It has been reported that introduction of building materials, not common in a certain geographical area, increase biodiversity by colonization of specific microorganisms (Ariño & Saiz-Jimenez, 1996).
Within the general stone cultural heritage sector, it can be considered several types of environments (Table 1), which play a considerable role in the development of specific and common biological agents (Albertano et al., 2008).

Environment	Characteristics	Biological colonizers
TERRESTRIAL ENVIRONMENTS		
A. Enclosed environments:	*reduced exchange with the outdoor environment*	
A1. museums	inside microclimate conditions (usually controlled and monitored) are strongly influenced by location and geography of the site, characteristics of the building and museum management	rarely heterotrophs (bacteria and fungi)
A2. churches and crypts	a certain stability of the internal microclimate conditions, not much influenced by the daily and seasonal variations, but influenced by their function (number of visitors, heating), structural and architectural characteristics	actinomycetes, cyanobacteria, microalgae, fungi
A3. tombs, catacombs and hypogean environments	located below the ground level, with a high and constant relative humidity level (>70%) and low and relative constant temperature (between 10-18°C); the stability of microclimate conditions is related with the size and depth of hypogean space, characteristics of the terrain above, the degree of pollution in the atmosphere, and absence of visitors	subaerial microflora – archaeobacteria, bacteria, actinomycetes, cyanobacteria, microalgae, fungi, phototrophic biofilms, mosses, insects, bats
B. *Outdoor environments:*	*different characteristics according to the climate context, geographical and topographical location*	
B1. urban environments	high population density, with human features expressed by urbanization processes, with a microclimate becoming warmer and drier, with increasing atmospheric pollution	black crust (bacteria, cyanobacteria, algae, fungi) and vascular plants, less lichens and mosses, insects and avifauna

Environment	Characteristics	Biological colonizers
B2. parks and rural environments, archaeological remains	microclimate conditions induced by forest and vegetation cover, with lower temperatures, lower chemical pollution, higher relative humidity, higher nutrients availability and biological contamination, with respect to urban environment	abundant and varied biological colonization forms, bacteria, cyanobacteria algae, fungi, lichens, mosses, vascular plants micro and macrofauna
B3. coastal environments (sandy coastline and maritime rocks)	influenced by the action of the sea, with high concentrations of marine salts both in the air and on the substrate, according to distance from the sea, with an urban or rural microclimate characteristics	halotolerant and halophytic species, algae, cyanobacteria, fungi, lichens, mosses, plants
B4. fountains and nymphea	usually located outdoor, in parks or urban environments, with a constantly or sporadically water supply which can wet totally, partly or not the stone surfaces, often with incrustations and eutrophication areas	aquatic communities, poikilohydric microorganisms, subaerial microflora, lichens, mosses, vascular plants, fishes
C. *Semienclosed environments:*	*partially circumscribed by natural materials (walls, rocks, soil) or by a roof cover*	
C1. loggia and porticoes	microenvironmental conditions can be uniform or with strong fluctuations, depending on the architectural structure, distance from the ground, height from the pavement, inclination, extensiveness of the roof cover	photoautotrophic microorganisms (cyanobacteria, algae), heterotrophic microflora, lichens, bryophytes, weeds
C2. rupestrian environments and caves	located in a natural environment, dug into the rock, generally in close contact with the soil, the microclimate conditions vary greatly in relation to the distance from outside, with similarities with hypogean environments	sciaphilic cyanobacteria, bacteria, microfungi, algae, insects
C3. sheltered archaeological sites	microclimate conditions vary according to size and type of the shelter, the moisture level usually is lower with respect of non-sheltered sites but it could generate a green-house effect	phototrophs, lichens, mosses, vascular plants, micro and macrofauna
MARINE AND FRESHWATER ENVIRONMENTS	microclimate conditions afforded by the specific aquatic environment	biofouling organisms

Environment	Characteristics	Biological colonizers
EDAPHIC ENVIRONMENTS	microclimate conditions afforded by the specific edaphic environment (e.g. clay seems to create an anoxic environment, sandy soils create a low water content environment, while permafrost a low temperature one)	microflora – bacteria (heterotrophic and chemoautotrophic), yeasts, actinomycetes, fungi, cyanobacteria

Table 1. Types of environments and their impact on biocolonization of art and building stone materials

