npj | Materials Degradation

www.nature.com/npjmatdeg

REVIEW ARTICLE OPEN

Check for updates

Microbiologically induced aesthetic and structural changes to dimension stone

Christine C. Gaylarde[1]✉ and Jose Antonio Baptista-Neto[2]

Dimension stone is natural rock prepared for building use. It is rapidly colonised by microorganisms that cause discoloration (mainly cyanobacteria, algae and fungi) and structural damage. Microbial mobilisation of ions leads to new superficial or internal deposits, weakening the structure. Cyanobacteria and fungi may penetrate, filling pores or creating new spaces. Lichens, fungus/phototroph associations, colonise surfaces and damage stone through ingrowing rhizines and acid production. Initial degradation produces conditions suitable for germination of seeds of higher plants and further destruction. Emerging techniques to elucidate stone-cell interactions and control of initial biofilm formation that eventuates in stone disintegration are discussed.

npj Materials Degradation (2021)5:33 ; https://doi.org/10.1038/s41529-021-00180-7

INTRODUCTION

Dimension stone is any type of natural rock employed for building; the main rocks used are calcareous: limestone, marble, travertine; and siliceous: granite, quartzite, sandstone, soapstone, brownstone, basalt and slate. They can be used for building facades, monuments, paving and even furniture, such as kitchen worktops. The cutting and polishing of the rock removes any surface imperfections and previous incrustations that may have formed during its lifetime of natural exposure. However, once installed in its new position, the polished stone immediately becomes subject to new colonisation, initially by microorganisms[1]. Since the environment has changed, it is obvious that the new microbial flora will be different from that on the original rock and this has been shown by Vlasov et al.[2], who investigated the microorganisms colonising Rapakivi granite on St. Petersburg monuments and the original rock quarries. Differences were found especially in the fungal communities, with dark-coloured genera like *Cladosporium* and *Alternaria* being dominant in the urban, built environment. These granites are unusual, however, and their microbial populations may differ from other types. In particular, they do not have high quartz content, which may be the main granite component conferring relative resistance to microbial colonization because of its non-porous structure[3,4]; this may limit entry of, especially, filamentous microorganisms, actinomycetes, cyanobacteria and fungi[5].

The primary colonisers of the polished stone are the phototrophs and chemotrophic bacteria, which require no organic food materials[6–8]. These pave the way for heterotrophic and more invasive higher organisms, as well as having their own disfiguring and destructive effects. The chemical and physical interactions between the stone and the colonising organisms (bacteria, algae, fungi and, eventually, higher plants) can cause both aesthetic and structural, or mechanical, damage. The first colonisers of the surface produce thin coating layers, known as biofilms, that contain not only the living organisms, but also their metabolic products, including acids, oxidising/reducing agents, osmolytes and extracellular polymeric substances (EPS), all of which can affect the structure of the stone[9–11].

This review critically examines some of the many publications in this area, synthesising the diverse information available, and considers not only how the most recent microbiological techniques may alter our understanding of the phenomenon, but also, briefly, the methods available for controlling stone colonisation.

AESTHETIC CHANGES

An undesired alteration in the appearance of the stone, which does not accompany any apparent weakening of its structure, is termed Biodeterioration without Biodegradation. In the case of dimension stone, this is a sufficiently severe change to give impetus to the development of non-damaging cleaning techniques and protective treatments, which will be discussed later.

Many microbial biofilms simply produce a grey/black discolouration on the stone surface. This may be due to black or brown pigments produced by the cells (see Table 1), or may be a mixture of variously coloured substances that have aged and become oxidised.

The cyanobacteria, for example, can produce a wide variety of pigments, but they are often seen as a black discolouration on the dried surfaces of stone (Fig. 1); when rehydrated, the biofilm can regain its basic green colour, as the cells begin to multiply once more.

The level of humidity of the surface can make a considerable difference, not only to the colour of the biofilm, but also to its constituents. Some microorganisms require a much more humid surface than others for growth. This was demonstrated by the contrasting biofilms on two areas of a fort in Niteroi, Rio de Janeiro, Brazil. The dark green/brown biofilm below a leaking pipe contained Chloroflexi, a phototrophic filamentous bacterial group, as the major organism, while the grey/pale green dry biofilm was mainly non-photosynthetic Proteobacteria[12]. These climatic and positional factors are exemplified also by the biofilms seen on the Rio Bec style Mayan buildings in Campeche state, Mexico. Red coloration produced by overgrowth of the carotenoid-producing alga *Trentepohlia* was seen on North- and East-facing walls or on other sites protected by tree canopies or architectural elements, while on more sun-exposed sites the typical grey/black growth of

[1]University of Oklahoma, Department of Microbiology and Plant Biology, Norman, OK, USA. [2]Universidade Federal Fluminense, Departamento de Geologia e Geofísica, Av. General Milton Tavares de Souza, Niterói, RJ, Brazil. ✉email: cgaylarde@gmail.com

Table 1. Pigments produced by lithobiontic algae, bacteria and fungi and their functions.

Pigment	Colour(s)	Function	Microorganism type	Examples
Chlorophylls	Green	Light absorption and photosynthesis	All phototrophs	Algae, cyanobacteria
Carotenoids	Red, orange, yellow, brown	Light absorption, photoprotection	Algae, fungi, bacteria (including cyanobacteria)	*Trentepohlia, Rhodotorula, Rhodobacter, Gloeobacter*
Phycobilins (phycocyanin, phycoerythrin)	Blue, red	Light absorption	Cyanobacteria, algae	*Gloeocapsa, Cyanidium*
Scytonemins	Dark brown/red	Protection against stress, including UV	Sheathed or encapsulated cyanobacteria	*Scytonema, Nostoc, Gloeocapsa*
Mycosporine-like amino acids (MAAs)	Dark brown	Protection against stress, including UV	Cyanobacteria, fungi, some algae	*Nostoc, Rhodotorula, Prasiola*
Melanins	Black/brown	Protection against stress, extreme environments	Fungi, bacteria	*Aspergillus*, "black fungi/yeasts", *Streptomyces*

dehydration- and UV-resistant cyanobacteria (mainly *Gloeocapsa* and *Chroococcidiopsis*) was present[13]. There was no evidence that stone degradation was occurring beneath the biofilms. Nevertheless, the mere presence of dark-coloured areas can result in stone degradation by differential expansion and contraction of the surface layers[14].

Figure 1 shows some of the colorations that can develop on stone surfaces following colonisation by various microorganisms.

STRUCTURAL AND MECHANICAL CHANGES

It can be difficult to distinguish non-biological weathering (degradation) of stone from that associated with microbiological growth. However, there can be no doubt that microorganisms are able to cause stone degradation. This has been demonstrated in laboratory simulation experiments that measure cation leaching[15], physical changes such as alteration in weight, porosity and surface hardness[16,17], or simple colonisation and concomitant biodeterioration[18,19].

