A review of the nature, role and control of lithobionts on stone cultural heritage: weighing-up and managing biodeterioration and bioprotection

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The nature, role and control of lithobionts on stone cultural heritage: weighing-up and managing biodeterioration and bioprotection

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Abstract

Lithobionts (rock-dwelling organisms) have been recognized as agents of aesthetic and physico-chemical deterioration of stonework, and removal of microorganisms from cultural heritage stone surfaces (CHSS) is widely considered a necessary step in conservation interventions. On the other hand, lithobiontic communities can help integrate CHSS with their environmental setting and enhance biodiversity. Moreover, in some cases bioprotective effects have been reported and even interpreted as potential biotechnological solutions for conservation.

This paper reviews the plethora of traditional and innovative methodologies to characterize lithobionts on CHSS in terms of biodiversity, interaction with the stone substrate and impacts on durability. In order to develop the best management and conservation strategies for CHSS, such diagnosis should be acquired on a case-by-case basis, as generalized approaches are unlikely to be suitable for all lithobionts, lithologies, environmental and cultural contexts or types of stonework.

Strategies to control biodeteriogenic lithobionts on CHSS should similarly be based on experimental evaluation of their efficacy, including long term monitoring of their effects on bioreceptivity. This review examines what is known about the efficacy of control methods based on traditional-commercial biocides, as well as those based on innovative application of substances of plant and microbial origin, and physical techniques. A framework for providing a balanced scientific assessment of the role of lithobionts on CHSS and integrating this knowledge into management and conservation decision-making is presented.

Keywords: biocide, biodiversity, biofilm, bioreceptivity, conservation of cultural heritage stone surfaces, stone cleaning, stone durability

The issue of lithobionts on the stone cultural heritage: an introduction

The preservation and transmission to future generations of stone cultural heritage (objects, buildings and sites carved in, or built from, stone), including rock art, archaeological and historical monuments and artistic stonework, are globally shared and signed duties, which need to be combined with the protection and conservation of natural heritage (Convention Concerning the Protection of the World Cultural and Natural Heritage 1972). As with other global issues in today's changing world, scientific advances can inform effective and environmentally conscious management, based on correct identification of threats, and development of sustainable solutions (Ferretti and Comino 2015; Charter and Tischner 2017). The growth of lithobionts (i.e. rock-dwelling organisms) on cultural heritage stone surfaces (CHSS; Fig. 1) has long been associated with biodeterioration, which can be defined as any undesirable change in the properties of a material caused by the vital activities of organisms (Hueck 1965), and recognized as a threat to conservation (Caneva et al. 2008). This is not surprising given widespread evidence of biogeophysical processes (such as mechanical fracturing and disruption of rocks and minerals by living structures) and biogeochemical processes (chemical destabilization and compositional change of rocks and minerals by metabolic processes and products), which are vital to pedogenesis (Silverman 1979; Totsche et al. 2010). Plenty of field and experimental data demonstrate rock weathering by macro- and micro-organisms (bioweathering), such as vascular plants (Pawlik et al. 2016) and bryophytes (Ricci and Altieri 2008), lichenized- (Seaward 2015) and non-lichenized fungi (Gadd 2017), photo- (Albertano 2012) and chemo- lithotrophic bacteria (Mapelli et al. 2012). Coupled with the desire to remove lithobionts from heritage stone surfaces for aesthetic reasons (because they can produce unsightly discoloration and obscure important carved details), such biodeteriorative roles strengthen the case for conservation interventions (Pinna 2017).

On the other hand, lithobiontic communities have also been seen as enhancing biodiversity, and in some cases bringing positive aesthetic characteristics to CHSS (see section *Factors affecting the opportunity to remove (or preserve) lithobionts*). Furthermore, the last two decades have seen an increasing interest in whether lithobiontic communities can in some circumstances also act as a bioprotective layer, covering stone surfaces and limiting weathering processes driven by abiotic factors, such as meteorological forces and air pollutants (Carter and Viles 2005; McIlroy de la Rosa et al. 2012). Such findings, mixed with advances in investigating biomediated approaches (microbial biocementation) which can promote the consolidation of stone (Fernandes 2006; Wang et al. 2016; Shraddha and Darshan 2019), have contributed to the recent proposal that bioprotection of buildings and cultural heritage by lithobionts may be a sustainable strategy (Gadd and Dyer 2017). It is important not to see contrasting research findings on biodeterioration and bioprotection as conflicting positions, as they likely represent different aspects of complex interactions within and between natural or cultural heritage ecosystems (see section *Biodeteriorative and/or bioprotective effects*). Misunderstandings on this point may indeed critically impact decisions on the management of lithobionts on CHSS, which should not be based on generalized views of biodeterioration vs bioprotection as ideologically opposed ‘schools of thought’, but on diagnostic analyses targeting each case. Advances in molecular, microscopy and spectroscopy methods since the 1990s have strongly improved the likelihood of characterizing the diversity of lithobionts on CHSS, and their related biogeophysical and biogeochemical processes (Piñar and Sterflinger 2018; Sanmartín et al. 2018; Schröder et al. 2019). In the following section, colonization patterns and physico-chemical processes driven by the major groups of lithobionts are exemplified, together with a critical evaluation of what diagnostic approach is required to reliably unveil their biodeterioration and/or bioprotection potential on CHSS. In the second part, approaches to manage lithobionts on CHSS are outlined, and a potential decision framework based on experimental assessment and monitoring of both the roles of lithobionts and the efficacy of control strategies is introduced.

Patterns and impact of lithobiontic colonization

Stone bioreceptivity and biogeophysical and biogeochemical impacts of lithobionts

Natural rock surfaces and CHSS provide interfaces between geological materials, air and water which are invariably colonised by lithobionts (Gorbushina and Broughton 2009). Intrinsic physico-chemical properties of stone materials (e.g. surface roughness, porosity, mineral, composition) are the primary factors determining their bioreceptivity, i.e. their aptitude to be colonized by living organisms (Guillitte 1995; Miller et al. 2010). The rate of supply of biological particles, usually resistant structures (spores, pollens) dispersed as bioaerosol, is the main complementary factor needed to start and support colonization dynamics (Mandrioli et al. 2003). Other extrinsic factors, such as climatic conditions and the availability of organic nutrients, influence the composition and structure of lithobiontic communities on CHSS, with their network of nutritional interactions (autotrophs/producers; heterotrophs/destroyers and consumers) (Caneva et al. 2008).

