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# Microbial Growth Prevented by Natural Biocides on Cultural Heritage

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**ABSTRACT:** Many historic, cultural and artistic objects and buildings are made of stone. Like all materials, stone is subject to inexorable deterioration. Along with chemical and physical weathering factors, microbial growth plays an important role in this process. Stone types and local climatic differences have a great impact on the bio-deterioration process and on their outcomes. Microbial metabolism products, as organic and inorganic acid, chelating agents, enzymes and extracellular polymeric substances (EPS), are responsible of bio-corrosion and of bio-mineralization; furthermore phototropic and heterotrophic microorganisms (e.g., Actinobacteria, Firmicutes and fungi) are able to penetrate into stone surface. In addition to structural injure, these microorganisms cause also aesthetic damage. Lithic artworks as churches, historical buildings and every usage object are our precious cultural heritage, memory of our past history step needed to build present and future. These artistic heritages with morphological, chemical and physical properties totally dissimilar make they a "unicum", characterized by a specific vulnerability. Their decay is unavoidable, but it is a challenge for the humankind to protect and preserve them. Thus far, solutions for the safeguard of cultural heritage are usually based on chemical procedures to remove biodeteriogen agents, but these substances can be hazardous to the environment, to public health and to stone materials itself because it is not known about the consequences of repeated applications. Microbial metabolism produces deteriorating agents such as organic and inorganic acid, chelating agents, enzymes and extracellular polymeric substances (EPS) causing e.g. bio-corrosion and bio-mineralization; furthermore phototropic and heterotropic microorganisms (e.g.Actinobacteria, Firmicutes and fungi) are able to penetrate into stone surfaces. In addition to structural damage, these microorganisms cause, also, aesthetic damage. Despite their toxicity, traditional biocides are still largely employed to contrast biodeterioration . However, biocidal treatments have a brief duration and must often be repeated frequently, creating a repeated threat to the heritage material and the environment . In addition, repeated biocidal treatments can cause resistance in target biological agents, and they can modify biofilm structures favoring the growth of more harmful biodeteriogens . Biocide application has indeed caused damage to non-target organisms.

**KEYWORDS :** biocides, microbes, algae, fungi, bacteria, acids, enzymes, biodeteriogen, organisms, heritage

## I. INTRODUCTION

Essential oils (EOs) have been known for a long time, and they are used in several fields such as medicine and aromatherapy, as well as in the food and pharmaceutical industries. In the last decade, EOs have also been applied to contrast the biodeterioration of cultural heritage, representing a powerful resource in green conservation strategies. In this study, an integrated approach based on microscopic observation, in vitro culture, and molecular investigation was preliminarily employed to identify biological systems colonizing wooden artworks. In order to contrast the biodeterioration processes[2] induced by fungal colonization (*Aspergillus flavus*) or insect infestation (*Anobium punctatum*), wooden artworks were exposed to the volatile compound of *Origanum vulgare* or *Thymus vulgaris* essential oils (EOs), the chemical composition of which was determined by GC–MS using both[1] polar and apolar columns. Artwork exposure was performed in ad-hoc-assembled “clean chambers.” Evaluating the effects on biological systems, the compatibility with artwork constitutive materials, and the lack of negative effects on human health and environmental pollution, the use of EOs as a valid alternative to traditional biocides must be considered. The identification of effectively biodeteriogenic agents and the design [3]of mitigation strategies directed to these agents without prejudice to historical materials, to the environment and to operators, taking into account the microbial community’s dynamics, is an important challenge to control biodeterioration of cultural heritage. Bacteria, in particular *Bacillus* spp. are worth for the creation of new green biocides solutions because they produce a great variety of secondary metabolites including ribosomally and non-ribosomally synthesized antimicrobial peptides, known to possess antagonistic activities against many biodeteriogenic fungi and bacteria. The discovery of new safe active compounds and green nanotechnology for direct application in cultural heritage safeguard can in a close future contribute to potentiate a new generation of biocides and safe sustainable methods for cultural heritage.[5]