In urban areas, especially, atmospheric pollution is a major agent leading to degradation of various types of rocks in building facades, and can be linked to chemical, physical and biological processes. Rocks from building facades in urban environments tend to react with and fix pollutants, while microbial cells colonizing a stone surface can intensify the degrading effects of pollution. The deposited pollutants may be used by the cells for growth and pigment production, as well as the production of corrosive metabolites such as acids[20,21].

Non-biological stone degradation may often be distinguished in polluted environments at those sites which are sheltered from direct rainwash, which may remove pollutants but not the more strongly adherent cells. Such stone areas will be exposed to more concentrated pollution attack in the form of occult (e.g. dew and frost), dry gaseous, and particulate deposition. This leads to the formation of a characteristic black damage layer, which usually gives rise to more severe decay that includes loss of stone material. These non-biological black crusts typically comprise interlocking tabular crystals of gypsum that entrap atmospheric particulates[22] and form a reactive surface on which other precipitates can form[23]. These ancillary components include inorganic airborne particulates (e.g. soil particles, dust, fly ash), organic airborne particulates (e.g. plant remains, pollen), inorganic precipitates, and organic growth in or on the crust surface (e.g. bacteria, fungi)[24]. Carbonaceous particles (such as flyash), derived from oil and coal combustion, are also frequently present, and these can act as active catalysts for the transformation of calcium carbonate in the stone to gypsum[25]. Another type of black crust also exists, however; this is the thin crust found on buildings in unpolluted environments, which have been shown to be composed entirely of cyanobacteria. Such microbially produced crusts have been found in the Mexican State of Campeche[26], as well as in Laos, the Brazilian State of Minas Gerais[27] and Guatemala[28].

In the polluted environment of the modern city of Rio de Janeiro the majority of the historically important buildings are composed mainly of augen gneiss, an extremely thick siliceous rock, with a large amount of ovoid and sub-rectangular k-feldspar megacrystals, varying in size from 2 to 10 cm. There is a smaller amount of megaplagioclase which, together with the k-feldspars, makes up 50 to 90% of the total rock volume. The matrix is quartz-plagioclase of varying proportions and the main mafic mineral (Mg and Fe-rich silicate) is biotite, at about 10%. There are small quantities of garnet (<2%) and trace amounts of hypersthene. Among the main accessories are zircon and apatite. The augen gneiss in the area has a high degree of metamorphism. Our previously unpublished geochemical analyses of stone from exposed augen gneiss facades of central Rio churches show high concentrations of Ca, Na, Cl and SO_4^{2-} (Table 2), highlighting the

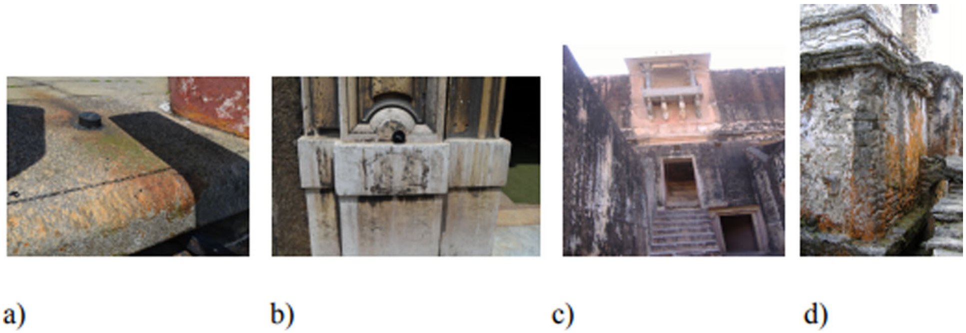

Fig. 1 Microbial discolouration of stone. Colours developed on stone surfaces by microbial colonisation. **a** Red and green growth of the alga *Trentepohlia* on gneiss; the orange/red coloration on the curved area indicates intracellular carotenoid droplets, possibly protecting the cells from lack of moisture or overexposure to sunlight. **b** Grey and light brown discoloration caused by cyanobacteria and fungal growth on limestone doorway of a church in Rio de Janeiro, Brazil. **c** Thick grey cyanobacterial and fungal biofilms on Amber fort, Rajasthan, India. The pink stone is the original colour of the sandstone. **d** Grey cyanobacteria-dominated biofilms next to red/brown growth of the alga, *Trentepohlia*, on the base of the limestone tower of the Palace, Palenque, Chiapas, Mexico.

presence of gypsum ($CaSO_4.2H_2O$), as expected from previous studies[21,23,29–31].

The presence of gypsum was confirmed by scanning electron microscopy (SEM) and petrographic thin section analyses (Figs. 2 and 3). The SEM analyses of the black crusts show high concentrations of gypsum occurring as needlelike crystals, with a lower concentration of halite. Gypsum crystals are accumulated in the inter- and intra-crystalline fractures of quartz and feldspar crystals and bridging gaps between open cleavage planes in micas, causing deformation and breaking (Fig. 2). In a previous analysis[21], neogypsum deposits were seen clustering around endolithic microbial cells, indicating the importance of microbial metabolism in deposition of new minerals. This is associated with the ability of microorganisms to solubilise deposits from the rock and transfer them to alternative locations, as discussed in subsequent sections.

Gypsum ($CaSO_4.2H_2O$) crystallises in the monoclinic system. Petrographic thin sections showed the formation of gypsum crystals in the rock microfractures (Fig. 3).

The fact that black gypsum crusts can develop over entire facades in a humid subtropical environment is testimony to the high levels of local pollution, especially particulate deposition. Reduced rainwash in the sheltered micro-environments of the narrow, canyon-like streets of central Rio de Janeiro overcomes the tendency for gypsum to be washed away. These observations further highlight that decay processes are primarily controlled by microclimatic conditions[23]. Gypsum is one of the most destructive of all salts[32]; it has been widely identified in stone monuments[33]. Cardell et al.[33] consider that the physical stress resulting from salt (halite and gypsite) crystallization in the rock pores is the most important mechanism of the deterioration of ornamental stone. Both of these salts are involved in disruption of the stone structure. Gypsum occurs mainly in areas associated with the black crust, or in fractures within the rock. The halite detected in the stone samples mainly relates to interaction with marine aerosols from the sea in Guanabara Bay; the crystals are found within the stone along with microbial cells[21] and, indeed, halite rocks have been shown to have a close relationship with microorganisms, especially cyanobacteria[34]. These two salts play a very important role in weathering of stonework, mainly when associated with the biological activities demonstrated by various authors[6,26]. The growth and metabolic activity of individual or complex microbial communities, algae, bacteria, cyanobacteria, fungi and lichens, influences the complex interaction between the various types of materials present in stone, leading to physical and chemical damage. Indeed, the simple formation of a thin biofilm on the stone surface can lead to damaging water retention, differential heating/cooling and removal of surface stone flakes by the drying and contracting adhesive gel[1,14]. Endolithic microorganisms growing within the stone can result in spalling of surface layers and endolithic fungi and cyanobacteria have often been detected in historic stone monuments[21,35–37]. Figure 4 shows green growth of mixed cyanobacteria discovered beneath a flake of sandstone removed from the surface of a church in Minas Gerais, Brazil.

