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(Article begins on next page)

Wood distillate as an alternative bio-based product against lichens on sandstone

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Abstract

The use of traditional biocides to halt or reduce biodeterioration is increasingly deterred, due to risks for human health and the environment, as well as for potential interference with stone materials. Alternative and eco-friendly substances are needed to limit these issues. Here we aim to evaluate the devitalization of lichens by a new bio-based product: wood distillate (also known as pyroligneous acid), a by-product of the use of plant biomass to produce bioenergy by pyrolysis without the addition of synthetic chemicals. We compared cellulose poultice applications of wood distillate at a concentration of 10% and two common chemical biocides against four epilithic lichen species on Pietra Serena, a sandstone widely used in Europe. The efficiency of devitalization was measured in terms of lichen vitality expressed by chlorophyll *a* fluorescence emission F_V/F_M and F_0. Furthermore, we evaluated the effects of wood distillate on physical properties of the stone material of relevance for its conservation, including colour, resistance to dissolution, and surface hardness. Wood distillate was as effective as chemical biocides in devitalizing the thalli and did not cause any relevant interference with the assayed sandstone, although a limited dissolution of its calcite cement was detected.

Keywords: CIELAB colour measurement; Circular economy; Devitalization; Lichen; Stone cleaning; Wood distillate.

1. Introduction

Natural and man-made building materials of ancient to contemporary exterior architectural structures are inevitably colonized by living organisms and susceptible to biodeterioration (Cwalina, 2011; Favero-Longo and Viles, 2020; Sanmartín et al., 2021). Among phototrophic colonizers, saxicolous (i.e. rock-dwelling) lichens play a primary role as agents of stone biodeterioration, causing aesthetic, chemical, and physical decay within a relatively short timescale (Caneva et al., 2008; Pinna, 2017). A debate is ongoing on the need to remove lichens, at least in cases where the deterioration effect is rather negligible and/or their presence positively contributes to the aesthetics or represents a biodiversity value (Pinna, 2014; Favero-Longo and Viles, 2020). A bioprotective effect has been even demonstrated for certain combinations of lichen species, rock substrates and climate conditions (Carter and Viles, 2005; Pinna, 2021) Nevertheless, their removal is still generally considered necessary for the conservation of archaeological and monumental sites as well as in the maintenance of exterior surfaces of every building (Seaward, 2015; Cappitelli et al., 2020). Although widely adopted in routine cleaning, the sole use of mechanical tools and (pressurized) water leave largely unremoved, and may even spread, lichen structures on and within the stone, and is thus usually followed by rapid recolonization dynamics (Pinna, 2017). In recent years, physical methods, such as laser treatments, have been proposed, showing promising results (e.g. Mascalchi et al., 2015; Sanz et al., 2015; Rivas et al., 2018). However, the optimization and practical applicability of these cleaning techniques are still pending, and some critical issue has emerged, including mineral melting and perceptible color change of stone surfaces (Sanmartín et al., 2019; Pozo-Antonio et al., 2019, 2022). Consequently, the effective practice most frequently used to remove lichens is based on their devitalization with chemical biocides (Kakakhel et al., 2019), followed by mechanical removal of thalli and, finally, the possible application of products (often the same biocides) aimed at limiting the recolonization (Pinna, 2017; Capitelli et al., 2020). However, biocides traditionally used in the control of biodeterioration, such as quaternary ammonium salts and isothiazolinones, give rise to increasing concerns due to health hazards, environmental persistence with consequent microbial adaptation, and/or potential nitrogen supply favouring recolonization (e.g. Bastian et al., 2012; Poursat et al., 2019; Silva et al., 2020).