Nanoparticles (NPs) of metal oxides, sometimes referred to as engineered nanoparticles have been used to protect building surfaces against biofilm formation for many years, but their history in the Cultural Heritage world is rather short. Their first reported use was in 2010. Thereafter, a wealth of reports can be found in the literature, with Ti, Ag and Zn oxides being the major protagonists. As with all surface treatments, NPs[4] can be leached into the surrounding environment, leading to potential ecotoxicity in soil and water and associated biota. Dissolution into metal ions is usually stated to be the main mode of toxic action and the toxic effects, when determined in the marine environment, decrease in the order $Au > Zn > Ag > Cu > Ti > C_{60}$, but direct action of NPs cannot be ruled out.[6] Although ecotoxicity has been assessed by a variety of techniques, it is important that a suitable standard test be developed and the European Unions's Biocidal Product Registration group is working on this, as well as a standard test for antimicrobial efficacy to determine their impact on ecological processes of surrounding non-target organisms and their transformation products under realistic scenarios.[7]

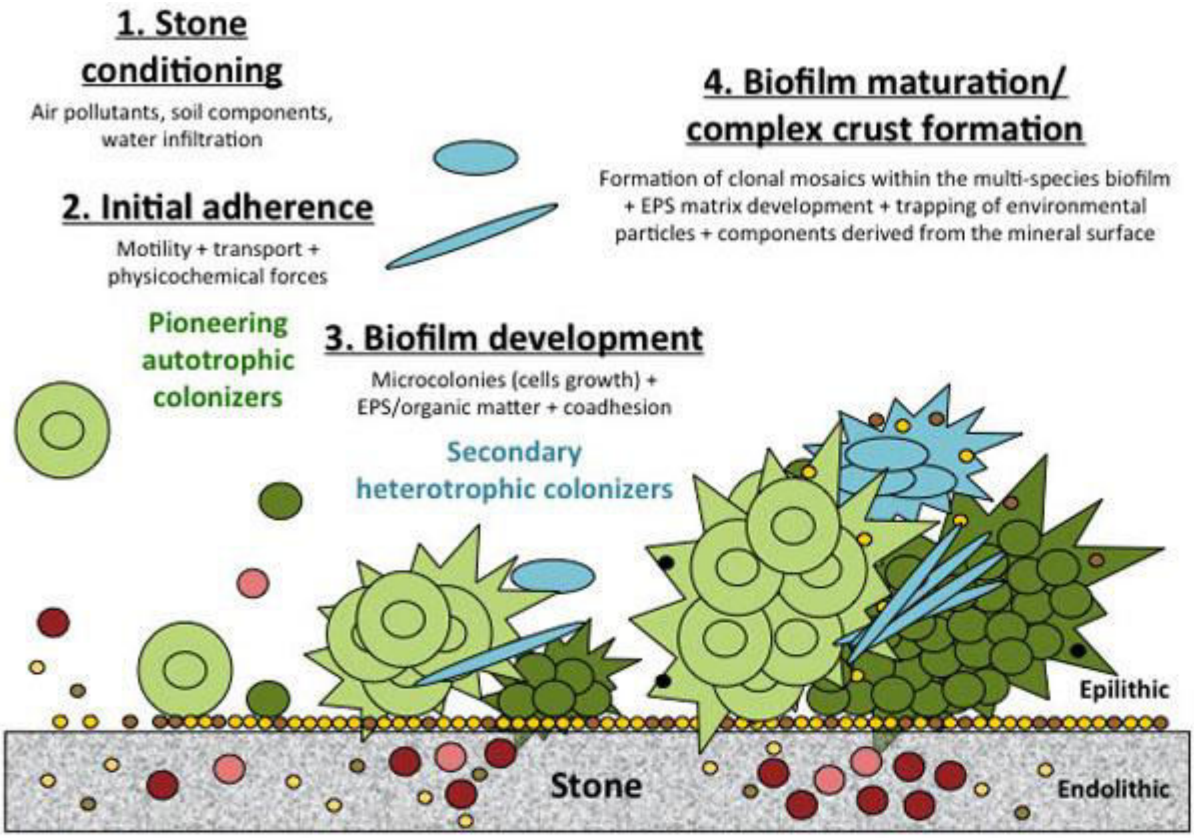


**Fig.1. Microbes causing destruction to heritage cultured monuments**

The growth control of microflora in cultural and built heritage is usually performed by treatments using chemicals that have high toxicity. Thus it is crucial to develop strategies to discover new bioactive molecules and establish effective approaches against the effectively biodeteriogenic agents, which are responsible for degradation of cultural and built heritage, without detriment to the environment. [8] *Bacillus* sp. produce a high number of secondary metabolites, some with antibiotic properties which can be used against biodeteriogenic filamentous fungi, source of serious damage in historic materials. These antagonistic proprieties are due to the production of bioactive lipopeptides which exhibit antifungal activity in the stationary phase of bacteria growth, being associated to secondary metabolism. A combined methodology using antifungal tests, chromatographic techniques, FTIR-ATR, microscopic approaches and simulations assays allowed the detection of antifungal potential and a rapid identification of these ground-breaking bioactive compounds without the need of previous total isolation. This novel green biocides show a great potential for future application in cultural and built heritage rehabilitation being an effective alternative to the chemical compounds usually applied.[9]

