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Medium-term *in situ* experiment by using organic biocides and titanium dioxide for the mitigation of microbial colonization on stone surfaces

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ABSTRACT

Organic biocides are commonly used to reduce the biocolonization on stone surfaces. However, it should be possible to prolong a new re-colonization by the use of a combined application of organic and inorganic active compounds. $TiO_{2,}$ thanks to its high chemical stability, non-toxicity, high photoreactivity and low cost, make it a potential effective molecule for long-term biocide activity against several biofoulers. In this research, a multi analytical approach, including microscopy, cultural and molecular analyses, has been applied to monitoring the treated surfaces of the southeast wall of Villa dei Papiri in Ercolano. In this study case, organic conventional biocide has been used, followed by the application of newly formulated products based on pure and doped titanium dioxide nanoparticles. As control, one part of the wall was treated only with organic biocides before applying only the binder.

Biological sampling was carried out during eight months, before and after the treatments with biocide, and after the treatments with undoped and doped TiO_2. The comparative analysis of results showed that microorganisms were drastically reduced after the biocide treatments, while the treatment with bare and doped TiO_2 reduced a potential new recolonization. However, the effectiveness of the treatments was dependent on distance from the ground since high humidity reduced the efficiency of the treatments.

1. Introduction

The growing awareness in conserving built heritage had stimulated an increase in the number of researches addressed to issues regarding stone degradation caused by biocolonisation. Microorganisms colonizing material surfaces can be considered as one of the most dangerous degrading agent for stone monuments exposed outdoors (Urzì, 2004; Scheerer et al., 2009; Adhikary et al., 2015; Karacal et al., 2015; Marano et al., 2016) and thus they need not only to be removed, but the recolonization process must be slowed down. The restoration intervention of such degraded surfaces is composed of different steps. The first one involves the removal of pre-existing alterations (i.e. deposits, crusts, biological patinas). After that, most of time, the stone needs to be consolidated. For this purpose suitable inorganic and organic products should be used. Cracks and fissures have to be filled with compatible products that do not alter the stability and the aesthetics of the monuments. The last stage involves the application of biocide products to avoid the re-colonization process (Urzì and De Leo, 2007; Crisci et al., 2010; Pinna et al., 2012; Sterflinger and Piñar, 2013). The use of organic compounds (Crisci et al., 2010; Urzì et al., 2016) would not assure long-term protection due to the quick degradation of such materials. For this reason, several studies have been carried out on the use of inorganic nanomaterials (Bruno et al., 2014; La Russa et al., 2014). Nanoparticles, if properly dispersed in coatings, can offer outstanding properties and performances. In this context, nanosized TiO_2 is one of the most promising metal oxides, as it has a

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high stability and photo-reactivity, as well as a broad-spectrum activation antibiosis and low cost. It has been successfully tested as biocide against various microorganisms (Maness et al., 1999; Hur and Koh, 2001; Yu et al., 2003; Rincón and Pulgarin, 2004; Nadtochenko et al., 2006; Gupta et al., 2013). This compound represents a novel eco-friendly alternative for disinfection (Stoyanova et al., 2012). Further its importance is due to the fact that it may decrease the bioreceptivity of treated surface because of its self-cleaning ability (Baneriee et al., 2015) and thus in a contaminated environment or in outdoor conditions, the rate of recolonization can be highly reduced by its application.

When TiO_2 is exposed to ultraviolet (UV) light ($\lambda < 400$ nm), holes (h+) and excited electrons (e-) are generated. The holes are capable of oxidizing water or hydroxide anions into hydroxyl radicals (-OH) (Zhang et al., 2008). The free radicals produced can decompose a wide range of organic compounds. The antimicrobial efficacy is determined by the competition among the recombination of electrons and holes after the excitation, and their interaction with the bacteria.

Titanium dioxide activity can be enhanced by the addition of small amounts of metals in the materials (Zaleska, 2008; Zhang et al., 2008; Fonseca et al., 2010; Hou et al., 2009; Iliev et al., 2006; Li et al., 2007). In particular, the Ag doping of TiO_2 can also cause the denaturation of proteins present in bacterial cell walls and slow down bacterial growth (Maness et al., 1999), leading to synergistic effect.