An index to quantify bioreceptivity has been proposed to evaluate the performance of any construction material and support management decisions in the ornamental stone industry which can be applied to CHSS (Vázquez-Nion et al. 2018). In the suggested protocol, the growth of a standard photoautotrophic community (bryophyta, green algae, cyanobacteria) on coupons of different lithologies is compared by fluorimetry (to quantify the amount of chlorophyll *a*) and spectrophotometry (to quantify colour change. Such an approach reflects the fact that aesthetic appearance (colour, visibility of artistic details) is important for perceptions of the value of CHSS (Brimblecombe and Grossi 2005). On the other hand, such an index does not take account of the three-dimensional phenomenon of lithobiontic colonization of CHSS, in which the hidden dimension, below the surface, represents the major interface between biotic and mineral components.

Across a range of spatial scales, higher plants, bryophytes, lichens and microorganisms anchor or adhere to mineral substrates, including CHSS, in order to provide stability, exploit water and/or nutrients, and as a consequence modify the physico-chemical properties of the substrates. The root systems of woody plants growing directly on pavements or masonries, and, in the case of tree, in proximity to building foundations and hypogean structures, can exert mechanical forces and cause superficial and structural damage, especially in archaeological sites (Caneva et al. 2009; Bartoli et al. 2017). The penetration of slender rhizoids and protonemata of mosses (even though not enforced by lignin) have been related to mechanical damage of mosaics and wall paintings (Saiz-Jimenez et al. 1991; Ricci and Altieri 2008). In the case of lichens (Fig. 2 a-d), the penetration of mycobiont hyphae beneath epilithic crustose thalli, and of the anchoring points of rhizinae and haptera of foliose and fruticose lichens, respectively, has been microscopically characterized within many different stone materials (de los Ríos and Ascaso 2005; Salvadori and Casanova-Municchia 2016). Different patterns of penetration are found depending on the mineralogical and microstructural features of different lithologies, and the species involved, with hyphal organization, spread, and depths ranging from a few microns to several millimeters (Favero-Longo et al. 2005; Scarciglia et al. 2012; Sohrabi et al. 2017). Moreover, some lichen species display an endolithic habit, with the thallus (including the photobiont layer) growing entirely within the rock substrate, exploiting internal cracks (chasmo-endolithic) or intrinsic porosities (crypto-endolithic), or actively dissolving minerals (eu-endolithic) (Pinna et al. 1998; Casanova-Municchia et al. 2014; Favero-Longo et al. 2015).

Similar ranges of growth, from fully epilithic to fully endolithic, also characterize autotrophic and heterotrophic microorganisms usually organized as biofilms, which are adapted to every kind and level of environmental stress in terms of temperature, water and nutrient availability, and solar irradiation (Gorbushina and Broughton 2009). Archaea, bacteria and eukaryotic microbes, as non-lichenized fungi, widely live embedded in an extracellular matrix of biopolymers, usually known as as EPS (Extracellular Polymeric Substances), which is dominated by polysaccharides, but also contains (glyco-)proteins, glycolipids and DNA (Flemming et al. 2007, 2017). High hydration of EPS makes biofilms functionally active and resistant environments, favouring the nutrition, communication and defence of microbes, and also drives their physico-chemical interactions with the substrate. Cyclic hydration and dehydration of biofilms cause volume changes and, as a consequence, pressures at their points of adhesion, causing disaggregation and detachments (Negi and Sarethy 2019). A similar mechanical action is exerted by mosses and lichens, whose water status varies passively with environmental conditions (poikilohydric organisms; Seaward 2015).

The covering of rock surfaces and CHSS by coloured dark patinas and thalli also influences the thermal behaviour of the surface and consequent physical stress (Garty 1990; Carter and Viles 2004). In parallel, water availability at the lithobiont-rock interface favours the mobilization of metabolites and mineral ions, supporting chemical modification and sometimes dissolution of the original mineral constituents and the precipitation of new (bio-)minerals (Banfield et al. 1999). Accordingly, protons excreted by plant root tips induce cationic exchange and contribute to ion mobilization from the contacted minerals, and their chemical modification (Caneva et al. 2008). Bryophytes, like algae and cyanobacteria, can favour the precipitation of carbonates, as a consequence of photosynthetic removal of CO_2 and their ability to solubilize and bind Ca, a phenomenon reported for natural springs, but also for fountains and monuments in humid regions (Bolívar and Sánchez-Castillo 1997; Ortega-Morales et al. 2000; Crispim and Gaylarde 2005; Ricci and Altieri 2008). Several lichenized and non-lichenized fungi secrete a large variety of primary and secondary metabolites with acidic and chelating functions, supporting acidolysis and complexolysis (Piervittori et al. 2009; Gadd 2017). The biomineralization at the lichen-substrate interface of different oxalates (e.g. Ca-, Mg-, Fe-, Mn-oxalate), depending on the rock metal contents, has frequently been cited as evidence of the direct influence of the mycobiont secretion of oxalic acid on colonized surfaces (Adamo and Violante 2000; Chen et al. 2000; Gadd et al. 2014). In particular, calcium oxalates (whewellite and weddellite) have been reported on a wide range of lithologies, even those poor in Ca, because of their very low solubility in comparison with other oxalate species (Fig. 2 e-f; Favero-Longo et al. 2005), and they are often found on CHSS colonized by certain lichens (Edwards et al. 2003; Pena-Pozo et al. 2018; Tonon et al. 2019). The insolubility of Ca-oxalates also accounts for their long persistence on CHSS, where their biological origin has been suggested as an alternative to a chemical origin (e.g. due to degradation of restoration products) even in the absence of viable lithobiontic communities (Caneva et al. 1993). Mineral leaching by other fungal primary metabolites (e.g. citric and malic acids) has also been documented (Wei et al. 2012; Sazanova et al. 2016), but they have generally received less attention in the field of CHSS in comparison to oxalates because of the absence of clear or persistent traces. Mineral leaching activity by complexation has also been reported for some depsides, depsidones and pulvinic acid derivatives (secondary metabolites exclusive to lichens) although their low solubility may limit their impact (Ascaso et al. 1976; Haas and Purvis 2006; Favero-Longo et al. 2013). All these metabolites, as well as the involvement of EPS, seem to contribute to the weathering of silicate minerals into clays, as TEM investigations have showed for the lichen-driven vermiculization of biotite and other cases (Cuadros 2017), and in several cases to the dissolution of carbonates (Pinheiro et al. 2019).

