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Original article

Biological colonization on stone monuments: A new low impact cleaning method

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ABSTRACT

In restoration and conservation practices, biocide treatments are considered one of the most practical approaches to remove biological colonization on artworks, including stone. Numerous studies have focused on the short- and long-term effects of these treatments and recently many alternative methods to reduce their potential hazards to human health and the environment have been proposed. In this study, a solvent gel containing dimethyl sulfoxide (DMSO), already used to clean paintings, was applied on colonized marble artifacts at the monumental cemetery of Bonaria (Cagliari – Italy) to remove biological patinas. The protocol efficiency was evaluated by scanning electronic microscopy, rugosimetric and colorimetric measurements and growth tests. A comparative study also was performed to validate the method using biocides currently used in conservation. The results demonstrate that DMSO solvent gel is efficient at removing patinas on stone, of low impact, easy to use, inexpensive and can be considered a more practical alternative to biocide treatments.

1. Introduction

Abiotic factors such as sun, frost, salts, wind, rain, and pollution all contribute to the weathering processes of monuments, sculptures and objects of art exposed to open air. However, even if bare lithic surfaces represent an exceptionally hostile niche for microbial settlement, due to extreme environmental conditions, they are inhabited by highly specialized organisms such as lichens, algae, cyanobacteria, chemolithotrophic and chemorganotrophic bacteria and fungi [1,2]. These organisms interact with each other, forming microbial matrixes of complex communities known as sub-aerial biofilm (SAB), held together and tightly bound to the underlying surfaces by extracellular polymeric substances (EPS) [3]. EPS are composed of different macromolecules including polysaccharides, proteins, some lipids and, to a lesser extent, nucleic acids and other biopolymers [4]. They play a crucial role in facilitating adhesion of organisms and airborne organic and inorganic nutrients, in retaining water over long periods, as osmoprotectant preserving cells under desiccation, and in enhancing resistance to chemicals [3,5]. Microbial attack not only causes aesthetic changes, modifying the colour and/or the shape of the object of art, but also produces mechanical and chemical damages affecting both surfaces and inner zones and in the long-term leading to their decay [1,6,7].

A significant contribution to deterioration is caused by endolithic microorganisms, such as meristematic melanized fungi, growing into the pores and crevices, tightly adhering to the matrix and able to withstand stressing conditions when dehydrated [8–12]. Cyanobacteria are also directly involved in most of the degradation of cultural heritage worldwide, being adapted to resist stressful conditions because of their thick outer envelopes and the presence of protective pigments [13,14]. The prevention and control of biodeteriogens from spreading are a demanding challenge for conservation of cultural heritage. The control of biodeteriogens is frequently conducted in the early phases of artwork cleaning treatments, and after restoration with conservative purposes, to prevent or slow the recolonization [15–20] using biocidal products

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and/or aggressive and strongly oxidizing chemicals (e.g. hydrogen peroxide).

Many studies report advantages and drawbacks of treatments on colonized rock substrates [21,22], highlighting the importance of the intervention design and the choice of products and protocols used [15,19,22–25]. Yet, these methods are not always effective in the long-term since biocides are not equally active on different SAB components [22–24,26].

Stone surfaces have been traditionally treated with chemical biocides, even if their use has been declining in recent years because of their risks to human health and the environment. Recently, new methods and products have been proposed for removing biofilms and lichens from outdoor monument surfaces including heat shock or enzyme treatments [27–29], metabolites produced by natural competitors of microorganisms occurring on stoneworks [30], and dry ice or laser cleaning techniques [31]. These treatments are eco-friendly or low impact procedures and reduce the health risks for the restorers, visitors and the environment [25,32,33]. In fact, studies on innovative and efficient methods are strongly encouraged.

Considering the cleaning results achieved by conservators and restorers using dimethyl sulfoxide (DMSO)-based gel on paintings since the early 2000s [34], as well as the advantages offered by the application of a gel on vertical surfaces and the DMSO properties, we investigated the potential that this gel would have to remove biological patinas growing on stone surfaces. Being a small amphipathic molecule, DMSO is used in a wide range of fields, including medical applications on humans and animals [35–37]. DMSO solubilizes a number of biomolecules commonly present in SAB, such as polysaccharides, proteins and organic pigments including chlorophyll and melanins [38–42]. Moreover, depending on its concentration, it can influence the stability and dynamics of biomembranes: at low concentrations it induces membrane thinning and increases fluidity of the membrane's hydrophobic core, at higher concentrations it induces transient water pores into the membrane and, at still higher concentrations, individual lipid molecules are desorbed from the membrane followed by disintegration of the bilayer structure [43,44].