In Europe, the use of biocidal products is regulated by the Regulation No 528/2012 of the European Parliament and of the Council (EU, 2012), which defines the implementation needed for the improvement of health and safety at work for humans and for the reduction of impacts on the environment. In particular, the removal or reduction of the use of hazardous products remains the primary objective in accordance with the United Nations' agenda for 2030 for sustainable development, namely the Third goal: "Ensuring health and security for all and for all ages" (United Nations, 2015). In addition, the EU’s chemicals strategy for sustainability towards a toxic-free environment foresees a specific action dedicated to boosting the investment and innovative capacity for production and use of chemicals that are safe and sustainable by design, and throughout their life cycle (European Commission, 2020). The proposal of biocompatible and “eco-friendly” strategies to control biodeterioration has thus increased, also considering the application of plant-derived bioproducts instead of synthetic chemicals, although their natural origin does not necessarily imply that they are not toxic to humans and the environment (Lo Schiavo et al., 2020; Cappitelli and Villa, 2021). Many alternative products are mostly based on essential oils and secondary metabolites produced by plants against pathogens and predators (Palla et al., 2016; Caneva and Tescari, 2017; Jeong et al., 2018), and their devitalizing effect on lichens has recently been investigated (Favero-Longo et al., 2021). Besides the devitalizing action, to exclude unacceptable corrosive or discolouring effects, a successful bioproduct should not interfere with the substrate (Pinna, 2017; Fidanza and Caneva, 2019).

Here we test a bio-based product, wood distillate (WD), also known as pyroligneous acid, a by-product of the use of plant biomass to produce bioenergy by pyrolysis. During this process, no synthetic chemical is added and only the physiological water present in the sapwood is used for the extraction and subsequent condensation (Wei et al., 2010; Mathew and Zakaria, 2015). Based on the different productive conditions (e.g. nature of the raw material, moisture content of biomass, temperature, contact time), WD may feature a different fine chemical composition, but the typical major constituents of WD are water, acetic acid, esters and phenolic compounds (Marumoto et al., 2012; Cai et al., 2012). In particular, the content of phenolic compounds, carbonyls and organic acids likely accounts for its known antimicrobial action (Velmurugan et al., 2009; Wei et al., 2010; Suresh et al., 2019). Anti-bacterial and insecticide effects have been shown at dilutions in the range 1:10-1:100 in deionized water (Mmojieje and Hornung, 2015) and 1:100 in 10 mM $MgSO_4$ (Misuri and Marri, 2021).

Although WD may be a promising biological alternative for the control of biodeterioration, to the best of our knowledge, the devitalization efficacy of WD against saxicolous lichens and its potential interference with stone materials have never been investigated. Hence, the aim of this study was to evaluate: i) the devitalization activity of WD using as reference two commercial chemical biocides widely used in Europe; ii) the interferences of WD with properties of relevance for the conservation of sandstone substrate, namely colour, resistance to dissolution, and surface hardness.

2. Materials and Methods

2.1 Sites and materials

WD and biocide applications on lichens were carried out, *in situ*, at the Botanical Garden of the University of Siena [Site A, Siena, Italy: WGS84N 43.858537, E 11.303751; 322 m a.s.l.] and the park of Pratolino at Vaglia [Site B, Florence, Italy: N 43.859492, E 11.304735; 451 m a.s.l.] (Fig. 1a, e). Treatments were performed on the epilithic crustose-placodioid species *Protoparmeliopsis muralis* (Schreb.) M. Choisy, for both sites, and the epilithic crustose-areolate *Verrucaria nigrescens* Pers., *Circinaria hoffmanniana* (S. Ekman and Fröberg ex R. Sant.) A. Nordin. and *Blastenia crenularia*

(With.) Arup, Søchting and Frödén, in the latter site only (Fig. S1). The selected species are common from the sub-Mediterranean to the montane belt of Italy on natural and man-made stone surfaces (Nimis et al., 2016).

In the first site, the substrate was a brick; in the second one the substrate was Pietra Serena, a sandstone lithology widely used in heritage sites as well as nowadays in Italy, composed of quartz with accessory plagioclase, calcite, K-feldspar, apatite, dolomite, and variable amounts of clay minerals and calcite cement as binders, and with an effective porosity around 3-5% (Fratini et al., 2014). Slabs of such rock material were obtained from a stone shop in Florence (Cosi & Bechelli S.N.C., Pontassieve) and used to assess the potential interference of WD with properties of relevance for conservation. The composition of the slabs was confirmed by X-ray powder diffraction, displaying quartz and subordinate calcite and plagioclase (Fig. S2); their effective porosity was estimated around 3.5% by water absorption under vacuum (Robin et al., 2016).