Different processes need to be invoked to explain the euendolithic behaviour of certain lichen species, as they do not secrete either oxalic acid or the above mentioned lichen secondary metabolites (Pinna et al. 1998). In some cases respiration-induced acidification of the substrate has been shown to be sufficient to provoke pitting activity (formation of sub-millimetric cavities on the surface) (Weber et al. 2011), but the involvement of siderophore-like compounds has also been hypothesized, as these complexing compounds, involved in iron nutrition, also scavenge calcium if iron availability is low (Favero-Longo et al. 2011). Similarly to endolithic lichens, microcolonial fungi (MCF), which are a group of fungi tolerant of the extreme stress of exposed substrates and thus colonizing bare rock surfaces, including CHSS (Sterflinger 2010), are responsible for dissolution and pitting phenomena on carbonate and silicate rocks, but the processes responsible still need to be clarified (De Leo et al. 2019). The rigidity of their cell wall due to melanin deposition has been related to their penetration ability (Sterflinger and Kumbein 1997), but ion mobilization by chelating molecules (Favero-Longo et al. 2011) and/or corrosive EPS containing pullulan and galactofuromannan as the main constituents (Breitenbach et al. 2018) may be involved.

The strong negative charge of cyanobacterial EPS, due to uronic acids and sulphate groups, also contributes to the dissolution of cations from colonized substrata and their microbial adsorption (Bellezza et al. 2006; Albertano 2012). Sulfuric, nitrous and nitric acids are released by chemolithotrophic (sulphur-oxidizing, nitrifying) bacteria and (ammonia-oxidizing) archaea, through enzyme catalyzed oxidation of inorganic compounds, such as ammonia, elemental sulphur and hydrogen sulphide, and are likely to have impacts on CHSS (Warscheid and Braams 2000; Zhang et al. 2019).

Biodeteriorative and/or bioprotective effects

All the above-mentioned (and many other) patterns of lithobiont-substrate interactions, in terms of biogeophysical and biogeochemical processes, have been increasingly detailed in the last two-three decades and supported by a huge number of study cases, including CHSS. This has certainly helped clarify the worldwide distribution and impacts of certain phenomena, but, in some cases, experimental evidence obtained by advanced, 21st century technologies simply confirms early insights dating back to the first part of the 20th century and before. For example, early descriptions and accurate microscopic observations of lichen growth within rocks (Gümbel, 1856; Fry 1924 1927), as well as the recognition of lichen-driven chemical processes due to oxalic acid secretion and/or a respiration-induced acidification (Slater, 1856; Uloth 1861; Sollas, 1880; Smith 1921), provided convincing evidence of lichen biogeophysical and biogeochemical processes and are still widely quoted today. However, the advent in recent years of a wide range of portable, non-destructive methods now permits sophisticated measurements (such as spectroscopic analysis) which can characterize the presence of metabolites and biominerals on/within CHSS in situ (Maguregui et al. 2012; Costantini et al. 2018). Advances in molecular biology, and in particular the diagnostic power of next-generation sequencing, have highlighted the huge variety of microorganisms which can occur together on and beneath rock surfaces and CHSS, only partially detectable by microscopy and culturing approaches, and not always directly related to more visible surface colonizers (Bjelland et al. 2011; Piñar and Sterflinger 2018; Trovão et al. 2019).

On the other hand, whilst the observation and detection of (micro-)organisms, their structures and metabolites, or their molecular signs, certainly implies present or past interactions with rock substrates and CHSS, it does not necessarily mean that they play a biodeteriorative role as is often routinely thought. In this context, DNA-metabarcoding analyses particularly need careful evaluation to decipher which microorganisms are growing on and actively interacting with the substrates, and which simply occur as past traces, dormant structures or episodic (dust) contaminants (Marvasi et al. 2019). Implementation of molecular analysis pipelines, including the storage and effective access of datasets dedicated to the biodeterioration research area, also represent an ongoing challenge (Sterflinger et al. 2018).

Whilst there is incontrovertible evidence of mineral disintegration, chemical modification and leaching by lithobionts, other studies have highlighted bioprotective activities, which were also hypothesized many years ago (Krumbein 1968). Such hypotheses were based on early macroscopic observations of differential erosion rates with and without lithobionts, suggesting an umbrella-like protective effect (Mottershead and Lucas 1990; Özvan et al. 2015). Such interpretations of lithobionts as forming a physical barrier against other weathering factors have been supported by the quantification of lower solutional weathering from lithobiont (lichen)-covered limestone slates in comparison with uncolonized controls after 1 year of exposure in the humid climate of Ireland (McIlroy de la Rosa 2014). Ivy (*Hedera helix)* has been found to also have an umbrella-like impact and protect historic limestone walls from pollutants (which can lead to soiling and deterioration) (Sternberg et al. 2011). Additionally, experimental studies illustrate that *Hedera helix* can also protect vulnerable historic stone walls from freeze-thaw damage through modifying microclimatic conditions (Coombes et al. 2018). In drier climates, rock surface microorganisms have also been demonstrated to be chemically involved in the development of rock coatings, which stabilize the rock surfaces and contribute to their long term preservation (Taylor-George et al. 1983; Dorn 2013).

Nanometer-scale transport of Fe and Mn accumulated in bacterial sheaths and fixed on clay minerals contributes to the polygenetic formation of rock varnish (Dorn 2013). In arid Jordan, biofilms of cyanobacteria and fungi have been shown to contribute to case-hardening of sandstone by aiding cementation (Viles and Goudie 2004), and in cold arid central Antarctica, endolithic lichens and their EPS have been found to biomineralize iron oxides (Guglielmin et al. 2011). Such effects are also found in wetter climates, for example in the UK where the deposition by epilithic lichens of silica-rich layers within cracks and along mineral boundaries has been observed on granite outcrops alongside biophysical and biochemical weathering impacts (Lee and Parsons 1999). Biomineralization of oxalates has also been considered to produce a potential protective shield against abiotic weathering agents, such as wind and runoff (Souza-Egipsy et al. 2004), and Ca-oxalates have often been proposed as producing potentially protective coatings for CHSS (e.g. Rampazzi 2019).

