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Review

Efficacy of different chemical mixtures against green algal growth on limestone: A case study with *Chlorella vulgaris*

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ABSTRACT

This study aimed to develop nine biocide mixtures that prevent the weathering and biofouling of stone monuments. Tetraethoxysilane (TEOS) was combined with one, two or three active components: chitosan (a biopolymer used for its antimicrobial potential), silver nitrate and hydrophobic silica.

A laboratory test was set up, consisting of the inoculation of untreated (control) and treated limestone ("Dom stone") slabs with an axenic suspension of the green alga *Chlorella vulgaris*. The biocide efficacy was evaluated by non-destructive methods such as colourimetry and chlorophyll *a* fluorescence analysis. The latter method supplements the information provided by the measurement of the colourimetric changes of stone surface and characterizes how the biocide acts on the alga PSII photosynthetic activity. Results revealed different patterns of algal development according to treatment efficacy. The combination of silver nitrate and hydrophobic silica, both at high dosages, provided the best biocide effect. When chitosan was added, a similar biocide effect was obtained using a lower concentration of chemicals. This synergy was not observed when hydrophobic silica was either absent or present at a higher dose.

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Introduction

Throughout history, stone has been the material of choice for cultural heritage because of its durability and beauty. However, in industrial countries and damp temperate climates, remedial steps must be taken to preserve the aesthetic appearance and historical value of stone. Biodeterioration starts with biological stains that lead to unsightly discolouration of the stone surface. Micro- and macroorganisms can deteriorate stone chemically and mechanically, causing irreversible damage (such as biocorrosion, pitting, cracking, detachment) at the surface and inside the stone (Urzi and Krumbein, 1994; Warscheid and Braams, 2000). The colonization and growth of the organisms depend on climatic and environmental conditions (Crispim et al., 2003) and on the bioreceptivity of the material, which is linked to its intrinsic properties (Guillitte and Dreesen, 1995; Miller et al., 2012; Manso et al., 2014).

The considerable diversity of micro-organisms and their ability to survive and to develop under varied environmental conditions (May et al., 2000) make it difficult to devise a cure-all product. In addition, the sustainability of treatments must be taken into consideration; they must be less toxic while remaining effective against biocolonisation.

In cultural heritage, *Chlorella* spp. is frequently identified in biofilm communities growing on buildings (Ortega-Calvo et al., 1995; Urzi and De Leo, 2007). It is one of the first pioneering green algae to colonize the substrate (Guillitte and Dreesen, 1995) and is widely used in laboratory to get a rapid colonization of material (Manso et al., 2014) or to assess the biocidal products used in restoration (Nugari and Salvadori, 2002).

Some treatments are used to clean biofouled surfaces while others are sometimes applied onto clean stone to prevent biological weathering. Some of the poor results arising from laboratory and in situ testing have been explained by the ineffective mixing of treatments (Urzi and De Leo, 2007; De Muynck et al., 2009) or by the interaction between treatments applied sequentially (Malagodi et al., 2000; Moreau et al., 2008). Quaternary ammonium compounds are the most widely used for their good efficacy against green algae (Nugari et al., 2009). Compounds such as plastic-based products have also been tested but they altered the substrate properties and were less efficient against green biofilms (Prieto et al., 2014). Other biocides, containing a combination of silver nanoparticles and water repellent, showed good algaecidal performance related to their silver concentrations (MacMullen et al., 2014) or the combinations with other chemicals (De Muynck et al., 2009). New non-toxic compounds such as anatase (TiO_2) had an efficient photocatalytic effect able to degrade organic matter and to inhibit recolonization (Fonseca et al., 2010; La Russa et al., 2014).

Many techniques have been developed to quantify the impact of toxic substances on photosynthetic organisms. Some are invasive and need sampling like spectrophotometric estimation of chlorophyll *a* concentration (Prieto et al., 2004), cell size measurement (Eggert et al., 2006), gas-exchanges (Bigot et al., 2007), epifluorescence (Tretiach et al., 2010) while others, such as surface colourimetry and chlorophyll *a* fluorescence measurement, are not destructive and are extremely useful in the field.

This study attempts to develop a preventive treatment using a sol–gel process applied onto a clean surface (Eyssautier-Chuine et al., 2014). Tetraethoxysilane (TEOS), commonly used as a consolidant, was used here as a precursor at low concentrations. Active components were then added. One of these is chitosan, used in many fields for its antibacterial activity (Runarsson et al., 2007; Raafat and Sahl, 2009). It is environmentally friendly, biodegradable and can work with inorganic materials such as TEOS through the sol–gel method (Yeh et al., 2007). Other components improve the chitosan effect: silver nitrate for its biocide effect and hydrophobic silica as a water-repellent. Nine treatments were developed using different concentrations of the four following ingredients: TEOS, chitosan, silver nitrate and hydrophobic silica. They were tested in laboratory conditions with a green alga of the widespread genus *Chlorella*.

In order to evaluate the biocide effect of the different experimental mixtures described above, two complementary techniques were used: colour measurement, commonly used in the conservation of stone monument, and chlorophyll *a* fluorescence. This last method, widely used in ecophysiological studies, has been used in the past few years in cultural heritage to characterize biofilm development through photosynthetic activity and to study the efficacy of biocide treatments. Fluorescence analysis can detect damage in the photosystem II earlier than other conventional measurements (Campbell et al., 1998). Damage is associated with a stress induced by changing natural environmental conditions (nutrient limitation, high light etc) (Choi et al., 2012) or by adding toxic elements (Lu et al., 2000).

Materials and methods

Stone

The material selected for this study was a limestone used widely in the buildings and monuments of northern France and southern Belgium. It is a Bajocian limestone (Middle Jurassic, 180 My) called Dom stone because it was extracted from quarries around the town of Dom-le-Mesnil (French Ardennes district) from Medieval times to the Second World War. In this area, it was called “Sun Stone” because of the russet colour produced by its high iron oxide content (0.5%).

The Dom stone is a bioclastic stone (Fronteau, 2000). In thin section, the facies is composed of calcitic debris: numerous echinoderm ossicles in a syntaxic cement, shell fragments and Foraminifera and a few quartz grains scattered throughout the rock. Considerable intergranular macroporosity remains due to only partial cementation and local dissolutions during the late-stage of diagenesis.

Petrophysics display a high total porosity (30 ± 2.7%) (European Committee for Standardization EN 1936). The capillary coefficient