A chestnut (*Castanea sativa* Mill.) wood distillate (WD) produced in Val di Chiana (Arezzo, Italy) by Esperia s.r.l. (RM Group Energy Solutions) and distributed by BioDea© was used at different dilutions, as subsequently detailed for each experiment. Our available data indicate a composition in line with the general richness of hundreds of organic compounds of this product, including acetic acid as main constituent (up to 30%) and several phenols, polyphenols, and tannins, with very low concentrations of toxic compounds such as PAHs and PCBs and trace elements like As and Cr (Wei et al., 2010; Filippelli et al., 2021). The following compounds were selected as reference chemical biocides: (i) benzalkonium chloride (BAC), prepared as 3% water solution of Preventol RI50 (alkyl dimethyl benzyl ammonium chloride, approx 50%, and isopropyl alcohol, 2%, in water; Lanxess, Köln, Germany), and (ii) N-octyl-isothiazolinone and didecyl-dimethyl ammonium chloride (OIT-DDAC), prepared as 3.0% solution of BiotinT (OIT, 7-10%, DDAC, 40-60%, formic acid 2.0-2.5%, and isopropyl alcohol, 15-20%, in water; CTS, Altavilla Vicentina, Italy). A bottled water with low mineral content (fixed residue at 180°C of 22-43 mg L^{-1}; Fonti di Vinadio, Vinadio, Italy) was used through the experiments, both for the dilutions and as negative control.

2.2 Wood distillate and biocides applications

A preliminary WD dose-effect experiment was carried out at site A, on bricks colonized by thalli of *P. muralis* (Fig. 1b-d). Different wood distillate concentrations (0.50%, 0.75%, 1%, 5%, 10%), selected with reference to the range of effectiveness reported against other biological targets (e.g. Mmojieje and Hornung, 2015; Misuri and Marri, 2021), were applied with a cellulose poultice (Arbocel BC 1000, JR Pharma, Rosenberg, Germany), approx. 1 cm thick, containing ca. 12 mL cm^{-3} of solutions, after having moistened the thalli with bottled water. To preserve the humidity, the cellulose poultice was covered with a sheet of aluminium foil for 4 hours and was later gently removed; thereafter, thalli were moistened with bottled water again (Favero-Longo et al., 2020).

To assess the species-specific effectiveness in comparison with traditional biocides, WD at the concentration of 10% -selected on the basis of the results of the above-described preliminary experiment (Table S1; Fig. 2)-, BAC and OIT-DDAC were applied at site B, on the horizontal sandstone balustrades of a monumental stairway (Fig. 1f-h). All the products were applied following the same protocol adopted at site A.

2.3 Lichen vitality

In both experiments, five thalli (statistical replicates) for each treatment were examined for their photosynthetic performance as target of the devitalization effectiveness. The vitality of lichens was checked by measurements of chlorophyll *a* fluorescence (Chl_aF), using a Handy-PEA fluorimeter (Plant Efficiency Analyser, Hansatech instruments Ltd., Norfolk, England). Measurements were performed on dark-adapted moistened thalli, previously humidified by bottled water, and covered with a black cotton fabric. Five measurements were taken for each thallus, positioning the sensor head at 90° over its surface, inducing Chl_aF by a red light (peak at 650 nm), and recording the data after a saturating light pulse (3000 μmol s^{-1} m^{-2}) of 1s (Bianchi et al., 2019). At site A, analyses were carried out 4 and 16 hours after the application of the wood distillate, to screen for the potential of the different dilutions on the short term. At site B, the analyses were performed one day (T1) and fifteen days (T2) after the treatments, to assess short term effects and the potential recovery after a couple of weeks (Tretiach et al., 2012; Favero-Longo et al., 2017). The maximum quantum efficiency of PSII, that is F_V/F_M (where $F_V=F_M-F_0$), where F_V is the variable fluorescence yield, F_M is the maximal fluorescence yield and F0 is the minimal fluorescence yield, was calculated (van Kooten and Snel, 1990). According to previous research on the effectiveness of biocidal treatments against lichens (e.g., Tretiach et al., 2012; Favero-Longo et al., 2020) the maximum quantum efficiency of PSII and variations in F_0, related to chlorophyll content of the light harvesting complex (Baruffo and Tretiach, 2007), were used to check the vitality of the thalli and PSII efficiency.