More specific degradation comes from the metabolic activities of the attached organisms.

Acid production

Acid attack was the first mechanism of microbial stone attack to be postulated; Munz (1890, cited in ref. [35]) suggested that nitric acid produced by nitrifying bacteria was the cause of stone erosion. This type of attack is often associated with the production of pits. For instance, the pitting caused in the limestone monuments of the Mayan culture at Edzna, Mexico, has been shown to be associated with colonies of *Trentepohlia*[38], a red-pigment-producing alga whose growth is normally considered simply as an aesthetic problem, although spalling of sandstone apparently caused by this organism has been reported earlier[39]. Since no bacteria were seen alongside the algal colonies in Edzna, it seems likely that the organic acids known to be produced by algae[40] were responsible for the pits. It is possible, but unlikely because of the very localised attack, that cation chelation (q.v.) was responsible for the degradation. It has been suggested that the main mode of stone attack by fungi is through the production of organic acids[41] and certainly fungi isolated from deteriorated limestone at the Mayan site of Uxmal, Mexico, produced oxalic acid, which reacted with solubilized calcium from the stone to produce crystals of whewellite and weddellite[42]. However, it is unlikely that organic acids degrade siliceous stones by direct acid attack; it is more likely that in this case they are acting as chelating agents (q.v.).

Song et al.[4] carried out quantitative analyses in vitro to show that *Bacillus subtilis* produced pits in a granite surface. Plagioclase or albite (aluminosilicates) were found to be the most vulnerable minerals in the rock, suggesting that the quartz component could be resistant because of its hard, non-porous structure. Barker et al.[43] had previously suggested that the aluminosilicate feldspar components were more prone to microbial attack because they contain ions that react strongly with organic acids.

Cation mobilisation

Most minerals are sparingly soluble in pure water, with equilibrium levels being reached before significant lattice damage can occur. Organic chelating agents, such as acids and polysaccharides, enhance cation solubility[40]. They react with the ions

Table 2. Average concentrations (mg.L^{-1}) of the main substances detected in stone samples from the facades of various churches in Rio de Janeiro.

Church	Component														
	F	Cl	NO^{3-}	PO^{4-}	SO^{4-}	Fe	Mn	Zn	Cu	Pb	Ni	Ca	Mg	Na	K
Nossa Senhora do Carmo	2.3	340.13	404.87	65.13	3074.22	1.87	0.73	0.32	3.1	0.1	0.1	2534	34	98	95
Candelária	2.9	311.78	350.23	11.03	4163.49	2.02	0.95	0.97	2.67	0.2	0.1	1809	42	76	120
São Francisco	1.7	473.89	178.98	9.6	598.13	0.70	0.51	0.2	1.02	0.1	–	524	24	64	140
Santa Rita	–	78.42	146.68	7.87	762.21	0.55	0.46	0.2	1.67	–	–	208	13	25	67
Mãe dos Homens	2.4	259.11	289.76	12.19	5972.37	1.23	0.92	0.77	2.2	0.12	0.1	1983	19.5	55	90
Detection Limits						0.04	0.03	0.01	0.03	0.10	0.04	0.09	0.01	0.01	0.03

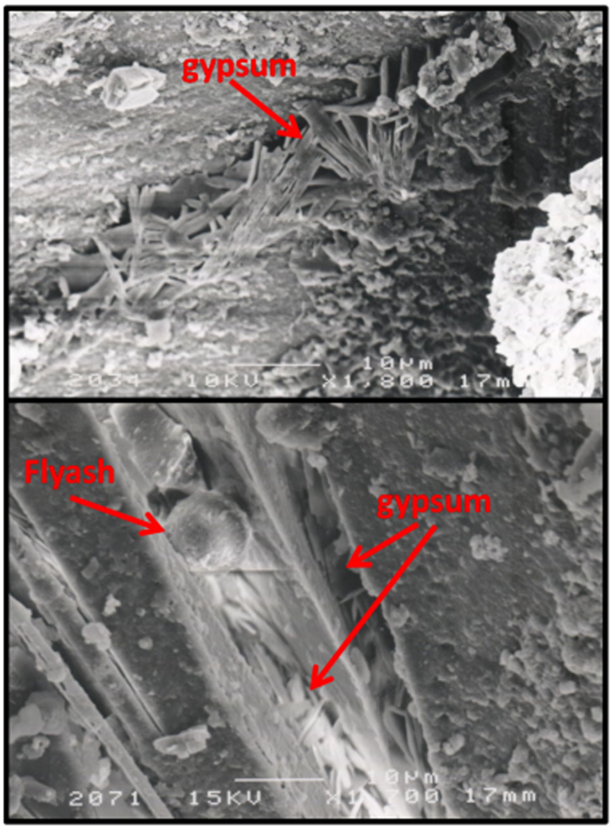

Fig. 2 Scanning electron micrographs of black crust. SEM micrographs of black crusts on the augen gneiss facade of a church in Rio de Janeiro, Brazil, showing needle-like crystals of gypsum accumulated in the stone facade; fly ash is also shown.

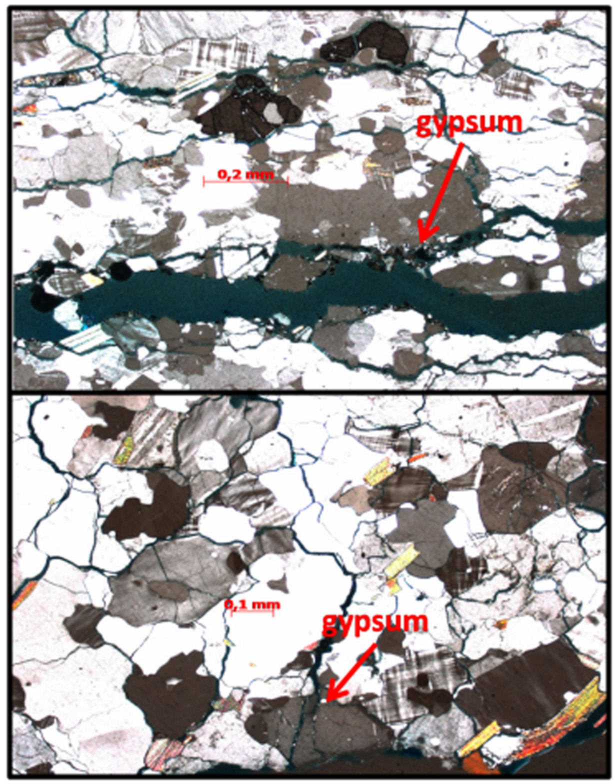

Fig. 3 Petrographic thin section of augen gneiss crusts. Petrographic thin section showing the formation of gypsum crystals in the microfractures of the stone below the crusts shown in Fig. 2.

as they are released, producing soluble organic complexes that can move through the pores of the stone and be deposited when they come into contact with a suitable precipitant, which may simply be oxygen at the surface or near-surface.