Multiple biodeteriorative and bioprotective roles of lithobionts have often been proposed. A notable example is the analysis of roles of the lichen *Verrucaria rubrocincta* on caliche plates in the Sonoran desert (Bungartz et al. 2004; Garvie et al. 2008): simultaneous biodeterioration of the rock substrate and a counterbalancing biomineralization of a upper protecting layer of fine-grained micrite have been recognized and characterized by isotopic analyses. Similarly, calcite dissolution and biomineralization of neoformed calcite around hyphae have been described for non-lichenized fungi (Fomina et al. 2010). Such recognition of both deteriorative and protective effects of lithobionts, and a deep evaluation of the prevailing process, may become crucial in future studies of lithobionts on CHSS (Bartoli et al. 2014). Observations of lithobiont (lichen) communities on tuff churches in Cappadocia showed the deteriogenic activity of penetrating hyphae, whilst some bioprotective effects were simultaneously envisaged, leaving uncertainty about whether or not the growths should be removed (Casanova-Municchia et al. 2018). Such a case study exemplifies the need for case-specific information on the multiple interactions of lithobionts and CHSS, their spatial patterning and their persistence over time, but also on their actual influence on stone durability, before appropriate management decisions can be made. Indices to evaluate and quantify the potential biodeteriogenic impact of plants (Signorini 1996), lichens (Gazzano et al. 2009) and microorganisms (Gazzano et al. 2011) have been proposed, but are more oriented to a comparative evaluation of the degree of physico-chemical interaction of different species with the substrate, rather than to quantitatively evaluate their impact on the CHSS (Pinna 2014). As a framework proposal, Table 1 summarizes a range of biodeteriorative and bioprotective roles reported for various types of lithobiontic organisms. It is worth remarking that each (potential) role has to be considered species-specific and cannot be generalized for all the members of each group; moreover it also depends on the lithology and the environmental conditions (see the next sub-section).

Lithobionts and stone durability

Biodeteriorative and bioprotective processes may or may not balance out, and thus produce complex impacts on the intrinsic stone properties relevant for durability, which can be defined as the capacity of a building material to maintain its size, shape, strength and aesthetic appearance over time (Bell 1993). In particular, knowledge of lithobiont-rock interactions should be combined with quantitative investigation of their impacts on petrophysical properties, including porosity, presence of swelling clay minerals, compactness, water absorption, presence and distribution of anisotropies, compressive strength, and surface hardness (Molina et al. 2013; Wilhelm et al. 2016). Relatively few studies have yet been made of the impacts of lithobionts on durability and resilience of CHSS, although some research has focused on (bio-)geomorphological studies of natural rock outcrops (Viles 2019). The results seem to depend strongly on lithology. For example, lichen colonization has been found to correlate with surface hardening and a reduction of water absorption on tuff (Garcia-Vallès et al. 2003), with a reduction of surface hardness on gneiss (McCarroll and Viles 1995; Favero-Longo et al. 2015), and with variable patterns of surface hardening or softening, and increased water absorption after the scraping of thalli from the surface on limestone (Morando et al. 2017). Such variability suggests the need for tailored investigations to cover a range of lithologies, different lichen species, and other, even more overlooked, lithobionts. Experimental datasets are needed to test conceptual models on the balance between biodeterioration and bioprotective processes and the net impact of lithobionts (lichens, in particular) on stone surfaces (McIlroy de la Rosa et al. 2012). In this regard, one particularly important future research direction is elucidating the impact of lithobionts on their substrate beyond their life-span. Surfaces once covered by lithobionts which have been removed by conservation interventions, or have simply died, may indeed display a long term protection effect due to past biogeochemical processes (e.g. protection by a biomineral deposit, cementation, pore-filling by biogenic silica or varnish). Conversely, such surfaces might also be more prone to disintegration following the loss of biological structures which bound the disentangled mineral fragments. The latter scenario appears particularly critical where stone surface details are a crucial component of the heritage values, as in the case of carved rock-art (Tratebas 2004; Favero-Longo et al. 2019).

Developments in laboratory experiments using cultured model lithobiontic organisms, genetically modified lineages, and the combination of closed and open experimental systems provide useful new approaches to disentangling the impacts of lithobionts on CHSS. For example, several studies have cultivated lithobionts on stone coupons and evaluated their impact on stone properties relevant for durability (Favero-Longo et al. 2009; Villa et al. 2015; Pokharel et al. 2017; Seiffert et al. 2016). In this context, it is very important to standardize protocols used in the characterization of biodeteriorative (and bioprotective) processes, using approaches such as microscopic, spectroscopic, culture-based, and ‘omics’methods, in order to make scientific data truly comparable and offer more reliable support for management decisions (Pinna 2014; Sterflinger et al. 2018). Research on lithobiontic impacts on CHSS should also consider predicted impacts of climate change on lithobiont colonization and growth (Davidson et al. 2018). Changes in temperature and precipitation regimes, beyond their direct impact on CHSS, have been hypothesized to affect lithobiont communities, causing potential shifts from biodeterioration to bioprotective impacts, and vice versa (Viles and Cutler 2012; Fatorić & Seekamp 2017). As an example, Gómez-Bolea et al.

(2012) predicted that lithobiontic biomass on CHSS should increase in northern Europe whilst decreasing in southern Europe because of contrasting trends in precipitation. Recent laboratory experiments simulating changing water regimes and increased CO_2 concentrations have confirmed the suggested impacts on biofilm composition and a shift towards biodeterioration effects (Prieto et al. 2020). From a different point of view, subaerial biofilms have also been noted as potentially important climate regulators, as agents of carbon sequestration, biogeochemical cycles and elemental transformations (Villa et al. 2016).

Approaches to managing lithobionts on CHSS

Factors affecting the opportunity to remove (or preserve) lithobionts

Aspects of culture and tradition have shaped and continue to shape perceptions of the growth of lithobionts on CHSS. Ruins covered by plants and other lithobionts, recognized as evidence of natural decay, are a symbol of the European Romantic taste of the 19th century (Stanford 2000; Huyssen 2006). Similarly, an abundance of lithobionts on some types of heritage sites, such as a Celtic graveyard or a Shinto shrine, can be seen in a positive light today, especially in places where local tradition tends to appreciate, rather than worry about, the merging of CHSS and nature, sharing a common acceptance of human impermanence (Fitzsimons et al. 2012). In most cases, however, alterations in CHSS caused by both biotic and abiotic drivers are considered to be negative, reducing heritage values (Brimblecombe and Grossi 2005; Prieto et al. 2007). As a consequence, removal of lithobionts is usually recognized as a necessary part of conservation interventions (Pinna 2017). However, scientific evidence justifying the removal of lithobionts is often missing.

