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Evaluation of treatments efficiency against lichens growing on monumental stones by electrical conductivity

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ABSTRACT

Lichens growth is frequently widespread on surfaces of monumental stones and archeological areas. These symbiotic organisms may be harmful to stone substrata due both to their metabolic activity and to the induced physical-mechanical stresses exerted by the thalli. The topic related to their elimination is under debate, and usually their removal is carried out applying biocide formulates or physical stresses. The effective biocidal action of such treatments can be evaluated by some methods such as chlorophyll fluorescence measurements or live/dead staining. Since a damaged organism changes its cell functionality, a simple method based on the measurements of these changes, at a physiological level, can be applied. The induced damage on the cell-membranes can be evaluated by measuring the amount of leached ions by the whole symbiotic organism (both photobiont and mycobiont components) using the electrical conductivity (EC) method. In this study different stress actions (e.g. temperature heating and chemical treatment using the biocide Rocima 103), were applied on lichens growing on monumental stone surfaces. The vitality of treated and untreated samples was evaluated by ions leakage and chlorophyll fluorescence measurements. The EC is a sensitive method for measuring the induced stress either on crustose lichens with a thick thallus or on foliose ones. This technique can be successfully used in the Cultural Heritage field, especially when the most suitable treatment conditions for implementing new control methods must be evaluated. In this paper, possible EC interferences of the lichens with stone substrata and with the applied biocide are discussed and solutions are suggested.

1. Introduction

Lichens are symbiotic organisms with an exceptional capability to adapt, survive and thrive under several climatic conditions on various substrates. Lichens biodiversity, their biodeteriogenic action on historical monuments and the possible preservation aspects for the substrata have been investigated by many authors (Nimis et al., 1987, 1992; Romão and Rattazzi, 1996; Pinna and Salvadori, 2000; Carballal et al., 2001; Villar et al., 2004; Rigamonti, 2008; Nascimbene et al., 2009).

In general, lichens are resistant to a wide range of environmental stresses, but they are very sensitive to air pollutants and temperature especially when fully hydrated (Lange, 1953; Heatwole, 1966; Shirazi et al., 1996; Häffner et al., 2001; Grube, 2010). It seems that lichens can self-adapt by optimizing the photobiont/mycobiont ratio (Friedmann and Sun, 2005). The most common methods used for controlling the unwanted lichenic growth on monumental stones are biocide treatments and mechanical removals of the dead biomass. As lichens are very resistant to harsh treatments in their dry stage, without losing their capacity to recover (Sancho et al., 2007; Vera et al., 2008), the chemical treatments have always shown better results when they were applied on metabolically active thalli. For this purpose the lichens are lightly moistened before the treatment, facilitating therefore the biocide uptake.

Some papers dealing with cultural heritage conservation have evaluated the health and vitality of lichens after a treatment taking mostly into account their photobiont component (Tretiach et al., 2007, 2010). These works focused on the analysis of changes in chlorophyll fluorescence (autofluorescence) or on the measurements of the efficiency of the photosynthethic system. The F_v/F_m ratio is commonly used as a reliable indicator of the maximum photochemical quantum efficiency of photosystem II (Nayaka et al., 2009) and for monitoring the stress effect in photosynthetic

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organisms. This non invasive technique is usually considered a useful tool for *in situ* measurements.

The study of lichens as bioindicators of the air quality has been a topic of great interest in the fields of environmental pollution and climatic changes (Nash, 1976; Pisani et al., 2007). Since 1985 (Pearson, 1985), the sensitivity of different foliose and fruticose lichens to atmospheric pollution was evaluated in many studies using the EC method (Alebic-Juretic and Arko-Pijevak, 1989; Shirazi et al., 1996; Garty et al., 1997, 2001; Godinho et al., 2004; Marques et al., 2005; Pisani et al., 2007; Paoli and Loppi, 2008; Munzi et al., 2009). The authors considered the integrity of lichen cell membranes as a suitable parameter for monitoring the effects of different air pollutants on the whole lichen (Munzi et al., 2009), relating these effects not only to the photosymbiont integrity. The physiological parameter is expressed by measuring the K^+ ions and other electrolytes which are released in a water solution through the plasma membrane. When lichens are damaged, the level of EC values increases considerably in comparison to a healthy lichen (Pearson, 1985).