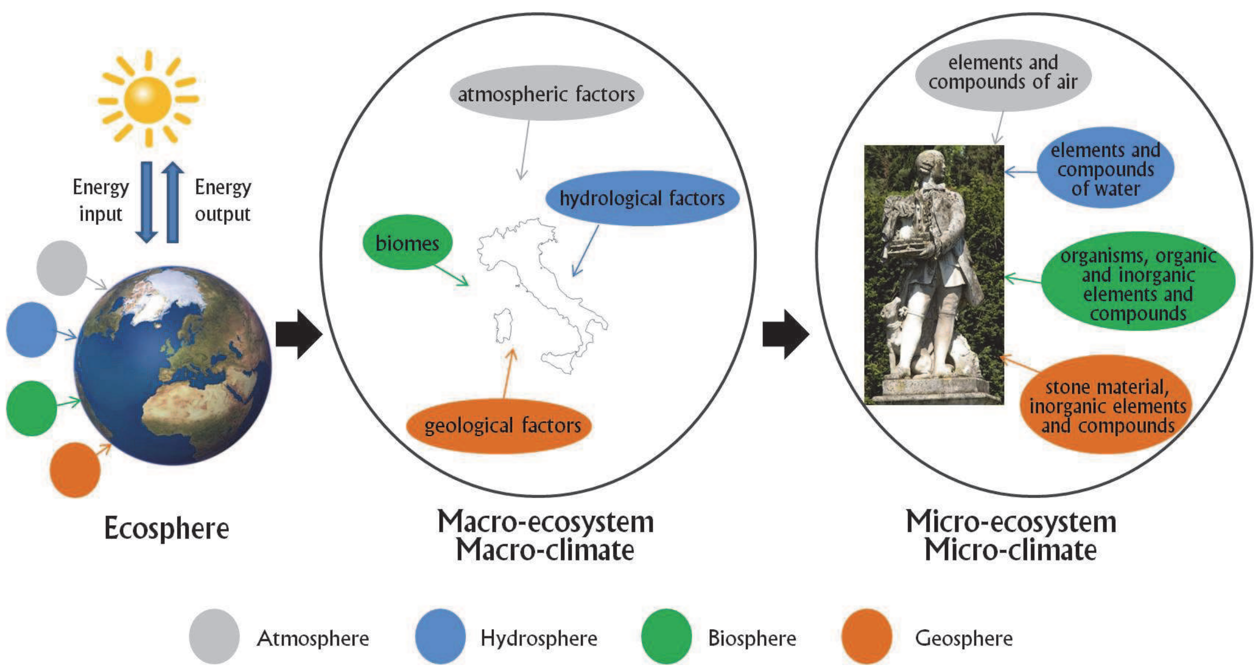

Fig. 3. Ecological hierarchy of an artwork ecosystem as part of the ecosphere

2.3 Mechanisms of colonization

The colonisation mechanism starts when one organism finds the optimal ecological conditions for dwelling, growth and multiplication. In fact, microorganisms may settle on the stone material during its entire life cycle, before and after its processing, from the quarry to the finished work of art (Garcia-Vallès et al., 2000; Cámara et al., 2011). This initial contact may result in a transitory chemical attraction that is difficult to characterize. Cell control attachment and release, are depending on the suitability of the microhabitat.

The photosynthetic microorganisms develop easily on the stone surfaces and, once established, allow the growth of more complex microbial consortia formed by heterotrophic microroganisms which can exercise stronger deteriorating activity (Tiano, 1993; Tomaselli et al., 2000; Crispim et al., 2003; Peraza Zurita et al., 2005). These phototrophic microrganisms are building up a so-called *biofilm*, enriched with organic and inorganic compounds.

As regard the stone colonization by lichens, their role of primary colonizers seems doubtful, especially for the crust like endolithic forms. According to Savoye & Lallemant, 1980, lichens start to develop on the substrate after it was partially enriched with nutrients from air and previous colonizers – bacteria (Lisci et al., 2003). The prime colonizers are in fact the single components (algae and microfungi) and if they are compatible can develop in a new symbiotic organism, the lichen (Joneson & Lutzoni, 2009).

At more advanced stage of stone colonization, the bryophyte communities and vascular plants can develop (Warscheid & Braams 2000). An important role for their growing is the presence of a protosoil (Gómez-Alarcón et al., 1995a), which is favoured by previously colonizations (cell debris) and by airborne particles retained on biofilms or accumulated in cracks or holes.

Biofilms occur on all solid surfaces in habitats where exists a constant availability of moisture. This complex biocenosis consist principally of water (70 to 95% of the fresh weight), extracellular polymeric substances (Flemming, 1993) and microorganisms such as phototrophs (algae, cyanobacteria, diatoms) and heterotrophs (bacteria, fungi, protozoa, nematods), which are embedded in this hydrated matrix. The proportion of each group is varying seasonally and it is influenced by different habitats (Underwood, 1984; Anderson, 1995; Roeselers et al., 2007). Furtermore, the biofilm contains cells debris, airborne particles and spores together with inorganic material adsorbed from the substratum (Warscheid, 2008). This complex structure also contains biopolymers such as exopolysaccharides (EPS) with adhesive properties, which are very relevant in the early development stage of a biofilm, since they facilitate the attachment of cells to the substrate (Decho, 2000, Barranguet et al., 2005).

The organization of a biofilm on a solid surface consists of three main levels: molecular fouling, micro-settlement and a macro-settlement. It has been noted that the formation of an organic molecular layer should be realized before the attachment of the microorganisms on a solid substrate. After that a reversible stage of adhesion of the primary colonisers starts, followed by their fixing and propagation, with an auto-organised three-dimensional structure. The adhesion of secondary colonizers (filamentous cyanobacteria, fungi) with further colonization of invertebrates, led to a mature biofilm with an expressed specific micro-ecosystem structure. This is a homeostatic phase of the biofilm, with a continuous growth and detachment of small parts. The mature biofilm has a complex genetic heterogeneity which confers stability and resistance to the biocenosis (Nikolaev & Plakunov, 2007). Biofilm evolution is also dependent by stochastic and mechanical processes, deterministic phenomena and temporal changes (Wimpenny et al., 2000). The formation of a visible biofilm on a stone surface in natural environment is a quite longer process (even months), and its proliferation is strongly dependent on the environmental conditions (especially light, temperature and water content) and on the concentration of the microorganisms that can adhere on it.

The observed patina composition on a marble specimen with a confocal microscope (CSLM) contains are the green algae, cyanobacteria and diatoms. These phototrophic pioneers are observed, as first stone colonizers in a young biofilm just after 6 days of incubation in contaminated water (Fig. 4) and it is possible to see that cyanobacteria prefer to colonize deeper irregularities of the stone substrate. The presence of the EPS and heterotrophs can be observed just after 22 days of immersion of the marble specimen in the contaminated water, under indoor laboratory conditions (Fig. 5).

3. Biological heterogeneity on stone artifacts

Biological development on stone monuments is strongly influenced by the whole complex interaction between biotic and abiotic factors. All together contribute to different biological developments. In fact, when it occurs, may give rise to a great variety of colonization patterns following dynamic phenomena and/or ecological succession.