The use of the advanced biotechnology of microbiological systems for the biological cleaning of Cultural Heritage (CH) has been recently improved and optimized taking into account different factors.[10] Biocleaning systems have been indeed applied to historic buildings, statue,s and frescoes. Such application has developed new techniques and optimised and refined the existing systems. These systems remove altered forms like sulfate and nitrate crusts and organic substances like animal glue in a more effective, less invasive way than the traditional cleaning techniques.[11] This review focuses on several delivery systems (sepiolite, hydrobiogel-97, cotton wool, carbogel, mortar and alginate beads, agar, and arbocel) used for the biocleaning of Cultural Heritage, comparing their main properties and characteristics, making a critical evaluation on how easy they can be applied, and on their future potentiality as ready-to-use and risk-free formulates. This will help conservation scientists, conservator-restorers, and researchers in the field to choose the most appropriate delivery system for any specific applications.[12]

## II. OBSERVATIONS

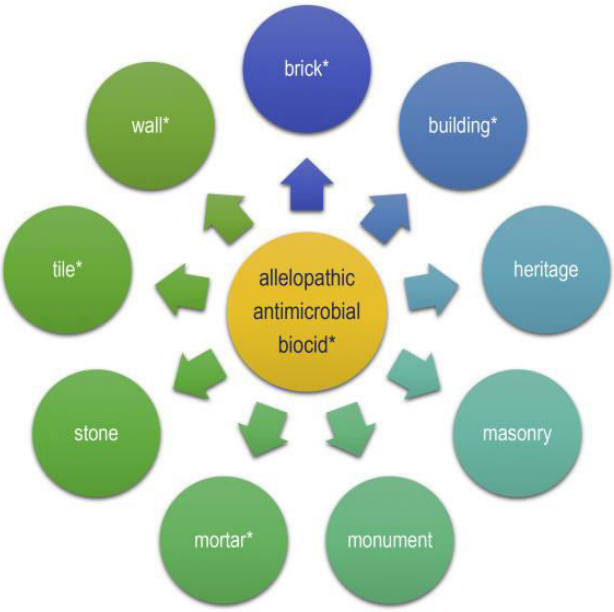
The hydroalcoholic extracts of *Arctium lappa* L. ("bardana" – "burdock") and *Centaurea cyanus* L. ("aciano o azuleno" – "garden cornflower") showed good biocide activity against microorganisms isolated from the indoor atmosphere of archives and from archive materials. These plants were selected for their antimicrobial, antiseptic and disinfectant actions, given by their content in coumarins (cichorin), flavonoids (kaempferol), organic acids (chlorogenic and isochlorogenic acids), polyacetilenic compounds, sesquiterpenes (arctiopicrin), organic acids (acetic and ascorbic acids), tannins, alkaloids, terpenes, steroids, lignans (arctiin and arctigenin) and phenols among other components.[13] These compounds were also present in the extracts evaluated in this study. These metabolites have a broad structural activity influencing:

i) the expression of their biological activity;

ii) solubility in polar dilution liquids like alcohol and water, (used both in the preparation of the extracts used in the present study), and