The aim of this study was to assess the sensitivity of the electrical conductivity (EC) method to evaluate the effects of physical (thermical) and chemical treatments (biocide) applied to some crustose lichens. The results were compared with chlorophyll fluorescence methodology and the pros and cons were discussed.

2. Materials and methods

2.1. Tested lichens and site description

The setup of the EC methodology has been made using fragments of the following lichens: *Aspicilia contorta* subsp. *hoffmanniana* S. Ekman & Fröberg and *Ochrolechia parella* (L.) A. Massal.), while the onsite test, using the Rocima 103 biocide, has been performed only on *A. contorta* subsp. *hoffmanniana* S. Ekman & Fröberg, because the latter species was not present in the selected treated area.

The archaeological area of Fiesole (Firenze) includes three monumental constructions: the Etruscan temple (III sec. B.C.), the Roman theater and the Roman baths (I sec. B.C.). The monuments were excavated at different times but in the early 1900 the area corresponded nearly to what is seen today (Caputo and Maetzke, 1959). It is located in a semi-natural hilly environment, limited to the South by the town of Fiesole. The natural and artificial stones which constituted the archaeological area are marble, sandstone and bricks, some of the latter covered by plasters. The Roman theater and the Etruscan temple are mostly made with a local sandstone, Pietra Serena, a feldspathic graywacke with a clay matrix and little carbonate cementing material (about 7%).

A sandstone area in the Etruscan temple was selected for the study. It has been almost completely colonized by lichens (Fig. 1). Two types of crustose lichens (*A. contorta* subsp. *hoffmanniana* S. Ekman & Fröberg and *O. parella* (L.) A. Massal.) were used for laboratory thermal stress experiments. The *in situ* experiments were performed on *A. contorta* subsp. *hoffmanniana* S. Ekman & Fröberg and *Diploschistes actinostomus* (Ach.) Zahlbr. The lichens were collected by a scalpel and were processed in the same day. The experiments were performed during the summer period (May–June).

Fig. 2. The biocide treatments.

2.2. Heating treatment

The lichens *A. contorta* subsp. *hoffmanniana* and *O. parella*, were placed into Eppendorff vials (about 200 mg of thallus with residual mineral particles). 200 μl of distilled water were added to make the thallus fully hydrated. After 1 h, the vials were sealed and put in a water bath (GFL 1002, Bioclass). The heating treatments were made by exposing samples at different temperatures (from 50° to 70 °C), for 1 and/or 3 min. A thermocouple thermometer (PT100) was used for checking the temperature inside the vials. The reference temperature was 25 °C.

2.3. Chemical treatment

The biocide ROCIMA 103 (Rohm and Haas; Phase, Italy) is a concentrated blend of 2-octyl-2H-isothiazolin-3-one and didecyldimethylammonium chloride in propane-2-ole and formic acid. It was applied by poultice (about 30 cm × 25 cm) for 48 h using a 10% v/v water solution (Fig. 2). The dose, time of treatment and application method of such biocide were previously optimized in a previous study (Pinna et al., 2012).

2-octyl-2H-isothiazolin-3-one is a heterocyclic N, S compound. These compounds are biocides containing N and S atoms in

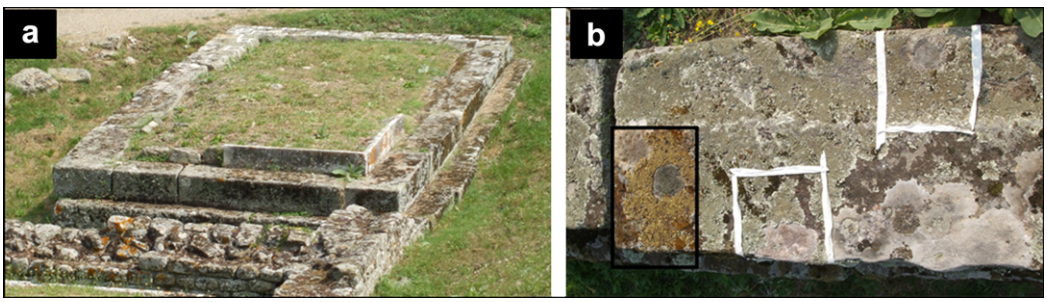

Fig. 1. Ruins of the Etruscan temple from archaeological site of Fiesole (a) and a detail of the selected area for the *in situ* experiments, 24 h after the treatment (b), with different test areas: treated with the biocide (black rectangle), treated with distilled water only (white rectangles) and the untreated lichens (all the rest).

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