As suggested by a number of authors [33] and according to the UNI Normal Italian guidelines, the interaction of DMSO-based gel with the stone substrate was first verified, then its efficiency against biological patinas was evaluated by optical and scanning electronic microscopy as well as by rugosimetric and colorimetric measurements and growth tests. The cleaning efficiencies of the DMSO-based gel and other currently used biocides are compared for validation.

2. Research aim

The prevention and control of biodeteriogens are a demanding challenge for conservation of cultural heritage. Chemical biocides in spite of their risks to human health and the environment, and the availability of eco-friendly and/or low impact procedures, still remain a frequent choice to control the biological colonization on outdoor stone artifacts. Aim of this interdisciplinary work is to propose a new low impact and inexpensive procedure that combines high efficiency with an easy and safe use in the field.

3. Materials and methods

3.1. Artifacts under study

A sample of ordinary, smooth but not glossy, uncolonized Carrara marble (ca. 20×20 cm) was used to monitor any possible change in physical features of the stone caused by the DMSO-based gel treatment.

Field tests were conducted on two cemetery marble monuments (authors unknown): a funerary cross (ordinary Carrara) and an angel (statuary Carrara), devoted to Giovanni Pisano and Frau – Carta's spouses respectively, and a hundred years old marble fragment (unknown affiliation; ordinary Carrara), diffusely colonized. All samples were from the Monumental Cemetery of Cagliari, whose position, climate and general historical notes are published [45].

The funerary cross was used as a preliminary test to verify the efficacy and the impact on the colonized marble surface by the proposed cleaning method.

The protocol was then tested on a larger surface, corresponding to the angel head, to verify its effectiveness in the long-term, since the angel is located in a shady and moist position that favours autotroph settlement. The re-growth of possible colonizers was studied by culture tests (see below).

The old marble fragment, due to its marked and uniform colonization, was used to compare the method proposed with a selection of biocide-based cleaning methods, by both colorimetric measurements and culture tests.

3.2. Sub-aerial biofilm characterization

SAB composition of each artifact was determined before treatments by both direct observation and culture methods. Non-destructive methods were used to collect aseptically small biological samples in sterile 1.5 mL tubes [46]. A small portion of each sample was observed by both stereo and light microscopes. Each sample was split into three portions and seeded on the following culture media, respectively: malt extract agar (MEA, Conda Laboratory, Spain) in Petri dishes at room temperature ($20 \pm 2\,^{\circ}$C) for fungi; 50 mL of BlueGreen-11 freshwater medium (BG11, Sigma Aldrich) for cyanobacteria; 50 mL of Bold Basal Medium (BBM, Sigma Aldrich) for micro-algae according with NORMAL 9/88 [47]. Cyanobacterial and algal cultures were maintained for 6–8 weeks under cool-white fluorescent illumination (Osram Dulux L 36W/840 Lumilux, 2900 lumens), with a 12-h photoperiod, at room temperature ($20 \pm 2\,^{\circ}$C).

After DMSO-based gel treatment, culture tests were repeated on further samples taken from the marble fragment (1 month later) and from the angel (12 months later) to verify short- and long-term microbial re-growth.

All isolated fungal strains are preserved at the Culture Collection of Fungi from Extreme Environments (CCFEE, Viterbo, Italy), mycological section of the Italian National Antarctic Museum "Felice Ippolito".

3.3. Solvent gel preparation and cleaning protocol

According to Wolbers [48,49] the solvent gel (Gel 1) was prepared using 100 mL of DMSO (CAS no 67-68-5, Carlo Erba, Milan, Italy; 89.15% w/w), 10 mL of Ethomeen® C25 (CAS no 61791-14-8; CTS, Altavilla Vicentina, Italy; 8.41% w/w), and 3 g of Carbopol® 934 (CAS no 600-07-7; Biochim, Milan, Italy; 2.44% w/w). Ethomeen® C25 is a tertiary amine polyethoxylate (slightly basic) that acts as a nonionic surfactant, here used to neutralize Carbopol® 934, a cross-linked polyacrylate polymer used in aqueous solutions and in polar organic solvents to produce formulations for gels, emulsions creams and suspensions [50].

The preparation involves the dispersion of the polymer in the solvent and further the addition of the surfactant. The pH, measured using a pH paper, was about 8.0.

As negative control a solvent gel (Gel 2) was prepared, where DMSO was replaced with distilled water.

All surfaces, regardless of the treatment applied, were preliminarily brushed and washed to remove loose deposits. Before

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