iii) the physiological characteristics of the plant at the moment of its collection. Similar results were found by Lima et al., 1993, when he evaluated an hydroalcoholic extract obtained at low temperature for maceration using aerial parts of *Artium lappa* but against *Staphylococcus aureus, Streptococcus pyogenes* type A and *Streptococcus pyogenes* type B. This effect was attributed to arctiopicrine (lactone sesquiterpenic).The slightly more active effect against gram-negative than grampositive bacteria is, furthermore, attributed to a substance with an unsaturated lactone group. This action is very important because some of these microorganisms evaluated here present cellulolytic, lignolytic, amilolytic, proteolytic activity.[14] Moreover, it has been frequently isolated from glues, old books, newspapers, parchments and documents with leather binding. Representatives of *Bacillus* genus can degrade cellulose, starch and proteins due to the action of its complex enzymatic system, can provoke purplish spots and acidification, and are also responsible for the Cancer of Parchment. On the other hand, *Streptomyces* genus comprises several *bibliophagus* species and it exerts a great destructive force upon cellulose, lignin and synthetic glues (containing urea-formalin). Not to mention, that they not only damage collections but their presence implies a risk factor of infections for all those in contact with contaminated materials as well.

In another study one research team tested hydraulic lime mortar to which they added carbendazim, a biocide compound generally widely used in paint, as it has low water solubility and is therefore more water resistant. In order to do so, they compared, on the one hand, the antimicrobial effectiveness of a lime mortar to which carbendazim was directly added and on the other hand, a lime mortar whose clay contained an anchored biocidal compound.Both underwent several microbiological tests in order to test their ability to fight microorganisms and a leaching process, in which the soluble parts of a material are removed, simulating various rain cycles in a short amount of time."In the first microbiological test, they verified that the first mortar, to which they directly added carbendazim, had a somewhat greater biocidal capacity. However, after the leaching processes, they verified that the second mortar, that had carbendazim anchored to the clay, showed better results since the biocide compound was released more slowly and therefore, its effect was more long-lasting.[12]



**Fig,2: Natural biocides for the conservation of stone cultural heritage**

Placing trust in biotechnology and microbiology to help conserve its cultural heritage—which has a significant impact on the economy of many nations—the European Union is supporting a number of innovative projects. These range from the development of new methods to preserve waterlogged archaeological wood to finding new inhibitors of microbial growth and preventive strategies to avoid the formation of biofilms, which damage stones in historical buildings and subterranean monuments.[10]

**Table 1. Projects involving novel microbiological tools for the conservation of cultural heritage funded under the European Union's Fifth Framework Programme**

| Project | Description | Cost (€) | Time span |
|---|---|---|---|
| BACPOLES | Preserving cultural heritage by preventing bacterial decay of wood in foundation poles and shipwrecks | 1.75 million | 2002–2005 |
| BIOBRUSH | Novel approaches to conserve our European heritage: bioremediation for building restoration of the urban stone heritage in European states | 1.74 million | 2002–2005 |
| BIODAM | Inhibitors of biofilm damage on mineral materials | 1.44 million | 2002–2005 |
| BIOREINFORCE | Biomediated calcite precipitation for monumental stone reinforcement | 5.48 million | 2001–2004 |
| CATS | Cyanobacteria attack rocks: control and preventive strategies to avoid damage caused by cyanobacteria and associated microorganisms in Roman | 1.68 million | 2001–2003 |

| Project | Description | Cost (€) | Time span |
|---|---|---|---|
| | subterranean monuments | | |
| COALITION | Concerted action on molecular microbiology as an innovative conservation strategy for indoor and outdoor cultural assets | 2.52 million | 2000–2003 |