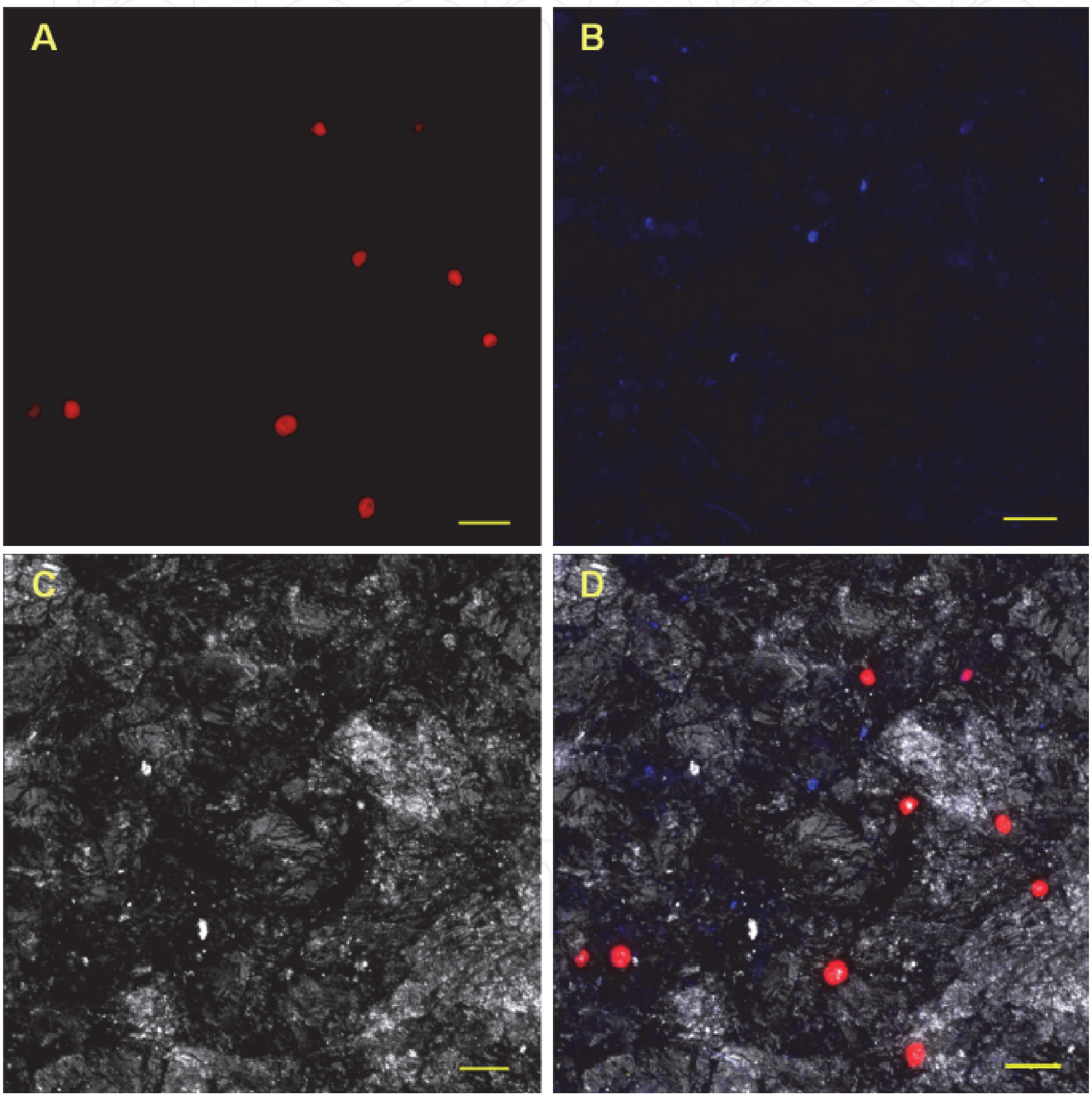

Fig. 4. Maximum intensity projection images of 6 days old biofilm developed on the marble sample examined by CLSM. Images are showing (A) the autofluorescence of individual algae in the red channel, (B) unidentified microorganisms in the blue channel, (C) reflection of the substratum in the blue channel, and (D) the resulting overlay of the four channel (in the green channel was not recorded any signal). Scale bar 20 µm. Colors are false colors