Barrionuevo et al.[44] found evidence of iron and manganese mobilisation from within the sandstones of the ruins of the Argentine missions, with a surface layer enriched in these minerals compared to the interior. There was a complex biofilm on these buildings, many components of which could be involved in such mobilisation and redeposition activities through cation chelation and acid production. A wide range of chelating agents are produced by bacteria[45], algae[46], cyanobacteria[47] and fungi[48]. Those that are involved in solubilisation of iron, facilitating uptake by the cells, have been termed siderophores. As they are secreted by the cells that synthesise them, they may also be used by other organisms[49] as well as for dissolution of iron from within the stone structure resulting in weakening and potential transport and reprecipitation in other areas.

Alkaline dissolution of Si and Al

The majority of the literature emphasises the damage caused by acidic microbial metabolites on built stone and this is doubtless one of the main degrading activities of bacteria and fungi. However, Gaylarde and Gaylarde[35] consider the susceptibility of siliceous stone to alkaline degradation. In concrete, the microfractures and spalling that can be induced when the silicate content of the aggregate particles reacts with alkali hydroxides that may be present in the cement itself, are well recognised[50] and this reaction has been the subject of considerable research. The first step in alkaline silicate weathering seems to be the acid-base reaction between the silica and the alkali to produce siloxane. The siloxane bridges are then attacked by alkali to cause disintegration of the stone. Ichikawa and Koizumi[51] found that irradiation increased the susceptibility of concrete to the alkali-silicate reaction; both crystalline and amorphous quartz were

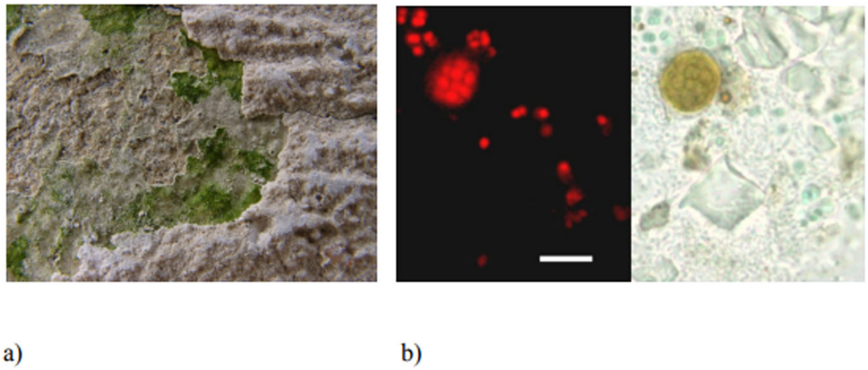

Fig. 4 Endoliths. Endolithic cyanobacteria growing within sandstone. In **a**, which is shown at approximately half-size, the green growth is seen; in **b**, the brown and green cells within the pulverised flake of stone are visualised under the light microscope and as autofluorescent red cells (indicating the presence of chlorophyll) under UV light. Bar marker = 10 µm.

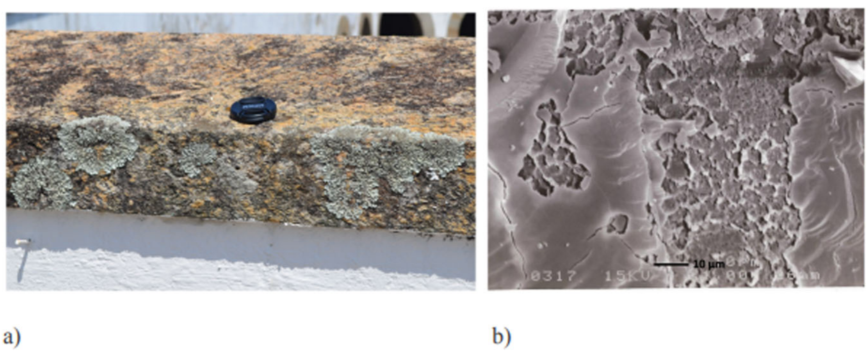

Fig. 5 Lichen growth. a The grey/green foliose lichen *Parmelia saxatilis* growing on factoidal gneiss in the Fortress of Santa Cruz da Barra, Niteroi, Brazil (size indicated by the lens cap), and **b** the etching beneath it, seen by scanning electron microscopy (bar marker 10 µm).

affected, the increased degradation being due to the formation of the more susceptible distorted amorphous quartz on the surface. Considering siliceous stone itself, rather than aggregate, the Si and Al content will be mobilised above pH 9.5, a pH value which is readily attained by phototrophic organisms during photosynthesis during the hours of sunlight[52,53]. Hence the alkaline degradation of siliceous stone by cyanobacterial and algal biofilms doubtless occurs. This may also be induced by cellular production of polyols.

Polyols are produced by all types of living organisms. They are organic compounds containing multiple hydroxyl groups, for example, sugar alcohols like mannitol and sorbitols (low molecular weight polyols) and polysaccharides (high molecular weight polyols). Such chemicals bind to siloxane layers in siliceous stone, causing them to expand and thus weakening the structure[35]. The findings of Song et al.[4] on differential degradation by *Bacillus subtilis* of quartz and aluminosilicates in granite could be explained by the action of bacterial polyols, known to be produced by this species[54]. Many microorganisms, bacteria, fungi[55,56], algae[57] and cyanobacteria[58] produce polyols. Vlasov et al.[2] found that polyols were higher in stone biofilms with a predominance of fungi. They found no important difference between quarry rocks and built monuments in St. Petersburg in terms of fungal/phototroph dominance, but the polyol content was higher in the urban samples; they concluded that this was a function of the taxonomic composition of the biofilms, but did not investigate any related biodegradation.

THE SPECIAL CASE OF LICHENS

Lichens are symbiotic associations of a filamentous fungus, the mycobiont, and one or more phototrophs, the photobiont. They take various physical forms, of which the crustose form is the most damaging to stone, being particularly strongly adherent to the surface. Other forms are foliose and fruticose. It is traditionally assumed that the phycobiont (an alga or cyanobacterium) donates organic carbon to the relationship, itself gaining minerals and protection from the fungus. Recently, however, it has become apparent that lichens may also contain yeasts[59] and non-photosynthetic bacteria[60] that also contribute to the relationship. It is difficult, therefore, to determine the exact origin of the destructive activities of lichens on stone surfaces, but there is clear evidence of such destruction on many historic stone buildings[61–63]. By lichenic dissolution of mineral elements from the stone, degradation occurs; for example, lichen growth on basalt was shown to produce ferromanganese minerals, leaving a calcium-rich surface (Jones et al., 1980, cited in ref. [64]), ready to react with sulfur compounds from polluted air, as previously described in this article.