In order to make more informed decisions about whether to remove (or preserve, or even encourage further) lithobionts, aesthetic evaluations should be combined with data on the impacts of lithobionts on stone durability, based on an analysis of the balance between biodeteriorative and bioprotective processes. Such an approach is not in conflict with the priority often given (at least in some countries) to aesthetic evaluations as the basis of management strategies for heritage sites, but enables a broader, more balanced assessments of risks vs benefits. By way of an example, in cases where lithobionts are shown to be predominantly bioprotective, their removal might cause a decrease in durability of CHSS: if the aesthetic damage they cause is more severe, however, and they are removed, strategies to substitute their bioprotective function should be considered. In cases where the aesthetic damage caused by lithobionts is minor, and there is evidence of a bioprotective effect, or of only minor biodeterioration impact, a recommendation could be made to preserve the lithobionts and promote their biodiversity as an additional cultural value of the heritage site (Nimis et al. 1992; Steinbauer et al. 2013). Such an approach may be particularly valuable in the case of CHSS immersed in the natural environment, where the removal of a mature-climactic lithobiontic community, similar to that found on surrounding natural outcrops, is often followed by a rapid recolonization by a simplified community of ‘banal’ species (Nascimbene et al. 2009). The inclusion of biodiversity in the concept of cultural value of a heritage site agrees with official measures addressing parallel conservation of cultural and natural heritage (e.g. Italian Ministry for Cultural Heritage and Activities 2004: the Italian code of cultural heritage and landscape; UNESCO 2014: Florence Declaration). This may be particularly important for cemeteries, monumental or archaeological sites which are hotspots of lithology or microhabitat diversity and consequently host strongly heterogeneous lithobiontic and plant communities, making them easily accessible to the public (e.g. Nimis et al. 1987; Cicinelli et al. 2018; Löki et al. 2019). In other cases, cultural heritage sites have been clearly identified as preferential sites for the preservation of threatened species or even their re-introduction (Valkó et al. 2018; BLS 2020).

CHSS management should consider all facets of lithobiontic growths before automatically removing lithobionts to conserve and present heritage sites. In all cases, once the decision has been taken to remove lithobionts, the adoption of an effective strategy to clean the CHSS is an absolute priority (Pinna 2017). A major issue for CHSS management is indeed the frequent need to repeat treatments to control lithobionts, which in most cases are stress-tolerant (micro-)organisms well-equipped to thwart human attempts to remove them and prevent their regrowth.

Direct and indirect strategies to control lithobionts

Two main strategies to control lithobionts on CHSS can be distinguished, although their choice should not be considered exclusive and they may be used in combination: first, an indirect approach focused on the control of microenvironmental parameters which favour/allow their growth, and second, direct intervention to remove them from CHSS by physical and/or mechanical and/or chemical methods (Pinna 2017). The first approach is based on the fact that the presence of lithobiontic communities, and, in particular, of certain biodeteriogenic species, depends on the availability of suitable microenvironmental conditions, allowing their establishment, expansion, and reproduction. At any one heritage site, different CHSS often represent diverse ecological micro-niches, hosting different lithobiontic communities which interact with the substrate and influence its conservation in different ways (Marques et al. 2016; Tonon et al. 2019). Knowledge and monitoring of (micro-)environmental parameters favouring or discouraging lithobiontic communities, and in particular, deteriogenic species, can help recognise and achieve the best conservation solutions (Caneva et al. 2016; Schumacher and Gorbushina 2020). For example, the development of phototrophic biofilms on hypogean CHSS, and even on outdoor surfaces lit by artificial lighting, may be controlled through careful illumination strategies, based on modifying the spectral emission of lamps to affect microbial biomass, colour and EPS production (Albertano and Bruno 2003; Sanmartín et al. 2017). Careful management of higher plants in heritage sites may limit their shading of CHSS, with the consequent increase in humidity, and their supply of nutrients, both of which favour lithobiontic growth (Salvadori and Charola 2011). Such ecologically-based approaches may also be used to choose the most appropriate contemporary building materials (Caneva et al. 2008).

Direct intervention to clean CHSS should satisfy the main aims of removing the lithobiontic communities and preventing (re-)colonization processes by limiting surface bioreceptivity. These objectives have been variously pursued by combining several mechanical, physical and chemical approaches, already reviewed in detail elsewhere (Pinna 2017). The crucial point is that lithobiontic colonization usually extends well beneath the rock surface and microbial structures generally show a strong capacity to recover from disturbance, and so many cleaning interventions without subsequent biocide treatment are usually followed by rapid recolonization (Sohrabi et al. 2016). Removal by (pressurized) water and brushing may be effective in the case of superficial, subaerial biofilms (Sanmartín et al. 2020), but in other situations they may spread microbial structures, pushing them deeper within the substrate (Pinna 2017). Valuable protocols for the physical removal of lithobionts by laser ablation have been drawn up in the last decade (Sanz et al. 2017), but even the combination of scalpel and laser may sometimes fail to eliminate lithobiontic structures within fissures (Rivas et al. 2018). Similarly, some laser ablation can cause damage to the mineral surface whilst not completely removing the lichens (Pozo-Antonio et al. 2019). As a result of these issues, biocide treatments often precede mechanical removal (Kakakhel et al. 2019). Although many of the more toxic biocides have been banned, safer compounds may have some drawbacks. For example, the effectiveness of quaternary ammonium salts (e.g benzalkonium chloride) to disrupt cell membranes and kill lithobionts has been well demonstrated (Wessels and Ingmer 2013; Vannini et al. 2018; Sanmartín et al. 2020), but their use can promote bacterial adaptation and antibiotic resistance (Kampf 2018; Kim et al. 2018; Poursat et al. 2019). Moreover, their degradation is suspected to contribute to nitrogen supply (Scheerer et al. 2009), thus favouring, rather than preventing, recolonization by aggressive nitrophytic species. Such alarming findings call for caution in biocide application (Stupar et al. 2014) and the need for non-chemical alternatives. As mentioned above, electromagnetic methods, which include laser irradiation as well as UV, microwave and gamma rays (Riminesi and Olmi 2016), are useful but still have limitations for large-scale applications to outdoor CHSS. Alternatively, heat shock treatments have been shown to provide a sustainable approach to killing lithobionts such as lichens and mosses (Tretiach et al. 2012; Bertuzzi et al. 2013). These poikilohydric organisms tolerate thermal stress well whilst dehydrated, but do not survive heating to 40-60°C for 6 hours when hydrated. Unfortunately, such an approach was less effective against microalgae (Bertuzzi et al. 2017) and the original proposal to exploit solar irradiation to provide the heating may be only suitable under stable weather conditions in warm countries, which limits its applicability. Nevertheless, the combination and improvement of all the cited and other approaches may be expected to shortly produce valuable strategies to moderate the use of biocides.