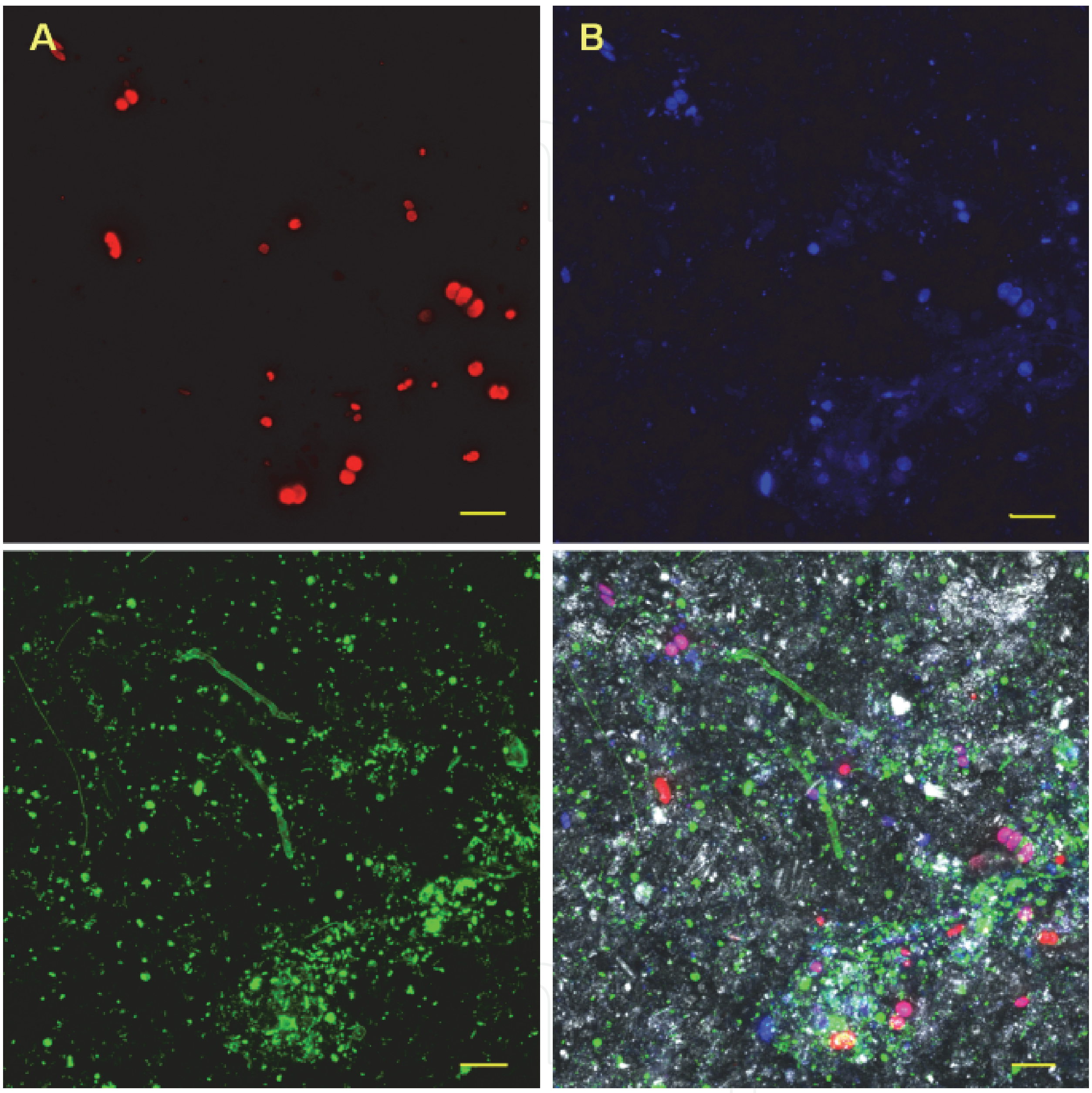

Fig. 5. A 22 days old biofilm developed on a marble sample. The single channel signals are showing (A) algae and diatoms in division – autofluorescence in the red channel, (B) algae and cyanobacteria - autofluorescence in the green channel, (C) EPS and fungal hyphae stained with concanavaline A cojugated with Alexa Fluor 488 - blue channel, and (D) the resulting overlaying of all four channels (including the channel used for capturing the substratum signal, in reflectance mode). Scale bar 20 μm

The biological growth can present different colours, morphologies and agents. The bio-patina or patina is the most general term for defining a epilithic microbiota spread on a stone surface. This, different from the "artistic patina", alters the aesthetical, physical and chemical aspects (Krumbein, 2003) of the substrate. When this patina exerts a protective role for the stone substrata, it can be called bioderma. It must be also considered that in the initial phase some biological agents can develop inside the stone with a concealed pattern. The type of organisms can be subdivided in chasmoendoliths (colonize cracks, fissures or pores, being in contact with the stone surface), cryptoendoliths (colonize pores inside rocks and/or develop in strata inside parallel to the stone surface) and euendoliths (actively dissolve and penetrate the stone surface, forming cavities with different morphologies). The bio-patina can support the development of other organisms such as protozoa, nematodes, insects and arthropods.

According to different typologies found in the literature, biological colonization can be divided in: **A.** *micro-colonial epilithic growths*, **B.** *epilithic formations with patina aspect* and **C.** *chasmo-endolithic developments*; each of these categories can be mono- or multispecies associations (Figs. 6 and 7). The epilithic organisms may be superficially attached to substratum, through synthesized exopolysaccharides (EPS), as the phototrophs, while fungi, lichens, mosses or plants, are owning specific structure (hyphae, rhizines or rhizoids, roots) that allow them to mechanically fix into the stone porosity. Mature biofilms or crusts, demonstrate great resistance to environmental stresses and conservation interventions.

Biological colonisations on monumental stone surface essentially assume the following forms:

A. Epilithic growth with micro-colonial aspect

A1. *Pustules* (Fig. 6a,b)

A pioneer stage of colonization, with hemispheric morphology, with a verrucose and gelatinous aspect, due to the EPS secretion, being formed by one or more phototrophic biotypes, with a compact pseudo-parenchimatic structure **(**Sánchez Castillo & Bolívar, 1997).

Organisms: green algae (*Palmella miniata*) and cyanobacteria (*Pleurocapsa minor, Chamaesiphon polonicus*) (Sánchez Castillo & Bolívar, 1997), diatoms (*Navicula, Achnanthes*).

Ecology: in wet or very humid areas, in the monumental fountains and nymphea, in hypogean environments.

A2. *Microbial concealed growth*

This microbial contamination (up to 5 cm deep), gives an eroded and/or pulverized aspect to the stone surface (Warscheid & Braams, 2000). Microbiological development difficult to observe were found on mural paintings. Some pigments can change colour due to their oxidation, reduction or metal ions transfer after chemical processes induced by bacteria (Petushkova & Lyalikova, 1986; Nugari et al., 2008).

Organisms: chemiotrophic bacteria (*Thiobacillus, Nitrobacter*) (Caneva et al., 1991; Warscheid & Braams, 2000), heterotrophic bacteria (*Bacillus*, *Arthrobacter*) (Petushkova & Lyalikova, 1986; Nugari et al., 2008).

Ecology: on coarse grained porous stones, sandstones, man-made stones and frescoes, in urban environments, churches and crypts, tombs and catacombs.