The etching produced by lichens on rocks and stones (Fig. 5) was commonly thought to be produced by so-called "lichenic acid"[65]; these authors also note the neoformation of calcium carbonate, calcium oxalates and gypsum under lichen action. Gadd[66] discussed the various acids produced by lichens that could degrade stone surfaces, the majority being organic acids such as oxalic.

This group of organisms also physically penetrate rocks with their rhizines, small, fungal 'roots', which probably require prior weakening of the stone surface for their entry. This effect gives the lichen a very strong physical hold on the surface. It has been suggested that it is inadvisable to remove such growths, not only because rough removal techniques can further damage the surface, but also because the actual physical presence of the lichen may protect against attack by other agents, including non-biological climatic influences[67]. Nevertheless, lichens are inherently a danger to stone integrity; not only do crustose lichens actively penetrate the rock, they may also harbour and protect degradative microorganisms beneath their thalli. Endolithic cyanobacteria beneath lichen growths have been shown to disaggregate the rock of the Tomb of Cyrus, in Pasargadae, Iran[61]. Stone degradation by lichens is complex and yet to be completely elucidated.

POTENTIAL CONTROL

As already noted, stone biodeterioration begins with its colonisation by microorganisms. The prevention of this process, therefore, presents a method of stopping biodeterioration before it begins. Coating the surface with a suitable protective layer and inhibiting the attachment and/or growth of the initial colonisers are potential strategies.

The use of a hydrophobic coating is a possible solution. This protects the stone from absorption of water and materials carried in it; it should also be resistant to wear and abrasion and aggressive chemicals like those produced by microorganisms. However, it will not necessarily be resistant to the attachment of microorganisms. The development of antibiofilm coatings is extremely challenging[68]. Traditional coatings are acrylic polymers, siloxanes, fluoropolyethers and fluorinated acrylic polymers. These may suffer from poor adhesion to the underlying substrate, may lose their protective effect quite rapidly with time, and also may, themselves, be colonised by microorganisms[69]. New and developing technologies, involving nanomaterials, biomimetic approaches and packaging/carrier systems (nanocapsules) may revolutionise the available protective systems, if the increased costs can be overcome; for example Jin et al.[70] have produced halloysite nanotubes that offer controlled release of a fungicide that could be useful for long-term control of fungal growth on stone. The group of Ruggiero, in Rome, has been very active in this field recently[71–73], developing antimicrobial and water-repellant nanocoatings for use on stone surfaces, while De Leo et al.[68] have developed surface-active ionic liquid-containing coatings that can both remove colonising organisms and prevent new biofilm formation. The latter is an important objective, as reapplication of removal treatments can be not only expensive, but also potentially damaging to the stone.

The need for more environmentally acceptable antimicrobial treatments has led to a number of research groups working on so-called "natural biocides" or "green conservation", using substances extracted from plants or other forms of life that kill or inhibit microorganisms. These are generally cheap to produce and are considered safe not only for the environment but also for the stone structure and the people working with it[9,73–76]. Their efficacy is, however, not always proven by application to existing buildings[75,77].

Once the biofilm (sometimes called a patina) has been formed it is necessary to use a removal technique that does not damage the underlying stone substrate; scrubbing and use of bleach, for example, are not recommended. Gabriele et al.[78] tested a hydrogel that incorporated hypochlorite into sodium alginate and found that it effectively removed the filamentous cyanobacteria and algae from calcarenite Lecce samples. Laser cleaning has some advantages over mechanical and chemical cleaning; it is gradual, selective, contactless and environmentally friendly. However, the wavelength must be carefully selected if damage is not to occur. Barreiro et al.[79] found that a wavelength of 532 nm produced the best biofilm removal from Vilachán granite, but was somewhat aggressive, producing greatest change in appearance through selective extraction of some weathering materials. Biotite melting occurred on all surfaces regardless of the wavelength.

While complete prevention of biofilm formation may not be possible at a reasonable financial and environmental cost, periodic inspection of stone buildings is essential. Ferreira et al.[80] carried out a study on 203 Portuguese buildings with natural stone claddings and determined that a system of cleaning and minor repair, when first signs of degradation were seen, promoted the durability of the structure. Although costs were increased, the strategy increased the predicted lifetime of the materials to a worthwhile extent. This, indeed, conforms to the 'good housekeeping' rationale promoted by the Biodeterioration community[81].

CONTRIBUTION OF NEW METHODOLOGY TO MICROBIOLOGICAL STUDIES ON STONE

The new techniques of genetic and metabolic analyses will profoundly influence our understanding of microbial interactions with stone in the future. Although speculations on the possible microbial activities that result in stone degradation exist (for example, chelation of Mn, solubilisation of Fe, sulfur and silicon metabolism, production of organic acids and pigments), there is no direct evidence to show that the corresponding changes in degraded stone were actually caused by microbial cells. Only rather recently have the so-called 'next-generation sequencing' (NGS) techniques been applied to stone[21,82]. These have allowed the detection of a much greater number of microbial taxa than was previously recognised. Now, metagenomic analyses have begun to be applied to these ecological systems; these can lead to the identification of functional genes in the microbial population, allowing us to detect cells with the abilities to produce chelating molecules, to metabolise sulfur, manganese, nitrogen, and to produce relevant organic acids and pigments. Esposito et al.[83] used shotgun metagenomic sequencing to analyse the microbial population of a siliceous rock varnish in the Matsch Valley, Italy. Interestingly, they found a high number of Archaea in the varnish. Functional genes of interest detected within the genomes showed the potential for CO_2 fixation, carbohydrate and nitrogen metabolism, siderophore biosynthesis and genes associated with photosynthesis. The very young science of metabolomics has not yet been applied to rocks, although Gutarowska et al.[84] have used untargeted metabolomics with ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry to detect the activated pathways of microbial cells in wood and brick. This group used the same method to detect putative activities in the degradation of a variety of building materials: sulfur metabolism, carbohydrate digestion, carotenoid biosynthesis and photosynthesis[85]. Sanmartin et al.[86] reviewed some of the metabolic profiling methods that had been used to analyse biodeterioration of various types of cultural heritage, including stone, up to that time. Together with older techniques, such as scanning electron microscopy, these may allow us to understand the real interactions between microorganisms and their stone substrate, which should lead to an increased ability to control the biodegradation of dimension stone in the future.