Whatever the chosen approach to kill lithobionts, it is necessary to monitor its efficacy on the target organisms and the examined CHSS: widely used biocides applied to lichens showed species- and site-specific effectiveness, likely depending on differences in the resistance of symbionts and the influence of different stone surfaces and climate/meteorological conditions (Favero-Longo et al. 2017). Biocides may have different effectiveness against epilithic and endolithic lithobionts (de los Ríos et al. 2012). Moreover, the method chosen to apply biocides, often defined for economic reasons, may strongly influence the final treatment success (Favero-Longo et al. 2017; Matteucci et al. 2019). A preliminary assessment of the viability of targeted microorganisms before and after treatment should be routinely performed by in situ methods, such as fluorimetry (Tretiach et al. 2010).

Although the effective devitalization of lithobionts contributes to keeping CHSS in a “clean” state for longer, (re-)colonization is inevitable (Fig. 3). However, longer term cleaning can be achieved by avoiding the application of compounds after cleaning which may increase bioreceptivity and promote recolonization, as demonstrated in the case of certain consolidants and other restoration products (Barriuso et al. 2017; Favero-Longo et al. 2018). In contrast, some other lithobiont control strategies may contribute to delay recolonization, including the application of photocatalytic nanoparticles (Fonseca et al. 2010; Sierra-Fernandez et al. 2017), and eco-friendly products, as essential oils and other biological substances exerting allelopathic effects (Fidanza and Caneva

2019). Monitoring programs are increasingly needed to monitor the long-term effectiveness of such approaches, in comparison with the traditional application of biocides following mechanical removal (Bruno et al. 2019; Favero-Longo et al. 2019; Sanmartín et al. 2020). Under certain climate conditions, however, even the most effective strategies may not fully prevent recolonization (Pinna et al. 2018), confirming the necessity of an integrated, site-specific approach (including microclimate control, where possible) to improve the success of management strategies for the conservation of CHSS.

Concluding remarks

Advances in technology and medical research have provided great tools to diagnose, control and prevent human diseases, and there is a clear integration between scientific research and managing health. This review has outlined that similarly integrated scientific approaches are available to diagnose the role of lithobionts on CHSS and inform management strategies. Lithobiont-CHSS systems display remarkable complexity, encompassing equilibria between lithosphere (i.e. the stone substrate) and biosphere (i.e. the lithobionts), as well as atmosphere/hydrosphere and anthroposphere, which are additional components affecting colonization and weathering dynamics. As in the case of human health, management decisions about the conservation of stone cultural heritage should increasingly take into consideration all the levels of knowledge available (or implementable) to decipher this complexity, and should be based on accurate diagnosis of each situation. Figure 4 presents a framework to include the above-reviewed spheres of investigation required for decision-making on lithobionts and CHSS, with a focus on whether to remove or preserve lithobionts and, if removal is chosen how to choose a suitable control strategy. As Figure 4 demonstrates, there is no ‘one size fits all’ approach because durability of different CHSS depends on the balance between biodeterioration and bioprotection effects driven by lithobiontic communities, which in turn depends on the species and lithology involved and the (micro)-environmental context. Moreover, these same factors (species-lithology-environment) also determine the effectiveness of control strategies. Thus, whatever the protocol used (innovative or based on traditional techniques) an experimental assessment of its suitability for the CHSS of interest is vital. Funding limitations may discourage scientific investigations on the role of lithobionts and the efficacy of control strategies, but such investigations are vital for cost-effective and environmentally-sustainable management of CHSS in future.

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Table

Table 1 - Biodeteriorative and bioprotective roles often reported (●) or hypothesized/debated (?) for various types of lithobiontic organisms. It is worth noting that each potential role has to be considered species-specific and cannot be generalized for all the members of each group.

				Higher plants	Bryophytes	Lichens	Microbial biofilms		
							Algae	Cyanobacteria	Microcolonial fungi
Biodeteriorative roles	During life	Biogeophysical	“Rooting”	●	●	●			●
			Wetting/ drying			●	●	●	
			Enhanced thermal stresses		?	●	●	●	●
		Biogeochemical	Biomineralization		●	●		●	
			Acid/ complex dissolution	●	●	●	●	●	?
		Aesthetic loss	Surface coverage/ soiling	●	●	●	●	●	●
	After death	Remnant biocrusts	Disfiguring patina	?	●	●	●	●	●
Bioprotective roles	During life	Shielding	Umbrella effect	●	●	●	?	?	
			Reduced thermal stress	●	●	●	●	?	
			From pollutants	●	?	?	?	?	
		Biogeochemical	Rock varnish & case hardening			●		●	●
			Biomineralization		●	●		●	
		Aesthetic/ biodiversity enhancement	‘Greening’ walls	●	●	●	●	●	
	After death	Remnant biocrusts	Protective patina	?	●	●	●	●	●

Figure captions

Fig. 1. Lithobionts on stone cultural heritage. **a** Residence of the Royal House of Savoy in Govone (Italy; year 2010); **b** Fushimi Inari-Taisha-Shrine in Kyoto (Japan; 2019); **c** Sanctuaire de Notre-Dame de Laghet in La Trinité (France; 2019); **d** Roman underground cistern down to Lithostrotos in Jerusalem Old Town (2017); **e** 16th century Kelmscott Manor near Oxford (UK; 2018); **f** Engravings of the 'Rock of the Map' in Valle Camonica (Italy; 2016); **g** St Andrew's Churchyard in the Isle of Portland (UK; 2018); **h** North Grotto Temple in the Gansu province (China; 2018); **i** The Mostaccini Fountain in the Boboli Gardens of Florence (Italy; 2016).

