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Species- and site-specific efficacy of commercial biocides and application solvents against lichens

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ABSTRACT

Control of lichens on stone cultural heritage is mostly achieved by a combination of mechanical removal with biocide applications. However, there is a lack of scientific evidence on the efficacy of different biocides on different species, and on the consistency of biocide effects on heritage sites in different environmental conditions. This results in some uncertainty when conservation interventions to control lichens are routinely defined on the basis of restoration tradition or empirical evaluation, without experimental measures of how lichens respond. In this work, we quantitatively evaluated (a) the efficacy of five commercially-available biocides, applied using a brush or with a cellulose poultice, against two species (*Protoparmeliopsis muralis*, *Verrucaria nigrescens*), and (b) whether the effects on the two species were consistent, per treatment, across three Italian heritage sites. Lichen vitality was quantified through analyses of chlorophyll *a* fluorescence (Chl_aF) and ergosterol content. The results indicated that all the tested biocides, and their organic solvents, affected the vitality of both the species. However, most of treatments displayed different efficacy on each species, across the different sites and between brush and poultice applications. Accordingly, when a conservation intervention to control lichen growth is planned, biocide treatments need both species- and site-specific calibrations and lichen vitality should be properly ascertained *in situ* by monitoring Chl_aF parameters (F_V/F_M and F_0) twenty days after trial biocide applications.

1. Introduction

The effects of lichens on stone monuments are nowadays considered a matter of debate, as researchers are increasingly contributing, and counterposing, evidence for lichen-related biodeterioration and bioprotection processes (Salvadori and Casanova-Municchia, 2016). The need to remove lichens in all cases may be reasonably questioned, as for example in cases where lichen colonization accounts for a negligible deterioration effect, shows some bioprotective attributes, contributes to the aesthetic of the monument and/or represents biodiversity value (Pinna, 2014). Nevertheless, in cultural heritage management a direct relationship between lichens and weathering is still usually envisaged, and lichen removal is generally planned as component of restoration interventions (Caneva et al., 2008).

In any cleaning interventions, devitalization of lichens is necessary to avoid them being undesirably scattered, rather than

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controlled, by the cleaning actions (Caneva et al., 2008). So far, the application of biocides has been the most followed approach to kill lichens, although chemical treatments give rise to concerns about their impact on the environment (e.g. Gromaire et al., 2015) and have already showed technical limitations (Speranza et al., 2013 with refs therein). Biocide application has indeed yielded mixed results, including poor treatment response, changes in community dynamics, persistence of dead thalli, and damage to substrate surfaces (Seaward, 2015). Accordingly, several innovative and promising approaches have been proposed in the last years to substitute for, or reduce, biocide application, including heat shock treatments (Tretiach et al., 2012), infrared and ultraviolet laser irradiation (Speranza et al., 2013; Sanz et al., 2015; Pozo-Antonio et al., 2016), and others, which still need to be better calibrated on lichens, such as anatase photocatalysis (Fonseca et al., 2010) or enzymatic treatments (Scarpa et al., 2016). Nevertheless, the adoption of these new techniques is generally limited by experimental time, extent of surfaces to be treated, and, in some cases, economic constraints, while the use of biocides persists as a routinely adopted approach, with protocols often based on traditions and empirical evaluations more than on experimental analyses of their efficacy in each case-study (Caneva et al., 2008).

Research on biocidal effects on lichens has been conducted since the 1970s and 1980s, with treatment success being mostly empirically defined *in situ* (Caneva et al., 1996, and references therein), while standardization of experimental techniques to assess lichen devitalization after biocide application (i.e. fluorescence microscopy) was established at the beginning of 1990s (Normal, 1994). Conservators have claimed some difficulties in directly testing a range of biocide and cleaning agents (Schnabel, 1991), and have noted the need for comprehensive reviews on commonly used biocidal materials (e.g. Caneva et al., 1996). However, as a response, lists of products rather than investigations into their efficacy have been produced, and some products have become outdated over the years, following the recognition of their toxicity-related environmental and health hazards (Nugari and Salvadori, 2003; European-Commission-Regulation, 2007; SCENIHR, 2009). More recent research has considered the biocidal effect(s) of restricted sets of products (e.g. Tretiach et al., 2007; de los Ríos et al., 2012), in comparison with physical treatments (e.g. Fonseca et al., 2010; Tretiach et al., 2012) or in combination with other restoration products (e.g. Pinna et al., 2012). Different approaches to assess the effects of the treatments have been considered, including microscopical observation of chlorophyll epifluorescence in photobionts (Nugari et al., 1993), SEM evaluation of the integrity of anatomical structures of both lichen partners (Speranza et al., 2012), fluorimetric analyses of biophotonic activity (Bajpai et al., 1992) and chlorophyll *a* fluorescence of photobionts (Chl_aF) (Tretiach et al., 2008, 2010), electrical conductivity of thalli (Cuzman et al., 2013) and molecular assessments (e.g. DGGE; Cámara et al., 2011). The diversity of methods used to assess lichen devitalization in these studies makes it hard to compare results. Moreover, although a species-specific lichen sensitivity to biocides has been suggested (Alstrup, 1992; Nimis and Salvadori, 1997), only few researchers have included a focus on this feature (Tretiach et al., 2007, 2010, 2012). More remarkably, researchers have neglected to evaluate the *in situ* reproducibility of devitalization results across different heritage sites, nor have they clarified if different biocidal approaches, in terms of active principle, preparation solvent and/or application method, may be more or less suitable against certain species, on certain stone substrates or under certain macro- and micro-climatic conditions. However, similar information, in parallel with research on alternative approaches for lichen control, would be of value to optimize routinely-adopted biocidal application, and, consequently, reduce related environmental contamination (Scheerer et al., 2009).