DATA AVAILABILITY

The datasets analysed during this study are included in this published article.

Received: 20 April 2021; Accepted: 27 May 2021;
Published online: 17 June 2021

REFERENCES

1. Scheerer, S., Ortega-Morales, O. & Gaylarde, C. Chapter 5: Microbial deterioration of stone monuments-an updated overview. *Adv. Appl. Microbiol.* https://doi.org/10.1016/S0065-2164(08)00805-8 (2009).
2. Vlasov, D. Y. et al. in *Processes and Phenomena on the Boundary Between Biogenic and Abiogenic Nature* (eds Frank-Kamenetskaya, O., Vlasov, D., Panova, E. & Lessovaia, S.), Lecture Notes in Earth System Sciences, https://doi.org/10.1007/978-3-030-21614-6_29 (Springer, 2020).

3. Rades-Rohkohl, E., Fränzle, O. & Hirsch, P. Behavior, activities, and effects of bacteria on synthetic quartz monocrystal surfaces. *Microb. Ecol.* **4**, 189–205 (1977).
4. Song, W., Ogawa, N., Takashima-Oguchi, C., Hatta, T. & Matsukura, Y. Laboratory experiments on bacterial weathering of granite and its constituent minerals. *Biogeomorphology* **16**, 327–336 (2010).
5. Abdulla, H. Bioweathering and biotransformation of granitic rock minerals by actinomycetes. *Microb. Ecol.* **58**, 753–761 (2009).
6. Crispim, C. & Gaylarde, C. Cyanobacteria and biodeterioration of cultural heritage: a review. *Microb. Ecol.* https://doi.org/10.1007/s00248-003-1052-5 (2005).
7. Di Carlo, E., Barresi, G. & Palla, F. in *Biotechnology and Conservation of Cultural Heritage* (eds Palla, F. & Barresi, G.) (Springer, 2017).
8. Wang, Y. & Liu, X. Sulfur-oxidizing bacteria involved in the blackening of basalt sculptures of the Leizhou Stone Dog. *Int. Biodeterior. Biodegrad.* https://doi.org/10.1016/j.ibiod.2021.105207 (2021).
9. Liu, X., Koestler, R. J., Warscheid, T., Katayama, Y. & Gu, J.-D. Microbial deterioration and sustainable conservation of stone monuments and buildings. *Nat. Sustain* https://doi.org/10.1038/s41893-020-00602-5 (2020).
10. Sakr, A. A., Ghaly, M. F., Edwards, H. G. M., Ali, M. F. & Abdel-Haliem, M. E. F. Involvement of *Streptomyces* in the deterioration of cultural heritage materials through biomineralization and bio-pigment production pathways: a review. *Geomicrobiol. J.* https://doi.org/10.1080/01490451.2020.1754533 (2020).
11. Bailón Moreno, R. et al. Granada, Spain, Vol. 2, 1177–1188 (University of Granada, 2018).
12. Ogawa, A., Celikkol-Aydin, S., Gaylarde, C., Baptista-Neto, J. A. & Beech, I. Microbial communities on painted wet and dry external surfaces of a historic fortress in Niteroi, Brazil. *Int. Biodeterior. Biodegrad.* **123**, 164–173 (2017).
13. Ortega-Morales, B. O. et al. Orientation affects *Trentepohlia*-dominated biofilms on Mayan monuments of the Rio Bec style. *Int. Biodeterior. Biodegrad.* https://doi.org/10.1016/j.ibiod.2012.07.014 (2013).
14. Warscheid, T. & Braams, J. Biodeterioration of stone: a review. *Int. Biodeterior. Biodegrad.* https://doi.org/10.1016/S0964-8305(00)00109-8 (2000).
15. McNamara, C. J., Perry, T. D., Bearce, K. A., Hernandez-Duque, G. & Mitchell, R. Measurement of limestone biodeterioration using the Ca^{2+} binding fluorochrome Rhod-5N. *J. Microbiol. Meth.* **61**, 245–250 (2005).
16. May, E., Papida, S., Hesham, A., Tayler, S. & Dewedar, A. in *Of Microbes and Art. The Role of Microbial Communities in the Degradation and Protection of Cultural Heritage* (eds Ciferri, O., Tiano, P. & Mastromei, G.) (Kluwer Academic/ Plenum, 2000).
17. Papida, S., Murphy, W. & May, E. Enhancement of physical weathering of building stones by microbial populations. *Int. Biodeterior. Biodegrad.* **46**, 305–317 (2000).
18. Favero-Longo, S. E., Borghi, A., Tretiach, M. & Piervittori, R. In vitro receptivity of carbonate rocks to endolithic lichen-forming aposymbionts. *Mycol. Res.* https://doi.org/10.1016/j.mycres.2009.08.006 (2009).
19. George, R. P., Ramya, S., Ramachandran, D. & Kamachi Mudali, U. Studies on biodegradation of normal concrete surfaces by fungus *Fusarium* sp. *Cem. Concr. Res.* https://doi.org/10.1016/j.cemconres.2013.01.010 (2013).
20. Balland-Boulou-Bi, C. et al. in *Science and Art: A Future for Stone: Proceedings of the 13th International Congress on the Deterioration and Conservation of Stone* (eds Hughes, J., Howind, T.) Vol. 1, 24–32 (University of the West of Scotland, 2016).
21. Gaylarde, C. et al. Epilithic and endolithic microorganisms and deterioration on stone church facades subject to urban pollution in a sub-tropical climate. *Biofouling* **33**, 113–127 (2017).
22. Saiz-Jimenez, C. Deposition of airborne organic pollutants on historic buildings. *Atmos. Environ.* **27B**, 77–85 (1993).
23. Baptista Neto, J. A. et al. Surface modification of a granite building stone in central Rio de Janeiro. *Acad. Bras. Cienc.* **78**, 317–330 (2006).
24. Whalley, W. B., Smith, B. J. & Magee, R. W. in *Stone Cleaning and the Nature, Soiling and Decay Mechanisms of Stone* (ed. Webster, R. G. M.) 227–238 (Donheat Publ., 1992).
25. Del Monte, M., Sabbioni, C., Ventura, A. & Zappia, G. Crystal growth from carbonaceous particles. *Sci. Total Environ.* **36**, 247–254 (1984).
26. Gaylarde, C. C., Ortega-Morales, B. O. & Bartolo-Perez, P. Biogenic black crusts on buildings in unpolluted environments. *Curr. Microbiol.* **54**, 162–166 (2007).
27. Ortega-Morales, O. et al. Deterioration and microbial colonization of cultural heritage stone buildings in polluted and unpolluted tropical and subtropical climates: a meta-analysis. *Int. Biodeter. Biodegr* https://doi.org/10.1016/j.ibiod.2019.104734 (2019).