Fig. 2. Lithobiont-related processes and spatial patterning on CHSS: the example of lichen-forming fungi. **a** rock flaking associated with lichen colonization of a sandstone (Pietra Serena) sculpted surface. **b** Pietra Serena colonized by the lichen *Aspicilia cinerea* (L.) Körb., displaying the thalline component (#) at the sandstone surface, and the hyphal penetration component (*) developing along the grain borders (polished cross section stained by PAS and observed under reflected light microscopy; bar: 500 μm); **c** pitting at the surface of a limestone previously colonized by an endolithic lichen; **d** Aurisina limestone colonized by the endolithic lichen *Bagliettoa baldensis* (A. Massal.) Vězda, displaying both the photobiont (red) and the mycobiont (green) developing within the substrate, and a pit occupied by a fruiting body (*) (polished cross section stained with FITC-Concanavalin A and observed under confocal laser scanning microscopy; bar: 500 μm); **e** biomineralization of calcium oxalates at the interface between a lichen thallus and a serpentinite rock (*, cross sectioned hyphae submerged by the oxalates; scanning electron microscopy; bar: 5 μm); **f** serpentinite colonized by the lichen *Lecidea atrobrunnea* (DC.) Schaer., displaying oxalates (milk-like high birefringence colours) in the medulla layer and at the rock interface (thin cross section observed under cross polarized transmitted light; bar: 500 μm).

Fig. 3. Colonization dynamics on CHSS. **a** lichens on a granite gravestone in the Walser graveyard of the alpine village Gressoney-la-Trinité (Italy; year 2010), **b** the same surface after cleaning by professional restorers (year 2011); **c** lichen recolonization after eight years (year 2019).

Fig. 4. Framework summarizing the spheres of knowledge dealing with the lithobiontic colonization of CHSS (white boxes) and the potential control strategies (green), with the related diagnostic approaches (blue). Such knowledge may properly support management decisions (orange) to improve the conservation of stone cultural heritage.