## III. DISCUSSION

Several research groups have shown that the anaerobic sulphate-reducing bacteria *Desulfovibrio desulfuricans* and *D. vulgaris* can efficiently remove the black sulphate crusts that often tarnish buildings . Other studies reported that the bioformation of oxalic acid could generate a protective calcium oxalate patina on stone surfaces . Biomineralization is another emerging [10] interdisciplinary research field with high applicative potential in the consolidation and restoration of deteriorated ornamental stone. Several bacterial strains have been shown to precipitate calcium carbonate—a process known as carbonatogenesis—mainly in the form of calcite, which can form a protective layer on the surface of weathered stones and even penetrate the stone matrix to act as a bioconsolidating cement .Other strains, such as the microbe *Shewanella oneidensis* MR-1, inhibit the rate of calcite dissolution under laboratory conditions .
The good news is we can ward off microbial infestations by storing artifacts in conditions that don't encourage spores to settle and germinate. This means keeping things cool and dry. “In an indoor environment, like a museum or a library…you can control the temperature, humidity, and light,” Conservators also use UV-C fans to filter spores from the air and zap them with ultraviolet light. Once a piece of artwork is colonized, it must be dried and cleaned before conservators can get to work restoring it. Artifacts can also be placed in a chamber filled with gases like argon. Eventually, the lack of oxygen will defeat most lingering microbes.[8]
For some treasures, long-term protection is in order. Meteorites are often stored under nitrogen to deter microbes. “They don't want to have to differentiate between Earth microbes and any signals from the origins of meteors,”. Conservators have less control over outdoor artifacts, although they can treat them with biocides that prevent microbes from growing.[12]
Thwarting nuisance microbes with extracts from plants. They are testing garlic, mint, and tea tree oils. These compounds are part of the plants' natural defenses against infestation, he says. The research team is currently testing plant oils on the mosaics. [14]

## IV. RESULTS

Extensive range of case studies of important world heritage artefacts and monuments as well as an overview of in situ preservation of historic ships " Provides background knowledge on the use and application of modern analytical techniques in conservation " Contains detailed information on molecular and synchrotron techniques to assist with identifying biological and chemical threats to heritage artefacts and monuments. Techniques cover the use of GIS image processing, molecular biological analysis of environmental samples including FISH, electrophoresis to remove corrosive ions and synchrotron radiation to detect chemicals present in artefacts. Biofilms are the dominant lifestyle of microorganisms in all environments, either natural or manmade, including heritage.[7] The development of effective strategies to combat biofilms is a challenging task. These emerging novel antibiofilm strategies are still in the nascent phase of development, and more research is urgently needed to validate these approaches, which may eventually lead to effective prevention and control of biofilms. Until now, the research and application of antibiofilm compounds have often been questioned owing to the diversity of the testing methods available and the variations of the results reported in the literature vs those obtained in-field.[10] Thus, numerous innovative antibiofilm approaches have been published, but it is difficult to reliably compare all these strategies. Some factors still hamper the testing and screening of antibiofilm compound such as, among others, the scarcity of homogenized testing protocols, the lack of normalized vocabulary, the difficulty of testing repeatability and reproducibly. Thus, a key aspect of future antibiofilm research is the need for standards: a unified terminology and well described protocols and guidelines are required to test the effectiveness of traditional or novel compounds against biofilms retrieved on heritage surfaces. These protocols and guidelines should be a preliminary step in the direction of a potential code of green practices.[12]