28. García de Miguel, J. M., Sánchez-Castillo, L., Ortega-Calvo, J. J., Gil, A. & Saiz-Jimenez, C. Deterioration of building materials from the Great jaguar pyramid at tikal, Guatemala. *Build. Environ.* **30**, 591–598 (1995).
29. Smith, B. J. & Magee, R. W. Granite weathering in an urban environment: an example from Rio de Janeiro. *Singap. J. Trop. Geogr.* **2**, 143–153 (1990).
30. Smith, B. J. et al. The decay of coastal forts in southeast Brazil and its implications for the conservation of colonial built heritage. *Environ. Geol.* **46**, 493–503 (2004).
31. Baptista Neto, J. A., Smith, B. J., McAlister, J. J., Silva, M. A. M. & Silva, A. L. C. in *Salt Weathering on Building and Stone Sculpture* 113–120 (University of Cyprus – Europe, 2011).
32. Duffy, A. P., Cooper, T. P. & Perry, S. H. Repointing mortars for conservation of a historic stone building in Trinity College, Dublin. *Mater. Struct*. 10.1007/BF02472952 (1993).
33. Cardell, C. et al. Salt-induced decay in calcareous stone monuments and buildings in a marine environment in SW France. *Constr. Build. Mats* https://doi.org/10.1016/S0950-0618(02)00104-6 (2003).
34. de Los Ríos, A. et al. Comparative analysis of the microbial communities inhabiting halite evaporites of the Atacama Desert. *Int. Microbiol*. https://doi.org/10.2436/20.1501.01.113 (2010).
35. Gaylarde, P. & Gaylarde, C. Deterioration of siliceous stone monuments in Latin America: microorganisms and mechanisms. *Corr. Rev.* **22**, 395–415 (2004).
36. Favero-Longo, S. E. & Viles, H. A. A review of the nature, role and control of lithobionts on stone cultural heritage: weighing-up and managing biodeterioration and bioprotection. *World J. Microbiol. Biotechnol.* https://doi.org/10.1007/s11274-020-02878-3 (2020).
37. Tonon, C. et al. Hyphal morphology and substrate porosity—rather than melanization—drive penetration of black fungi into carbonate substrates. *J. Cult. Herit.* https://doi.org/10.1016/j.culher.2020.11.003 (2021).
38. Gaylarde, P., Englert, G., Ortega-Morales, O. & Gaylarde, C. Lichen-like colonies of pure *Trentepohlia* on limestone monuments. *Int. Biodeterior. Biodegrad.* **58**, 248–253 (2006).
39. Wakefield, R. D. et al. Investigations of decayed sandstone colonised by a species of *Trentepohlia*. *Aerobiologia* https://doi.org/10.1007/BF02248119 (1996).
40. Mustoe, G. E. Biogenic weathering: Solubilization of iron from minerals by epilithic freshwater algae and cyanobacteria. *Microorganisms* https://doi.org/10.3390/microorganisms6010008 (2018).
41. Gadd, G. M. Geomycology: Biogeochemical transformations of rocks, minerals and radionucleotides by fungi, bioweathering and bioremediation. *Mycol. Res.* **111**, 3–49 (2007).
42. Ortega-Morales, B. O. et al. Bioweathering potential of cultivable fungi associated with semi-arid surface microhabitats of Mayan buildings. *Front. Microbiol.* https://doi.org/10.3389/fmicb.2016.00201 (2016).
43. Barker, W. W., Welch, S. A., Banfield, J. F. in *Geomicrobiology: Interactions between Microbes and Minerals* (eds Banfield, J. F. & Nealson, K. H.) 391–428 (Mineralogical Society of America, 1997).
44. Barrionuevo, M. R. E., Englert, G. E. & Gaylarde, C. C. Physical and microbiological analysis of sandstone deterioration in the Argentine Jesuit missions. *Geomicrobiol. J.* https://doi.org/10.1080/01490451.2015.1079668 (2016).
45. Leventhal, G. E., Ackermann, M. & Schiessl, K. T. Why microbes secrete molecules to modify their environment: The case of iron-chelating siderophores. *J. R. Soc. Interface* https://doi.org/10.1098/rsif.2018.0674 (2019).
46. Priyadarshini, E., Priyadarshini, S. S. & Pradhan, N. Heavy metal resistance in algae and its application for metal nanoparticle synthesis. *Appl. Microbiol. Biotechnol.* https://doi.org/10.1007/s00253-019-09685-3 (2019).
47. Nishanth, S. et al. 2021. in *Microbial and Natural Macromolecules* (eds Das, S. & Dash, H. R.) 349–369 (Academic Press, 2021).
48. Pathak, A., Kothari, R., Vinoba, M., Habibi, N. & Tyagi, V. V. Fungal bioleaching of metals from refinery spent catalysts: A critical review of current research, challenges, and future directions. *J. Environ. Manag.* https://doi.org/10.1016/j.jenvman.2020.111789 (2021).
49. Kramer, J., Özkaya, Ö. & Kümmerli, R. Bacterial siderophores in community and host interactions. *Nat. Rev. Microbiol.* https://doi.org/10.1038/s41579-019-0284-4 (2020).
50. Swamy, R. N. *The Alkali-Silica Reaction in Concrete* (CRC Press, 1991).
51. Ichikawa, T. & Koizumi, H. Possibility of radiation-induced degradation of concrete by alkali-silica reaction of aggregates. *J. Nucl. Sci. Technol.* https://doi.org/10.1080/18811248.2002.9715272 (2002).
52. Miller, A. G., Espie, G. S. & Canvin, D. C. Physiological aspects of CO_2 and HCO_3- transport by cyanobacteria: a review. *Can. J. Bot.* https://doi.org/10.1139/b90-165 (1990).
53. Ataeian, M. et al. Direct capture and conversion of CO_2 from air by growing a cyanobacterial consortium at pH up to 11.2. *Biotechnol. Bioeng.* https://doi.org/10.1002/bit.26974 (2019).
54. Rice, T., Zannini, E., Arendt, E. K. & Coffey, A. A review of polyols—biotechnological production, food applications, regulation, labeling and health effects. *Crit. Rev. Food Sci. Nutr.* https://doi.org/10.1080/10408398.2019.1625859 (2020).
55. Zakaria, A. Production of natural and rare pentoses using microorganisms and their enzymes. *Electron. J. Biotechnol.* **4**, 103–111 (2001).
56. Filippousi, R. et al. Isolation, identification and screening of yeasts towards their ability to assimilate biodiesel-derived crude glycerol: microbial production of polyols, endopolysaccharides and lipid. *J. Appl. Microbiol.* https://doi.org/10.1111/jam.14373 (2019).