A3. *Irregular stains*

The stains are related to the pigments belonging to the stone colonizers: *black stains* (melanin, melanoidins, products of chlorophyll degradation) (Fig. 6f); *green and greenish*

Fig. 6. Various typologies of biological colonization found on monumental stones: (a) green algae pustules and (d) microbial mat on artistic fountain from Alhambra complex, Granada (Spain); (b) diatoms brown pustules and (c) phototrophic biofilm developed on Tacca's fountains from Florence (Italy); (e) irregular whitish stains produced by actinomycetes in an Etruscan tomb from Chiusi (Italy); (f) microcolonial fungal growth on marble stone, from Trespiano monumental cemetery, Florence (Italy)

stains (photosynthetic pigments from algae and cyanobacteria) (Fig. 6a); *yellow-orange-brownish stains* (carotens and carotenoids and degradation products of chlorophyll such as phycobiliproteins) (Fig. 6b); *bright orange, pink and red stains* deriving from pigments of chemoorganotrophic (halophilic) bacteria and degradation products of cyanobacteria and algae with iron enrichment (Warscheid & Braams, 2000). They may have a powdered, mucous or compact aspect, according to environmental conditions and constituting organisms.
Organisms: black fungi (*Ulocladium, Alternaria, Scolecobasidium, Phytomyces*); yeasts (*Rhodotorula minuta*); heterotrophic pigmented bacteria (*Micrococcus roseus, Flavobacterium*) (Tiano & Tomaselli, 1989), actinomycetes (*Streptomyces, Nocardia*) (Fig. 6e); chemoorganotrophic bacteria (*Bacillus, Arthrobacter, Rhodococcus, Brevibacterium*) (Warscheid & Braams, 2000; Pinna & Salvadori, 2008);
Ecology: in tombs, catacombs and hypogean environment, in artistic fountains and nymphea

B. Epilithic formations with patina aspect

B1. *Films*

Films are formed by being thin unistratified sheets fairly homogeneous, generally a further development of the pustules, without an increase in species complexity or represented biotypes. It can become a more complex community throughout its development under favourable environmental conditions (Sánchez Castillo & Bolívar, 1997). They are located superficial or along natural crackers and fissures, mostly unilamellar thin structure of mono- or multispecies (Warscheid & Braams, 2000).
Organisms: bacteria, phototrophic microflora (*Chamaesiphon, Pleurocapsa, Pleurastrum, Chlorogea*), microfungi.
Ecology: in wet or very humid areas, in the monumental fountains and nymphea.

B2. *Biofilms, subaerial biofilms*

Biofilms are aggregates of microorganisms embedded in a mucilaginous organic matrix (EPS) found in aqueous environments, usually intended as a spread microbial growth at the water-substratum interface, such as the basins and artistic fountains pools or the architectural elements under a continuous water flow (Cuzman et al., 2010). According to the quantity, turbulence and water spraying the stone surface can partially or totally in direct contact with the air as well. The *subaerial biofilms* develop on surfaces, at the atmosphere-rock interface, but in very damp environmental conditions. They are reported in hypogean monuments, catacombs (Gorbushina, 2007), on the walls or statues with north orientation and surrounded by vegetation, or areas with water percolations. Subaerial biofilms if colonize not only the irregularities of the stone surface but actively penetrate deeper into the substratum, are called *endolithic subaerial biofilms* (see C3).
These formations are characterized by patchy growth, usually in presence of high humidity conditions, being more complex than a film or pustules grouping, but thinner and less complex than a microbial mat. They may have a various coloured patinas aspect, staining the stone surface with organic pigments (e.g. chlorophylls, carotenoids, melanins) (Warscheid & Braams, 2000). The colour is depending on the type of biocenosis, its development stage and of the growth phase of the prevailing species. Individuals within these communities avoid sexual reproduction, but cooperate extensively with one another especially to avoid loss of energy and nutrients (Gorbushina, 2007). They can survive as

vegetative cells or tissue-like structure for long periods in case of stressing conditions (Gorbushina & Broughton, 2009), being very resistant to control treatments.

Organisms: mixed association of different biological groups, such as coccoid cyanobacteria (*Gloeocapsa, Chlorococcum, Myxosarcyna*), filamentous cyanobacteria (*Phormidium, Leptolyngbya*), coccoid algae (*Chlorella, Muriella terestris*), filamentous algae (*Trentepohlia*, *Mougeotia*), heterotrophic bacteria (*Rubrobacter radiotolerans*), and fungi (*Acremonium, Fusarium, Penicillium, Aspergillus*).

Ecology: in wet, very humid, with water condensation or percolation areas, in the monumental fountains and nymphea, in tombs, catacombs and hypogean environment, crypts, marine and fresh water environments

B3. *Microbial mats*

Phototrophic formations with a considerable thickness, characterized by prevailing filamentous species, developed at both water or atmosphere–stone interface. They may present filamentous fungi or lichens as well (Sánchez Castillo & Bolívar, 1997). These aggregations are a type of stratified and complex biofilm, laminated or forming stromatolites, being bound to the substrate and held together by slimy EPS. They are found especially in extreme environments (Riding, 2000). Usually the uppermost layers are dominated by aerobic phototrophs, while the lowest layers are dominated by anaerobic bacteria. The microbial mat may be *mucous* (made of microorganisms with sheath) or *fibrous* (made of microorganisms without sheets) (Sánchez Castillo & Bolívar, 1997).

Organisms: mucous mats (Fig. 6d): *Phormidium, Diadesmis, Apatococcus*; fibrous mats: *Cladophora, Gongrosira, Pleurastrum, Melosira* (Fig. 7e).

Ecology: in areas with long dry-wet alternation cycles, in the monumental fountains and nymphea, in the coastal environments.

B4. *Crusts*

The formation of biogenic crust is due to a chemical-physical process (calcite precipitation) mixed with the biological growth present on stone surface, or can be constituted by the spreading of the organisms that form crust-like structures, such as the crustose lichens and some type of mosses.