2.4 Colour measurements

In the laboratory, WD at the concentration of 10% was applied on the Pietra Serena sandstone slabs, cut to a dimension of 4×3×1 cm using a diamond saw. The application was performed with cellulose poultice as previously described. Moreover, pure WD and tap water were also applied for comparison. The pH of pure and 10% solution of WD, measured using a pH-meter ORP - HI2002 (Hanna Instruments, Italy), were 3.2 and 3.6, respectively.

Colour measurements on sandstone (Pietra Serena) were carried out by a portable spectrophotometer (Konica Minolta CM-23d), under the following conditions: D65 illuminant, 2° observer and a target area of 8 mm diameter, following Prieto and colleagues (2010). The CIELAB colour system (CIE, 1986) was used to analyse the data: each colour is defined by three Cartesian or scalar coordinates: the L* parameter represents the lightness, ranging from 0 (absolute black) to 100 (absolute white); a* represents the chromatic variations from red to green; and b* represents the chromatic variations from yellow to blue. To analyse the colour after WD treatment and after washing with bottled water, the total colour difference (ΔE^*_{ab}) was calculated as follows:

$$\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2},$$

where: $\Delta L^* = L^*_i - L^*_0$; $\Delta a^* = a^*_i - a^*_0$; $\Delta b^* = b^*_i - b^*_0$, the subscript i denotes the colour parameter after 4 hours and after washing, and the subscript 0 denotes the colour parameter at the beginning of experiment. Seven measurements were made directly on the threatened surface of three slabs per treatment (*n*=21).

Saturation [$C^*_{ab}=(a^{*2}+b^{*2})^{1/2}$] and hue [$h^*_{ab}=\arctan(b^*/a^*)$], together with their respective Δ values, were also calculated.

To perceive differences in colour, the following ranges were considered based on Mokrzycki and Tatol (2012): E1: $0 < \Delta E^*_{ab} < 1$ CIELAB units, observer does not notice the difference; E2: $1 < \Delta E^*_{ab} < 2$ CIELAB units, only experienced observer can notice the difference; E3: $2 < \Delta E^*_{ab} < 3.5$ CIELAB units, unexperienced observer also notices the difference; E4: $3.5 < \Delta E^*_{ab} < 5$ CIELAB units, clear difference in colour is noticed; E5: $\Delta E^*_{ab} > 5$ CIELAB units, observer notices two different colours.

2.5 Resistance to dissolution

To evaluate the impact of the WD acidity on the durability of Pietra Serena sandstone, incubations assays were performed. Four slabs (4×3×1 cm) were weighted with a Kern EG420-3NM (Kern and Sohn Gmbh, Balingen, Germany) before and after their immersion for 4 hours in a static 10% WD solution and their subsequent drying on a heating plate, thus quantifying the acidolysis-driven mass loss. Slab immersion in distilled deionized water was carried out as negative control.

To evaluate the persistence of mineral constituents, observations of the surface and cross sections of the slabs were performed using cathodoluminescence microscopy (CL). CL was carried out using a CITL 8200 mk3 equipment (operating conditions of about 17 kV and 400 μA). Moreover, CL observations were also performed on slabs incubated in stirred 10% solutions of WD and on slabs on which the 10% solution of WD was applied with cellulose poultice.