In this research, we compared the effects of five commercial biocides, nowadays widely used in Europe (BiotinR, BiotinT, Des-Novo, Lichenicida 464, Preventol RI80), and their application solvents (water, acetone, White Spirit) on the vitality of two epilithic lichens [*Protoparmeliopsis muralis* (Schreb.) M. Choisy and *Verrucaria nigrescens* Pers.] commonly found on stone cultural heritage in Europe and beyond (Nimis et al., 1992). The effects of the herbicide glyphosate (Glifene SL) and of the lichen secondary metabolite usnic acid, having biocidal potential against other deteriogenic lithobionts (Gazzano et al., 2013), were also assayed. All the products were applied *in situ*, with single brush and poultice applications at concentrations following the producers' recommended ranges, on lichen thalli growing on sedimentary rocks in three Italian heritage sites located in different (phyto-)climatic areas (as defined in Nimis and Martellos, 2008). The research did not aim to rank the performance of the different products, as each product was not tested in all possible concentrations, application methods and treatment cycles. The aims of the study were to quantify, for a series of biocide treatments, (a) if each approach (i.e. biocide × application method) showed a similar efficacy against different lichen species, and (b) if efficacy results were consistent, per species per treatment, between different sites. To accomplish these aims, we examined in each study site the vitality of lichen thalli before and after the treatments in terms of chlorophyll *a* fluorescence (Chl_aF) of the photobiont, recognized as an ideal tool for checking the vitality of photosynthetic organisms, including lichens (Tretiach et al., 2012; Malaspina et al., 2014). Additional analyses were also, in turn, performed to clarify the lichen response to biocide treatments, including microscopic assessment of chlorophyll epifluorescence in photobionts and the assessment of mycobiont vitality in terms of ergosterol content.

2. Materials and methods

2.1. Sites and lichen species

Biocide applications on lichens were performed, *in situ*, at three heritage sites distributed in different (phyto-)climatic areas of Italy: (A) the Roman Archaeological site of Industria [Monteu da Po, Torino; UTM ED50, N 5001078, E 422890; 170 m], in the dry sub-Mediterranean area; (B) the Roman Archaeological site of Luni [Ortonovo, La Spezia; UTM ED50, N 4879338, E 581882; 3 m], in the humid Mediterranean area; (C) the Boboli Gardens [Firenze; UTM ED50, N 4847851, E 680788; 49 m], in the humid sub-Mediterranean area (Fig. S1). Treatments were performed on mature thalli of the epilithic crustose placodiomorph *Protoparmeliopsis muralis* (Schreb.) M. Choisy and the epilithic crustose areolate *Verrucaria nigrescens* Pers. (Fig. S1), which were identified following Smith et al. (2009). These two subcosmopolitan species are extremely common both in urban and natural habitats (Nimis and Martellos, 2008), and on stone cultural heritage (Nimis et al., 1992). In particular, 60 thalli per species for each site were selected and treated: (A) on local sandstone masonry blocks at Industria, (B) on sandstone (Macigno sandstone from Lunigiana) blocks, and the adjacent mortar, at the amphitheatre of Luni, and (C) on the sandstone (Pietra Serena) pavement slabs of the monumental Fontana dell'Isola in the Boboli Gardens, at approx. 50 cm from the fountain water.

2.2. Biocide application

Biocides were applied by a professional restorer (site A) or under his supervision (sites B and C). Each biocide was prepared following the manufacturer's instructions (Table 1, including biocide