Crusts can be found in the uppermost layers of the stone, up to 1 cm depth, being composed by a complex and stable microflora (Warscheid & Braams, 2000). This mineralization process can occur in biofilms, subaerial biofilms or mats. It offers to subaerial biofilms protection from the environment and allows the accumulation and agglutination of airborne particles and deposition of minerals (Gorbushina & Broughton, 2009). The encrusted biofilm is able to penetrate inside the rock by hyphal growth and by biocorrosive activity (Warscheid & Braams, 2000). In monumental fountains the mineralization formations (calcareous deposits) may present a flat or nodular aspect (Bolívar & Sánchez Castillo, 1997; Sánchez Castillo & Bolívar, 1997).

Lichenic crust is closely adhering to the substrate through the hyphae of inferior cortex that are penetrating to varying degrees into the substratum. The lichenic crust can have different aspects, according to morphological variation of visible part of the lichens: leprous, continuous, areolate, verrucose, peltate, or placodiomorphic. (Piervittori et al., 2008).

Mosses crusts are not so often found on monumental stone surfaces. It is well known that many mosses are involved with the formation of calcareous deposits, being usually

associated with other microorganisms, such as cyanobacteria, green microalgae and diatoms. Crusts forming mosses play an important role in artistic fountains degradations, and can led to incrustation several decimetres thick, completely obliterating the legibility of the work, as was observed on Fountain of the Dragons in Villa d'Este in Tivoli (Ricci, 2008).
Organisms: encrusted biofilms: many cyanobacteria and algae (*Schizothrix, Symploca, Chlorosarcinopsis* minor, *Apatococcus, Scenedesmus, Trentepohlia; Scytonema julianum, Geitleria calcarea, Loriella osteophila, Herpizonema pulverulentum*) (Ortega-Calvo et al., 1995; Sánchez Castillo & Bolívar, 1997); chemoorganotrophic fungi (*Exophiala, Penicillium, Cladosporium, Aspergillus, Phoma, Ulocladium)* (Warscheid & Braams, 2000); crust forming lichens (*Caloplaca, Dirinia massiliensis, Lecanora, Pertusaria*); crust forming mosses (*Eucladium verticillatum, Barbula tophacea, Cratoneuron, Philonotis*).
Ecology: may occur in areas with dry-wet alternation cycles which favour the mineralization process, but with enough humidity, in monumental fountains and nymphea, in parks, rural and urban environments, in coastal environments and in hypogeal monuments.

B5. *Black crusts (Fig. 7a,c)*

The black sulphated crust, due to the interaction between calcareous substrate and the polluted atmosphere, differs from the biogenic one as being usually formed in areas sheltered from rainfall. The biological black crusts originate in a humid environment and in stone areas with running water (Gómez-Alarcón et al., 1995b; Lewin, 2006). Many heterotrophic and phototrophic organisms can adhere on the gypsum-rich black crusts. These biological agents are able to metabolize the organic (phenanthrene) or inorganic compounds (sulphur) found on it (Ortega-Calvo et al., 1995; Saiz-Jimenez, 1997). A current study reports in unpolluted environments, the presence of thin black crusts with biogenic origin, composed by a massive presence of filamentous cyanobacteria (Gaylarde et al., 2007).
Organisms: in non-biogenic black crust: bacteria (*Bacillus licheniformis, B. subtilis, B. brevis, Corynebacterium glutamicum, Actinomyces, Flavobacterium breve, Pseudomonas stutzeri, Nocardia*) (Turtura et al., 2000), fungi (*Papulaspora-like, Engyodontium album, Aureobasidium pullulans, Cladosporium sphaerospermum*) (Saiz-Jimenez, 1997; Frank-Kamenetskaya et al., 2009), and cyanobacteria (*Gloeothece, Chlorosarcinopsis*) (Ortega-Calvo et al., 1994); in biogenic black-crust: cyanobacteria (*Gloeocapsa, Nostoc flagelliforme, Chloroglea microcystoides, Scytomena, Oscillatoria*), black lichens (*Verrucaria*), and black fungi (*Ulocladium, Phoma, Alternaria*), meristematic fungi (*Aureobasidium, Sarcinomyces petricola*).
Ecology: in drier and occasionally wetted habitats and exposed to sunlight, in urban and rural environments.

B6. *Whitish efflorescence (Fig. 6e)*

Irregular heterotrophic colonial growth with puffy (due to white fungal mycelia) or powdering aspect (due to the bacterial sorted colonies). The actinomycetes development give rise to extensive, thin and pulverulent whitish patinas or to thicker and more localized forms which closely resemble saline efflorescences (Nugari et al., 2008).
Organisms: fungi (*Fusarium solani*) (Bastian & Alabouvette, 2009), actinomycetes (*Streptomyces, Micromonospora, Nocardia*) and bacteria (*Pseudomonas fluorescens*) (Nugari et al., 2008; Nugari et al., 2009).
Ecology: in habitats with organic matter, in shadow and humid areas, on wall paintings in tombs, catacombs, hypogean and rupestrian environment.