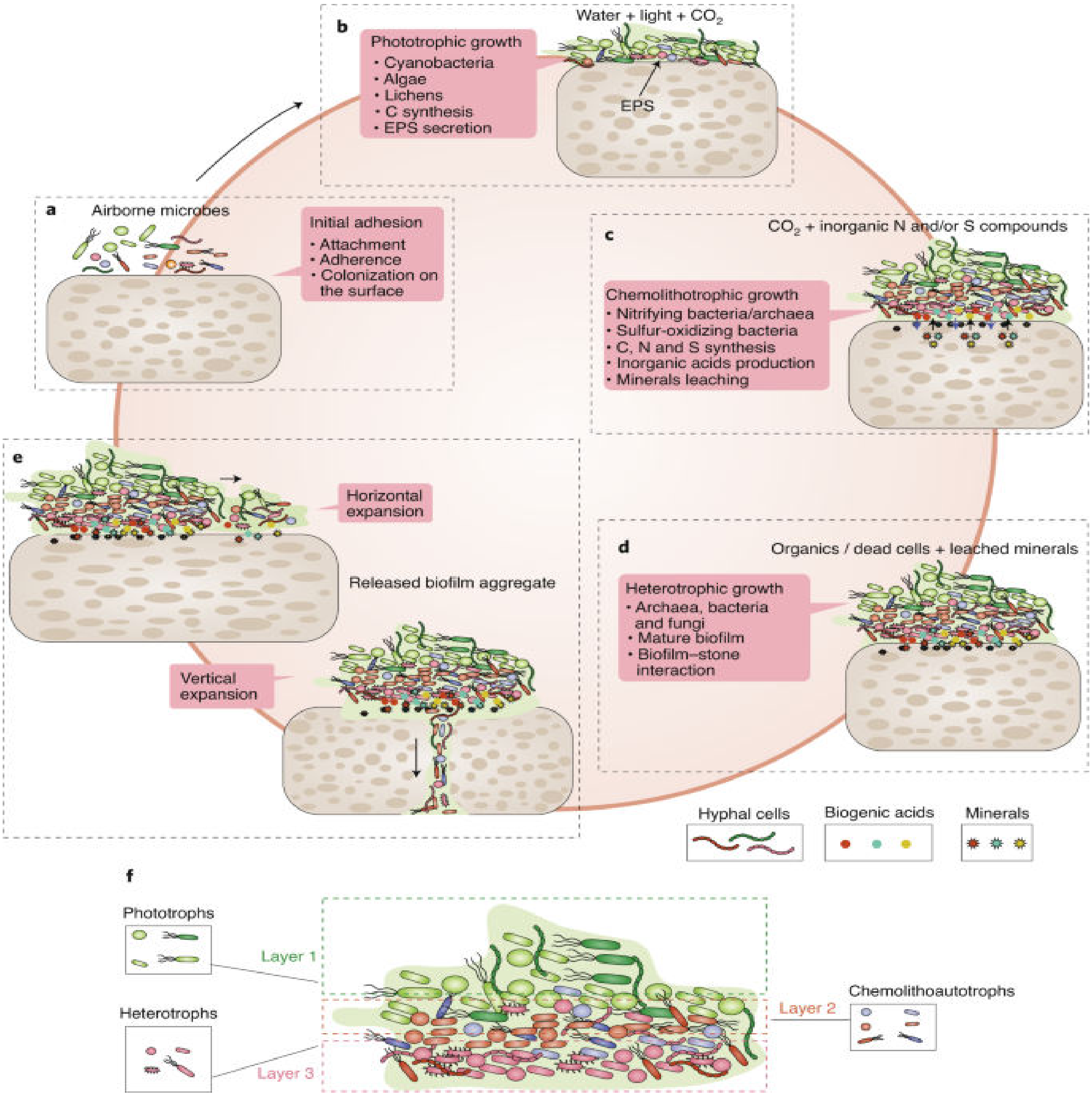


**Fig.3 Microbial deterioration and sustainable conservation of stone monuments and buildings**

## V. CONCLUSION

The combination of several analysis techniques is compulsory in the field of cultural heritage in order to develop and design new and effective conservation strategies to prevent, control and minimize biodeterioration. In this study, digital image analysis, confocal laser scanning microscopy, in vitro chlorophyll a quantification and colourimetry were applied in order to evaluate a new procedure of stone bio-cleaning consisting of the application of cells free filtrates of *Trichoderna harzianum* and *Burkholderia gladioli* pv. *agaricicola*, and glycoalkaloids from spontaneous Solanaceae with biocide properties. These techniques have shown a good direct correlation with the data obtained from these approaches, revealing that cells free filtrate of *Trichoderma harzianum* has an antagonistic capacity against the multispecies phototrophic culture tested. Nevertheless, [13] the efficiency of this compound on colonized stone surfaces needs further experiments to assess their mid- and long-term efficiency since efficient mitigation should inactivate the organisms and prevent their re-growth for an acceptable period of time. Moreover, colour variations greater than the generally accepted value represents a drawback scenario. In spite of the vast literature on the successful application of these potential natural biocides for controlling plant pathogens, their application on stone cultural heritage as an alternative approach to conventional biocides is still in its infancy. In fact, there is still a paucity of knowledge on natural products for biocontrolling purposes in the field of cultural heritage, and consequently, conservation interventions do not always obtain the expected result, and sometimes they even hasten the biodeterioration process. Thus, with a view towards the future conservation of deteriorated stone monuments,[10] laboratory-based experiments should be frequently developed since they allow the management of preventive conservation strategies and help to choose the appropriate treatments and conservation strategies. Laboratory experiments present the advantage of controlling environmental variables which simplifies the answering of important questions, particularly in the field of stone biodeterioration. [9] These experiments are prerequisite in the diagnosis of monuments and in the design of effective treatments for eliminating active microbial communities, since they allow an affordable evaluation of the efficacy of biocides, as showed in this work.

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