a
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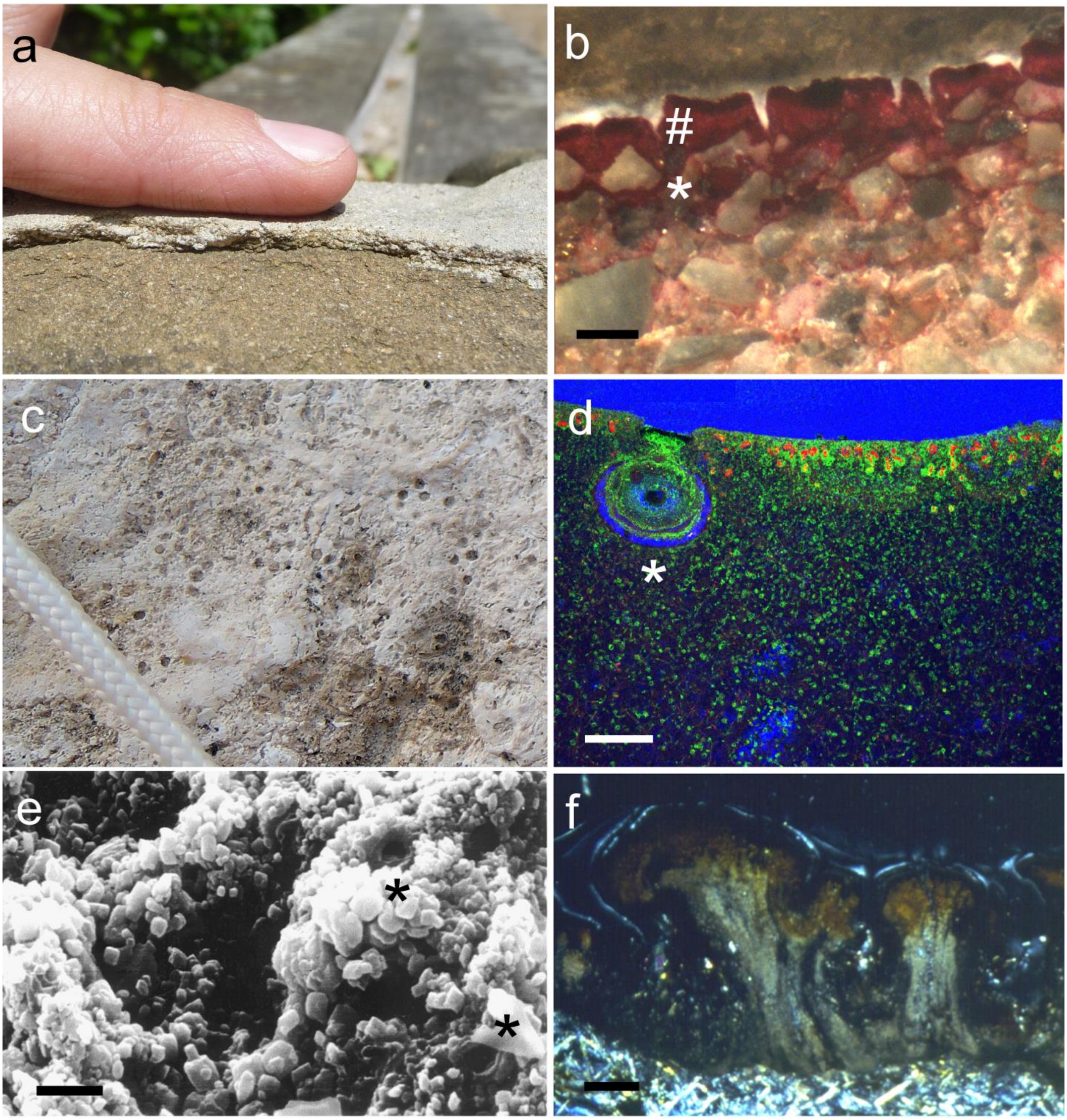
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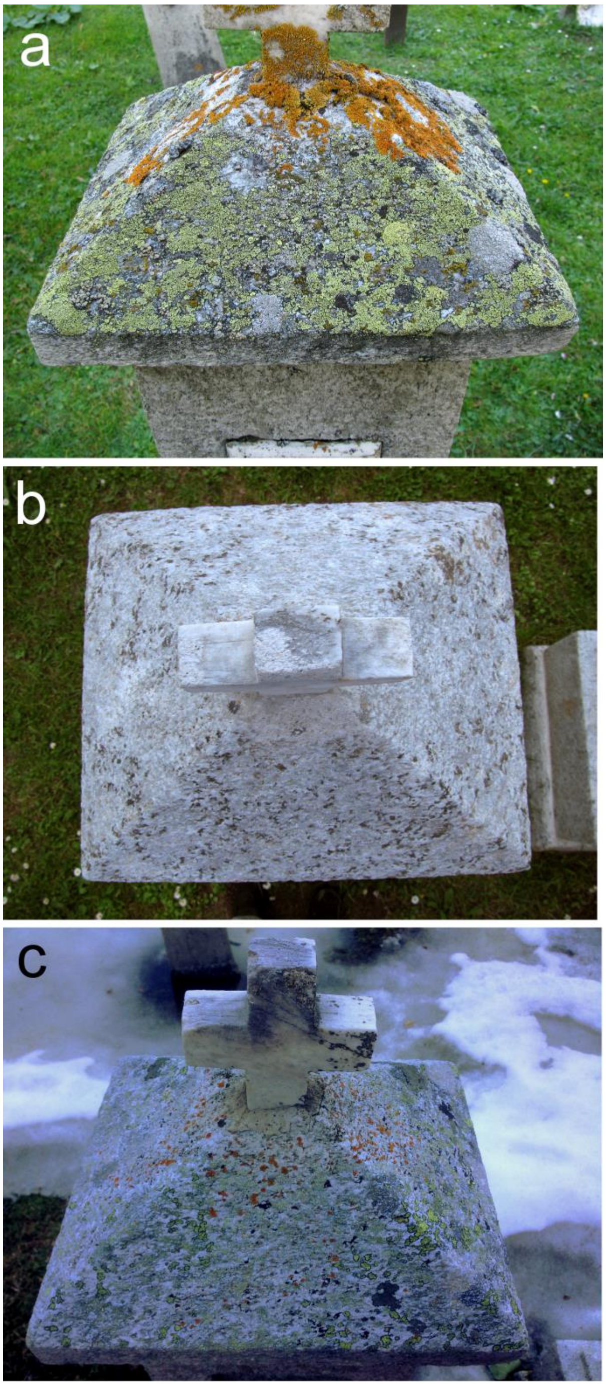
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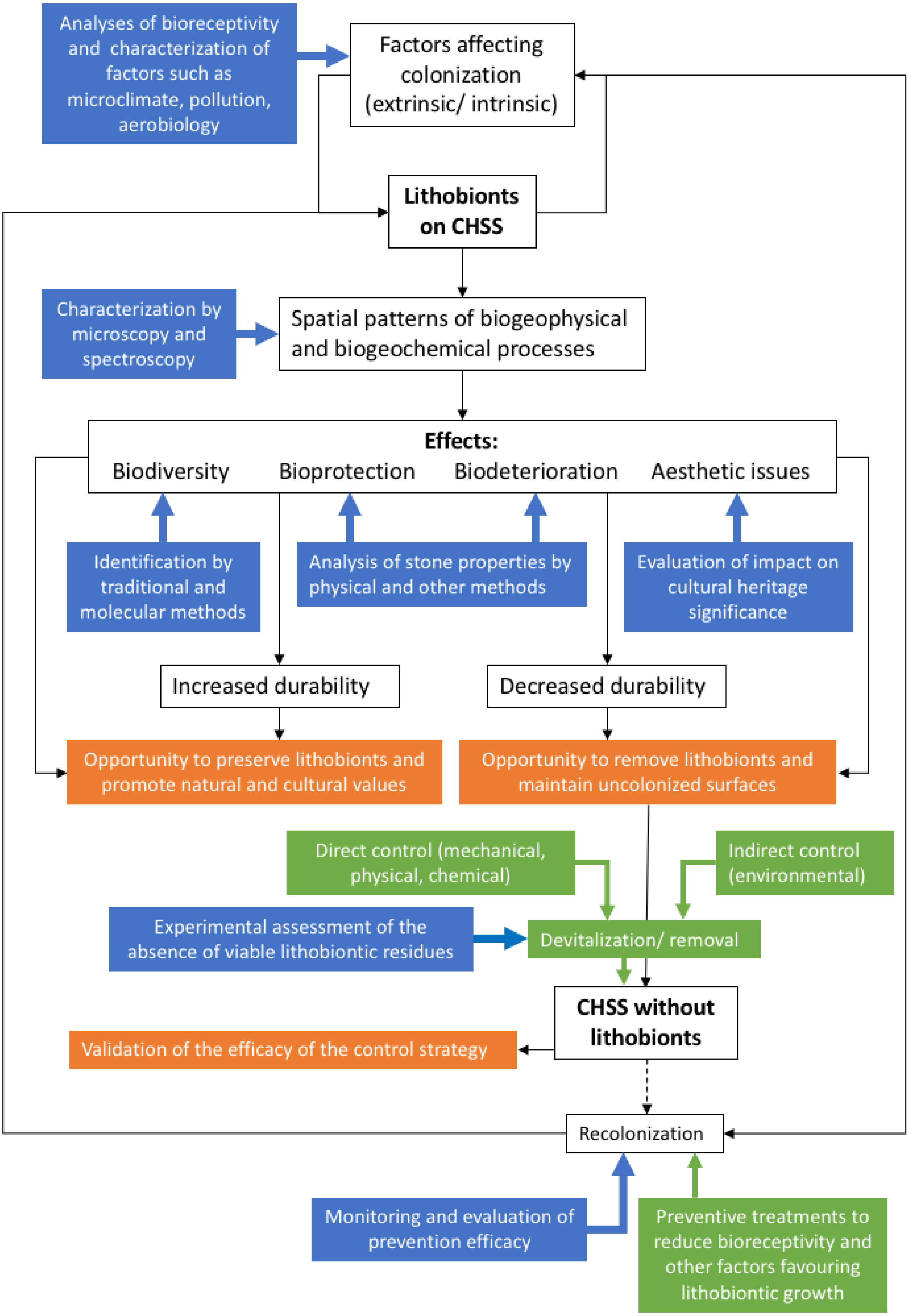
Analyses of bioreceptivity and characterization of factors such as microclimate, pollution, aerobiology
Factors affecting colonization (extrinsic/ intrinsic)
Lithobionts on CHSS
Characterization by microscopy and spectroscopy
Spatial patterns of biogeophysical and biogeochemical processes
Effects:
Biodiversity
Bioprotection
Biodeterioration
Aesthetic issues
Identification by traditional and molecular methods
Analysis of stone properties by physical and other methods
Evaluation of impact on cultural heritage significance
Increased durability
Decreased durability
Opportunity to preserve lithobionts and promote natural and cultural values
Opportunity to remove lithobionts and maintain uncolonized surfaces
Direct control (mechanical, physical, chemical)
Indirect control (environmental)
Experimental assessment of the absence of viable lithobiontic residues
Devitalization/ removal
CHSS without lithobionts
Validation of the efficacy of the control strategy
Recolonization
Monitoring and evaluation of prevention efficacy
Preventive treatments to reduce bioreceptivity and other factors favouring lithobiontic growth