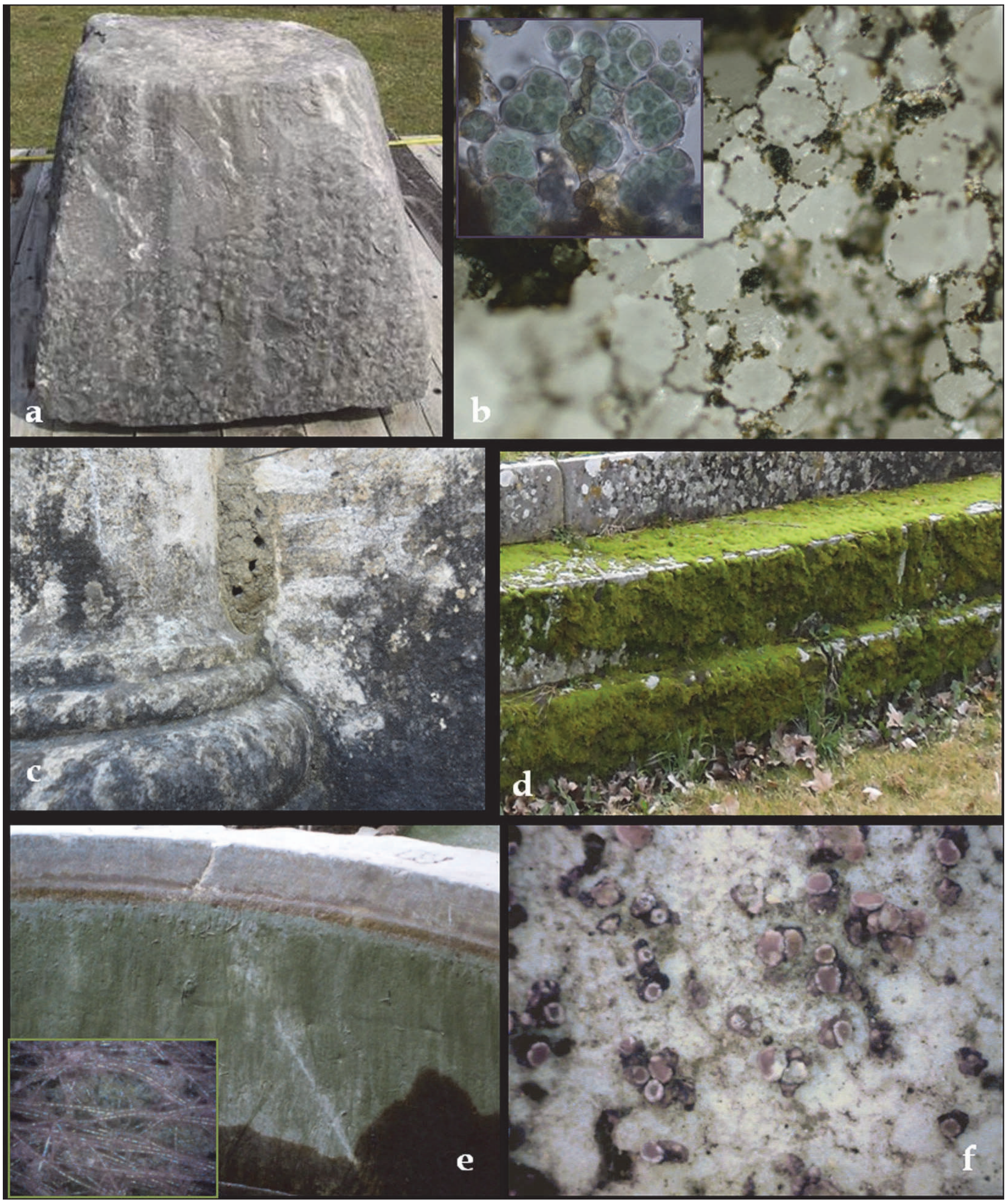

Fig. 7. Different patterns of biological growths found on monumental stones: (a) homogenous black crust with a detail (b) of interganular development of threadlike microcolonies containing cyanobacteria and black fungi, as revealed by microscopic insight, Carrara Marble Museum, Italy; (c) heterogenous lichenic black crust with a wasp nest on a limestone tomb, Trespiano monumental cemetery; (d) bryophite extended carpets, Fiesole archeological site, Italy; (e) fibrous microbial mat with a filamentous diatom (*Melosira* sp.) as a dominant species, Lindaraja Fountain from Alhambra complex, Granada (Spain); (f) micropits containing the apotheci of an endolithic lichen, Trespiano monumental cemetery

B7. *Carpet and cushion forms (Fig. 7d)*

These formations are composed by bryophyte associations, and rarely by foliose lichens developing in carpets-like form. The *carpets* are extended on wide stone surfaces, with individuals growing parallel to one another, either with an uniaxial or a branched growth habit. The *cushions* can be found in sheltered areas of a monument, where soil particles easily accumulate, with individuals placed radiate and having lateral ramifications with a hemispheric structure (Ricci, 2008). These biological formations can favour the seeds germination of small size vascular plants.
Organisms: saxicolous mosses (*Tortula muralis, Eucladium verticillatum*), foliose lichens (*Parmelia, Xanthoria*).
Ecology: in damp habitats, in archaeological sites, parks and rural environments.

C. Chasmo-endolithic growth

C1. *Micropits*

Small cavities created by active substrate dissolution. The euendolithic cyanobacteria may have different boring patterns, according to the various species (Caneva et al., 1991; Hoppert et al., 2004). The endolithic lichens on carbonate matrix can colonize to a depth of few millimetres and in exceptional cases deeper than one centimeter (Piervittori et al., 2008). The biopitting phenomena can be easily confused with abiotic alterations, such as soiling, due to the fact that appears as a simply variation of the stone's natural colour, often green or black (Pinna & Salvadori, 2008).
Organisms: blue-green algae (*Hyella balani, Hyella caespitose, Hormatonema paulocellulare, Kyrtuthrix dalmatica*), endolithic fungi (*Lichenothelia*), endolithic lichens (Fig. 7f) (*Lecidea, Caloplaca*) (Caneva et al., 1991).
Ecology: in humid and dim habitats, sometimes on surfaces sporadically wet and exposed to direct sunlight, in urban, rural and coastal environments.