2.6 Surface hardness measurements

Hardness measurements were carried out on Pietra Serena sandstone slabs (9×8×4 cm), before and after the application of WD at the concentration 10% with the cellulose poultice (n=3). Stone surface hardness was measured using a Proceq Equotip Piccolo 2, DL-type (Proceq, Switzerland). A combination of two measuring procedures [single impact method (SIM) and repeated impact method (RIM)] was adopted for each stone sample measuring area (Yilmaz, 2013; Wilhelm et al., 2016). Firstly, to evaluate the elastic and plastic properties of the rock surfaces after the treatment, a series of 45 randomly distributed readings (SIM) was carried out. Furthermore, a second series of 20 repeated measurements (RIM) on ten points was taken to characterize the elastic and plastic properties of the surface and subsurface of the rock, informative on strength characteristics such as the consolidation of mineral grains, the looseness of the original rock surface, and the degree of compaction due to repeated impacts (Aoki and Matsukura, 2007). The robust hybrid dynamic hardness (HDH_{robust}, sensu Wilhelm et al., 2016) was calculated as follows:

$$HDH_{robust} = (HLDL_{S:med})^2 / (HLDL_{R:med})$$

where: $HLDL_{S.med}$ is the median value of the SIM series and $HLDL_{R.med}$ represents the median of the maximum values of the ten RIM series.

2.7 Data analysis

A dose-response regression model was applied to describe the effect of WD at different concentrations on photobiont vitality of *P. muralis* thalli at Site A, using WD concentration as independent variable and the effect on F_V/F_M values as dependent variable. The effective dose was checked using the *drc* R package version 3.0-1 (Ritz et al., 2015). In *drc* the function ED was used to calculate arbitrary effective dose values ED10, ED50, ED90 and ED95 based on the model fit, where 95% confidence intervals are obtained using the delta method. A logistic curve was used to describe the response of fluorescence measurements against WD doses. A Linear Mixed Effect Model (LMEM), as a Repeated Measurement ANOVA design, was applied for each lichen species to describe the effects of the WD and biocide treatments on photobiont vitality (F_V/F_M and F_0), using thallus identity as a random effect factor. F_V/F_M and F_0 were used as response variables and treatment (WD and traditional biocides), species, and time as explanatory variables in a full factorial design. We evaluated the significance of the fixed effects and of associated interaction factors using a type III ANOVA, using the Satterthwaite approximation. For each analysis, data normality of the residuals was checked with the Shapiro-Wilk test. LMEM computations were performed using the lmer function of the *lmerTest* R package version 3.1-3 for fitting the models. The means and standard deviations (SD) of colours and the medians of hardness measurements on sandstone were checked by one-way ANOVA and Tukey post hoc test was used for a post-hoc comparison of individual means in all analysis (with at least $p<0.05$ as the significance level). The analysis was run using the statistical program R Core Team (2021).

3. Results

3.1 Efficacy of devitalization treatments in situ

Wood distillate at the concentration of 10% was effective at devitalizing 95% (ED95) of thalli of *P. muralis* (Table S1) almost zeroing F_V/F_M values after 4 hours from treatment at site A, with no recovery being observed after 16 hours (Fig. 2).

Treatments at site B with 10% WD, BAC and OIT-DDAC induced several physiological alterations in all species over time as shown by the results of LMEM analysis (Table S2a). F_V/F_M values of all species were significantly lower than those of controls both at T1 and T2 (Fig. 3), showing values below the viability threshold of 0.15 (Favero-Longo et al., 2017). Only in the cases of application of OIT-DDAC on *B. crenularia* and BAC on *P. muralis* at T2, F_V/F_M values showed a partial recovery over the viability threshold compared to the other treatments (mean±SD: 0.244±0.18 and 0.162±0.12, respectively; Fig. 3). At T2, the physiological parameters of all species treated with WD showed the strongest decrease, with F_V/F_M of WD-treated *B. crenularia and C. hoffmanniana* significantly ($p<0.05$) lower than values reached with traditional biocides (Fig. 3).

After all treatments, F_0 values changed significantly over time in all species (Table S2b). Upon WD treatment, at T2, F_0 values of all species were significantly ($p<0.05$) lower than those observed in controls (Fig. 4), but significantly ($p<0.05$) higher than those obtained with traditional biocides (Fig. 4).