57. Gustavs, L., Görs, M. & Karsten, U. Polyol patterns in biofilm-forming aero-terrestrial green algae (Trebouxiophyceae, Chlorophyta). *J. Phycol.* https://doi.org/10.1111/j.1529-8817.2011.00979.x (2011).
58. Pade, N. & Hagemann, M. Salt acclimation of cyanobacteria and their application in biotechnology. *Life* https://doi.org/10.3390/life5010025 (2015).
59. Mark, K. et al. Contrasting co-occurrence patterns of photobiont and cystobasidiomycete yeast associated with common epiphytic lichen species. *N. Phytol.* https://doi.org/10.1111/nph.16475 (2020).
60. Biosca, E. G., Flores, R., Santander, R. D., Díez-Gil, J. L. & Barreno, E. Innovative approaches using lichen enriched media to improve isolation and culturability of lichen associated bacteria. *PLoS ONE*, e0160328, https://doi.org/10.1371/journal.pone.0160328 (2016)
61. Sohrabi, M. et al. Lichen colonization and associated deterioration processes in Pasargadae, UNESCO world heritage site, Iran. *Int. Biodeterior. Biodegrad.* https://doi.org/10.1016/j.ibiod.2016.12.012 (2017).
62. de los Ríos, A. & Ascaso, C. Contributions of in situ microscopy to the current understanding of stone biodeterioration. *Int. Microbiol.* **8**, 181–188 (2005).
63. Sırt Çıplak, E. & Akoğlu, K. G. Enzymatic activity as a measure of total microbial activity on historical stone. *Heritage* https://doi.org/10.3390/heritage3030038 (2020).
64. Chen, J. et al. Weathering of rocks induced by lichen colonization: a review. *CATENA* **39**, 121–146 (2000).
65. Silva, B. & Prieto, B. in *Biodeterioration of Stone Surfaces* (eds St. Clair, L. L. & Seaward, M. R. D.) (Springer, 2004).
66. Gadd, G. M. Fungi, rocks, and minerals. *Elements* https://doi.org/10.2113/gselements.13.3.171 (2017).
67. Gadd, G. M. & Dyer, T. D. Bioprotection of the built environment and cultural heritage. *Micro. Biotechnol.* **10**, 1152–1156 (2017).
68. De Leo, F. et al. Surface active ionic liquids based coatings as subaerial anti-biofilms for stone built cultural heritage. *Coatings* https://doi.org/10.3390/coatings11010026 (2021).
69. Ruffolo, S. A. & La Russa, M. F. Nanostructured coatings for stone protection: An overview. *Front. Mater.* https://doi.org/10.3389/fmats.2019.00147 (2019).
70. Jin, X. et al. Functionalization of halloysite nanotubes by enlargement and layer-by-layer assembly for controlled release of the fungicide iodopropynyl butylcarbamate. *RSC Adv.* https://doi.org/10.1039/c9ra07593c (2019).
71. Ruggiero, L. et al. Silica nanosystems for active antifouling protection: nanocapsules and mesoporous nanoparticles in controlled release applications. *J. Alloy. Compd.* https://doi.org/10.1016/j.jallcom.2019.05.215 (2019).
72. Ruggiero, L. Synthesis and characterization of TEOS coating added with innovative antifouling silica nanocontainers and TiO2 nanoparticles. *Frontiers* **7**, 1–11 (2020).
73. Ruggiero, L. et al. Encapsulation of environmentally-friendly biocides in silica nanosystems for multifunctional coatings. *Appl. Surf. Sci.* https://doi.org/10.1016/j.apsusc.2020.145908 (2020).
74. Genova, C., Fuentes, E., Sanmartín, P., Favero, G. & Prieto, B. Phytochemical compounds as cleaning agents on granite colonized by phototrophic subaerial biofilms. *Coatings* https://doi.org/10.3390/coatings10030295 (2020).
75. Fidanza, M. R. & Caneva, G. Natural biocides for the conservation of stone cultural heritage: a review. *J. Cult. Herit.* https://doi.org/10.1016/j.culher.2019.01.005 (2019).
76. Gagliano Candela, R., Maggi, F., Lazzara, G., Rosselli, S. & Bruno, M. The essential oil of *Thymbra capitata* and its application as a biocide on stone and derived surfaces. *Plants* https://doi.org/10.3390/plants8090300 (2019).
77. Sasso, S. et al. Potential of natural biocides for biocontrolling phototrophic colonization on limestone. *Int. Biodeterior. Biodegrad.* https://doi.org/10.1016/j.ibiod.2015.11.017 (2016).
78. Gabriele, F. et al. Alginate-biocide hydrogel for the removal of biofilms from calcareous stone artworks. *J. Cult. Herit.* https://doi.org/10.1016/j.culher.2021.02.009 (2021).
79. Barreiro, P., Andreotti, A., Colombini, M. P., González, P. & Pozo-Antonio, J. S. Influence of the laser wavelength on harmful effects on granite due to biofilm removal. *Coatings* https://doi.org/10.3390/coatings10030196 (2020).
80. Ferreira, C., Silva, A., de Brito, J., Dias, I. S. & Flores-Colen, I. Definition of a condition-based model for natural stone claddings. *J. Build. Eng.* https://doi.org/10.1016/j.jobe.2020.101643 (2021).
81. Allsopp, D., Seal, K. & Gaylarde, C. *Introduction to Biodeterioration* 162 (Cambridge University Press, 2004).
82. Vázquez-Nion, D., Rodríguez-Castro, J., López-Rodríguez, M. C., Fernández-Silva, I. & Prieto, B. Subaerial biofilms on granitic historic buildings: microbial diversity and development of phototrophic multi-species cultures. *Biofouling* https://doi.org/10.1080/08927014.2016.1183121 (2016).
83. Esposito, A., Borruso, L., Rattray, J. E., Brusetti, L. & Ahmed, E. Taxonomic and functional insights into rock varnish microbiome using shotgun metagenomics. *FEMS Microbiol. Ecol.* https://doi.org/10.1093/femsec/fiz180 (2019).
84. Gutarowska, B. et al. Metabolomic and high-throughput sequencing analysis—modern approach for the assessment of biodeterioration of materials from historic buildings. *Front. Microbiol.* https://doi.org/10.3389/fmicb.2015.00979 (2015).
85. Adamiak, J. et al. Untargeted metabolomics approach in halophiles: Understanding the biodeterioration process of building materials. *Front. Microbiol.* https://doi.org/10.3389/fmicb.2017.02448 (2017).
86. Sanmartín, P., DeAraujo, A. & Vasanthakumar, A. Melding the old with the new: Trends in methods used to identify, monitor, and control microorganisms on cultural heritage materials. *Microb. Ecol.* https://doi.org/10.1007/s00248-016-0770-4 (2018).

AUTHOR CONTRIBUTIONS

Both authors contributed equally to the production of this article.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to C.C.G.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.