The use of bare or doped TiO_2 as biocide for stone protection has been successfully tested in Cultural Heritage restoration (Quagliarini et al., 2012, 2013; La Russa et al., 2012, 2014; 2016; Ruffolo et al., 2013; Munafò et al., 2015). However, such studies lack long- or medium-term in situ experimentation, since they deal mainly with laboratory tests aimed to assess the products' efficacy and suitability for stone protection.

This paper deals with an eight months medium-term in situ experimentation carried out in the archaeological site of "Villa dei Papiri" within the excavation of Ercolano site (Naples, Italy) (Fig. 1).

Coatings based on TiO_2 have been applied in situ. Before these treatments, organic conventional biocide has been used, followed by the application of newly formulated products based on pure and doped TiO_2.

Biological monitoring was carried out during this period of time, before and after the treatments with biocide, and after the treatments with TiO_2. The mitigation of microbial colonization was evaluated through the comparative analysis of results obtained during the three different sampling campaigns.

2. Materials and methods

2.1. Case study

Villa dei Papiri is one of the main examples of architecture in ancient Herculanum, Naples -Italy-, destroyed by the volcanic eruption of 79 A.D. The villa was discovered almost by accident in April 1750 during the digging of a well.

The selected area for the treatment was a southeast wall of the Villa dei Papiri covered with plaster as shown in Fig. 1. The presence of a little underground river leads to constant water rising from the ground, causing a dramatic stone degradation, especially in terms of a heavy colonization of the surface.

2.2. Preparation of formulations

Formulations to be applied on stone surfaces have been prepared as follows. Pure TiO_2 (anatase 25 nm, Degussa) and TiO_2 mixed with silver nanoparticles (size 100 nm, Sigma Aldrich) (TiO_2/Ag ratio 100/1) have been dispersed in aqueous dispersion of nanosilica (CTS). Blends having different concentrations of nanosilica have been prepared (B5% and B10% wt), while the TiO_2 concentration was always 1% wt. Each formulation was applied on the stone surface by brush in the amount of about 400 ml/m^2. As controls, the binder was applied alone and the TiO_2 was applied alone. These treatments were carried out on the southeast wall of Villa dei Papiri.

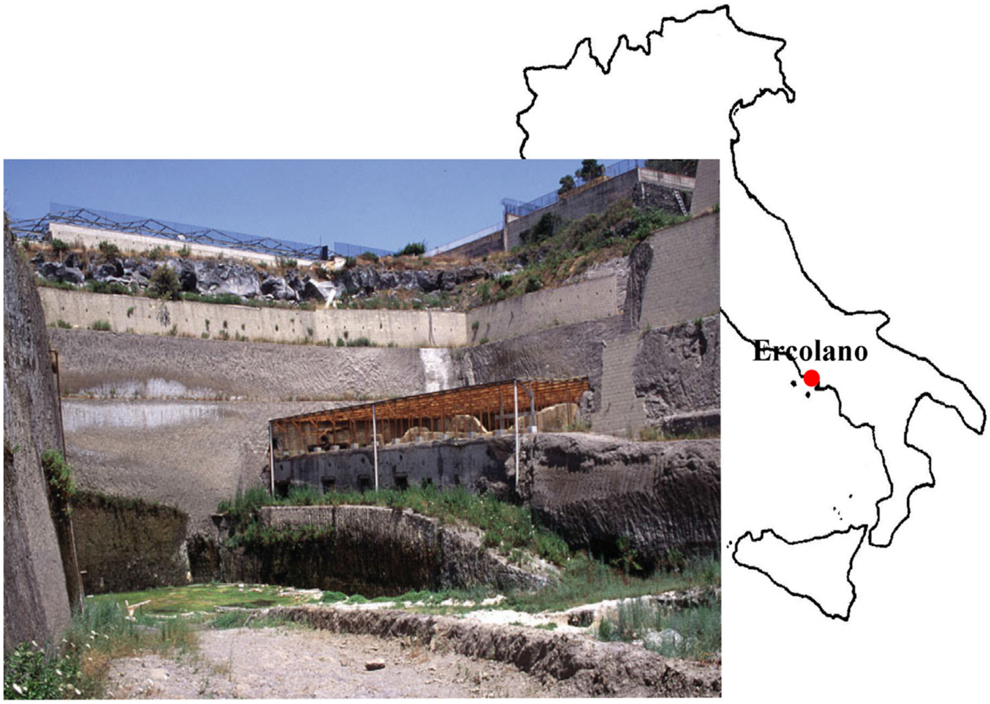

Fig. 1. Localization of Ercolano site (Naples, Italy).