3.2 Effects of wood distillate on the properties of sandstone

Table 1 reports the quantitative description of surface colourimetry measures on pure and 10% WD treated Pietra Serena sandstone and of those treated with tap water (TW) as control. Parameter ΔL*, related to the lightness of the colour, indicated a general darkening of stone samples after both pure (-4.25 CIELAB units) and 10% (-2.35 CIELAB units) WD applications with respect to TW (-1.27 CIELAB units). The washing step caused a significant reduction in darkening in stone samples treated with pure WD changing ΔL* to -2.51 CIELAB units while the values of those treated with TW and 10% WD only showed a slight variation to -1.84 and -2.79 CIELAB units, respectively. About the parameter Δa*, associated with greenness (-) – redness (+) changes, samples treated with TW showed values around zero, while samples treated with WD showed similar values around 0.50 CIELAB units. In both cases, Δa* after the washing step slightly lowered to 0.35 and 0.42 CIELAB units respectively.

Δb^* values, associated with blueness (-) – yellowness (+) changes, increased to 1.64 after pure WD and to 2.53 CIELAB units after 10% WD, significantly higher than the values of the samples treated with TW (around 0.20 CIELAB units). A significant reduction in yellowing began after the stone was cleaned with tap water (0.47 and 0.70 CIELAB units, respectively). Saturation (C^*_{ab}) and hue (h_{ab}) showed higher variation after 1:10 WD application (ΔC^*_{ab} = 2.42, Δh^*_{ab} -14.82 CIELAB units) than with pure WD (ΔC^*_{ab} = 1.52, Δh^*_{ab} -10.78 CIELAB units), but a significant recovery towards the original values was observed for both the treatments after the washing step. The total colour change (ΔE^*_{ab}) after pure WD treatment was 4.58 CIELAB units (range E4; *sensu* Mokrzycki and Tatol, 2012) and significantly decreased to 2.58 after the washing step (E3) The ΔE^*_{ab} in sandstone treated with 10% WD was 3.51, and 2.93 CIELAB units (E3) after cleaning, while for samples treated with TW it was 1.28 CIELAB units and reached 1.84 after the washing step (E2). In each case, ΔE^*_{ab} parameter did not exceed the threshold of 5 CIELAB units (E5).

Changes were not observed in the weight of sandstone samples soaked in static 10% WD solution (-0.05%±0.02) compared to the control ones soaked in water (-0.04±0.03%; Table S3). Nevertheless, CL observations showed partial dissolution of calcite cement at the surface, recognizable in terms of loss of orange luminescence signal with respect to controls in deionized water (Fig. 5a-b). Similarly, the slabs treated with 10% WD applied with cellulose poultice showed a partial calcite dissolution (Fig. 5c), while in the slabs incubated in the stirred solution the calcite cement at the surface completely disappeared (Fig. 5a). However, no treatment determined a microscopically detectable dissolution through the cross-sectioned slab profiles (Fig. 5d-g).

Equotip measurements showed that 10% WD applied with poultice cellulose did not affect the Pietra Serena sandstone surfaces showing also no significant variation compared with TW-treated slabs (Fig. S3).

4. Discussion

The search for innovative natural products to devitalize biodeteriogens is one of the hottest areas of interest for the cleaning of stone, particularly in the field of cultural heritage, and has led to several studies exploring their potential application (e.g. Lo Schiavo et al., 2020; Cappitelli et al., 2020). In this work, we showed that WD effectively devitalizes thalli of different lichen species, which are commonly found on architectural stone surfaces, showing results comparable with those obtained with conventional chemical biocides based on QACs and OIT. Such effectiveness was demonstrated when

WD was applied at a concentration of 10%, whereas more diluted concentrations were less effective for the devitalization of vitality parameters of *P. muralis,* although this lichen species has somewhat been recognized as less resistant to biocide treatments than others (Favero-Longo et al., 2017). A 10% WD treatment has been shown to have an inhibition effect against insects and some gram-positive bacteria (Lee et al., 2010; Mmojieje and Hornung, 2015), but other laboratory studies showed that even lower concentrations of 1-2% may be sufficient to inhibit the growth of some fungi and bacteria (Jung et al., 2007; Lee et al., 2010; Misuri and Marri, 2021). With this regard, the poor sensitivity of *P. muralis* to 5% and lower WD concentrations may depend on a higher resistance of saxicolous lichens with respect to other (micro-)organisms, but also on the fact that our experiments were run in the field under real conditions.