C2. *Microcolonial structure*

The microcolonial development can have three main patterns: (a) inter-crystalline growth (Fig. 7b), (b) biopitting growth (see *C1*) and (c) growth in already formed cracks and fissures (Urzì et al., 2000). The biologic agents forming this kind of development are usually cyanobacteria and/or black meristematic fungi. It seems that only the fungal filaments closest to the rock surface are pigmented, whereas filaments grown deeper in the stone are colourless (Hoppert et al., 2004).
Organisms: lichen-forming fungi (*Verrucariales, Lichenothelia*), black yeast and meristematic fungi (*Sarcynomyces,Exophiala, Aureobasidium, Capnobotryella, Phaeococcus, Trimmatostroma*); cyanobacteria (*Gloeocapsa*).
Ecology: usually on surfaces exposed to direct sunlight, but with enough humidity when phototrophs are present, in urban, rural and coastal environments.

C3. *Endolithic subaerial biofilms*

In the long-established subaerial biofilms, the biofilm communities are not only on the stone surface but they have crept into deeper layers of the substrate and offered themselves an endolithical niche in which the environmental extremes are better buffered. It has a complex spatial pattern of mineral grains, pores and fissures (Gorbushina & Broughton, 2009). Sometimes this biocenosis has a sandwich aspect, with compact and

distinct green layer beneath the stone surface (Gorbushina, 2007; Saiz-Jimenez et al., 1990).
Organisms: cyanobacteria (*Chroococcidiopsis, Synechococcus*) (Saiz-Jimenez et al., 1990), meristematic fungi, endolithic lichens.
Ecology: in humid and/or sporadically dry areas, in urban, rural and coastal environments.

4. Consequences of biological presence on artistic stone material

Beside the weathering agents, a synergistic biodeteriorative effect on stone surfaces can be started by the concomitant growth of phototrophic and heterotrophic populations. The biodamages produced on stone substrata are especially related from one side to the metabolic activity of living organisms and from the other side to aesthetical changes of the stone surface. The biological agents dwelling on stone monuments are involved directly or indirectly in the weathering of stones and constituent minerals (Warscheid & Braams, 2000), and can induce various alterations forms, as can be seen in Table 2.

Cause	Alteration type	Potential biological development
mechanical stresses such as swelling-shrinking and physical penetration inside the rock	decohesion fissures fractures	biofilms and subaerial biofilms, lichenic crusts, physical penetration of fungal hyphae or specialized organs such as rhizines (lichens), rhizoids (mosses) or roots (vascular plants)
reaction with atmospheric pollutants, correlated with condensation phenomena	peeling swelling pitting	black crusts
solubilization due to metabolic processes, acid attacks	disintegration pulverization flakes	microbial concealed growth (bacteria), lichens
deposits of different kinds of materials, crystallization of minerals	encrustation, concretions, film peeling	biofilms, subaerial biofilms, microbial mats, lichenic and mosses crusts
active penetration by chemical dissolution	pitting	endolithic cyanobacteria and lichens
presence of various natural pigments and degradation products	chromatic alteration spotting	phototrophic microorganisms black fungi chemoorganotrophic bacteria

Table 2. Different weathering processes associated with biological colonization and related alterations on artistic stone material

The main types of biodeterioration processes, often intercorrelated, can be classified as (Amoroso, 1995; Tiano, 1998):

- *physical-mechanical*: abrasion, detachments, disaggregation, fissures and crevices formation due to organisms growth or movement.
- *chemical*: chemical changing of the stone substrata due to interaction with metabolic products, solubilisation, biopitting, and formation of new-reaction products.
- *aesthetical*: coloured patches, patinas, crusts.

Generally speaking, besides the discoloration and staining processes produced by biogenic pigments, the microflora leads to the change of materials characteristics with regard to their mechanical properties, superficial absorbency/hydrophobicity, stability, density, diffusivity and thermal-hydric behavior (Warscheid, 1996, 2008). For example, the biological formations with high water content (films, biofilms, subaerial biofilms, mats) can induce combined alteration processes, due to the fluid retention, to the repeated wetting and drying cycles with the subsequent expansions and contractions of the biocenosis, to the aggressive action of both metabolic products and to atmospheric pollutants entrapped in the EPS, by increasing chemical corrosion process. Therefore, the stony structure can present pitting, ion transfer, leaching processes and dwindling (Tiano, 1998).

A particular aspect of biodeterioration of stone monuments is linked to endolithic organisms colonizing the interior of rocks (Salvadori, 2000). The presence of endolithic formations (crusts, micropits, endolithic subaerial biofilms, plant root systems), which can penetrate up to depths of several millimetres, with the diffusion of their excreted products into the intergranular matrix, enhance the weathering reactions and decrease mechanical properties of the stone material.

The fauna has also an important role in stone biodeterioration. Microfauna, such as various arthropods which are feeding fungi and lichens, contributes in their diffusion with the transport of propagules and spores. Macrofauna, especially the birds, have a decay action due both their movement and organic material accumulation, which favors the growth of heterotrophic organisms.

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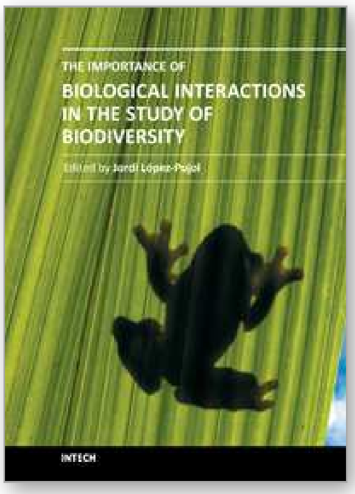

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The term biodiversity defines not only all the variety of life in the Earth but also their complex interactions. Under the current scenario of biodiversity loss, and in order to preserve it, it is essential to achieve a deep understanding on all the aspects related to the biological interactions, including their functioning and significance. This volume contains several contributions (nineteen in total) that illustrate the state of the art of the academic research in the field of biological interactions in its widest sense; that is, not only the interactions between living organisms are considered, but also those between living organisms and abiotic elements of the environment as well as those between living organisms and the humans.

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