With the application of 10% WD no signs of recovery were seen after 15 days from treatment, consistently with cellulose poultice application of QACs and OIT-based traditional biocides (Favero-Longo et al., 2017). In addition, in the case of *B. crenularia* and *C. hoffmanniana*, the best devitalization performance was obtained with WD, which always maintained F_V/F_M below the viability threshold set at 0.15.

Some difference between traditional biocides and WD in F_V/F_M and F_0 at T1 and T2 may be suggestive of a different toxicity process. In the case of BAC and OIT-DDAC, the zeroing of F_V/F_M at T1 was associated with a remarkable, but incomplete, drop of F_0, which instead more remarkably decreased at T2. Such pattern is compatible with a loss of chlorophyll determined by damage to thylakoidal membrane caused by QACs, as BAC and DDAC (Wessels and Ingmer, 2013). However, the non-disrupted photobionts maintained some potential for recovery, as indicated by the slight increase of F_V/F_M at T2 with respect to T0 observed for all the species here, in agreement with the observations of e.g., Tretiach et al. (2012). Differently, in the case of WD, F_0 showed a slight increase at T2 with respect to T1 (except for the less resistant lichen *P. muralis*), which is the same pattern observed for controls, likely related to different environmental conditions at the time of measuring. F_V/F_M values did not show any recovery, suggesting that the photobionts were fully inactivated. This pattern suggests an inhibitory effect on the physiological functionality of the photobiont, which is compatible with the high content of (poly-)phenolic compounds in WD and their known effects on other (micro-)organisms (Pimenta et al., 2018). This does not exclude a WD-driven structural damage influencing the chlorophyll content, which is likely prominent in the case of *P. muralis*. The identification of WD components responsible for its devitalization effectiveness, which is currently a shared objective in the case of its potential herbicidal, fungicidal, and bactericidal properties considered of valuable interest for agriculture (e.g., Pimenta et al., 2018; Aguirre et al., 2020), will be a future research step also in the case of lichens. Although the hazard profile of WD for humans and the environment similarly goes far beyond the aim of this study, our unpublished data indicate that levels of substances of possible toxicological concern such as PAHs and PCBs, as well as some toxic trace element like As and Cr, are very low in the used WD. Moreover, based on the effects on different human cell lines, a safe profile of WD emerged for short time usage, but caution is necessary following persistent product exposure (Filippelli et al., 2021).

In this work we also surveyed the interference of WD with the physical properties of Pietra Serena. Despite the dark brown colour of pure WD, the treated stone surfaces did not show important changes in the chromatic appearance, being in the range of those noticed only by experienced observers (Mokrzycki and Tatol, 2011). In particular, the ΔE^*_{ab} values for slabs treated with 10% WD (and even pure) decreased after the application and subsequent washing with blotted water to values in the E2-E3 ranges (sensu Mokrzycki and Tatol, 2011). They were thus well beneath the threshold of 5.0 CIELAB units over which an observer perceives two different colours and which is the normal limit of perception considered in industrial or technical applications (Palazzi, 1995; Eyssautier-Chuine et al., 2016). They were also beneath the more stringent threshold of 3.0 CIELAB units recently considered as upper limit of rigorous colour tolerance or noticeable change in colour following cleaning intervention on stone heritage surfaces (Sanmartín et al., 2020), and may be acceptable according to the Italian guidelines for the restoration of stone buildings (Bergamonti et al., 2018).

Incubation assays showed that WD, despite its low pH, did not determine any remarkable weight loss of the assayed sandstone slabs due to acidolysis, although the expected dissolution of calcite was detected with CL observations. Indeed, calcite dissolution appeared a remarkable feature at the surface of slabs incubated in stirred solutions, but poorly detectable in their interior. The phenomenon was even more contained for both the static incubation and the, similarly static, poultice application. Accordingly, calcite dissolution in static conditions is remarkably lower than in flowing solutions, particularly in the case of rock structural features limiting a reactive fluid infiltration (e.g. Brand et al. 2017; Pearce et al. 2019). Therefore, combination of single and repeated impact measures with Equotip did not show changes in Pietra Serena sandstone after the poultice application of 10% WD, compared with those treated with tap water. Such a stability of hybrid dynamic hardness, which is informative on

elastic and plastic properties of stone surfaces and sub surfaces and is a proxy of open porosity (Wilhelm et al., 2016), further accounts for a low impact of WD on the physical durability of the examined sandstone material.
By summarizing negative and positive issues, the low pH and the consequent (expected) dissolution of calcite cement in the examined sandstone, although contained by the static application, reasonably discourage the WD application on sculpted surfaces and fine details of the stone cultural heritage. However, the high devitalization efficacy and the limited impact on physical properties of the examined sandstone as colour and surface hardness may be compatible with the WD treatment of less delicate architectural elements, as pavements or unrefined stone blocks, overcoming emerging drawbacks of traditional biocidal products (see section 1) and the pending technical limits of physical approaches. Such potency may by particularly supported if the WD treated experimental surfaces will show a long-term preservative effect of WD against recolonization processes (monitoring of the assayed parcels in progress), which may exclude the necessity of repeated applications.
Based on its acidity, more remarkable negative interferences may be instead expected on carbonate substrata, as marble and limestone. It is nevertheless remarkable and encouraging that the neutralization of WD does not affect its activity against biological targets others than lichens (Mmojieje and Hornung, 2015).

5. Conclusions

This work showed that 10% chestnut wood distillate is effective for the devitalization of lichens on sandstone surfaces, and that its application is compatible with keeping stone colour and surface hardness, and thus appears as a promising plant-based product for the control of biodeterioration on similar lithologies, although some dissolution of calcite cement suggests to exclude its application on delicate architectural elements.

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Tables

Table 1. Values of CIELAB colour parameters (L*, a*, b*, C^*_{ab}, h_{ab}) and CIE total colour difference (ΔL*, Δa*, Δb*, ΔE^*_{ab}, ΔC^*_{ab}, Δh^*_{ab}) of Pietra Serena sandstone after tap water (TW) and WD (1:1 and 1:10). For each treatment measurements were carried out before (T0) and after treatment (After TW, After WD) and after washing step with blotted water (After WW). Different letters indicate statistically significant differences ($p<0.05$) for each treatment (lowercase letters) and between treatments (uppercase letters). For ΔE^*_{ab} values, E1-E5 ranges are indicated following Mokrzycki and Tatol (2011).

	CIELAB colour **parameters**										
	L*	**a***	**b***	**C^*_{ab}**	**h^*_{ab}**	**ΔL***	**Δa***	**Δb***	**ΔC^*_{ab}**	**Δh^*_{ab}**	**ΔE^*_{ab}**
TW											
T0	66.67 ± 1.42 a	-0.79 ± 0.04 a	1.92 ± 0.17 a	2.08 ± 0.15 a	112.70 ± 1.92 a						
After TW	65.40 ± 1.33 a	-0.79 ± 0.02 a	2.12 ± 0.19 a	2.26 ± 0.18 a	110.50 ±1.91 a	-1.27 ± 1.11 a, A	0.00 ± 0.03 a, A	0.20 ± 0.18 a, A	0.18 ± 0.17 a, A	-2.13 ± 1.90 a, A	1.28 ± 1.08 a, A (E2)
After WW	64.83 ± 1.36 b	-0.82 ± 0.05 b	2.10 ± 0.14 a	2.26 ± 0.14 a	111.50 ± 2.44 a	-2.84 ± 1.52 a, A'	-0.03 ± 0.07 a, A'	0.18 ± 0.23 a, A'	0.18 ± 0.20 a, A'	-1.19 ± 3.59 a, A'	1.84 ± 1.45 a, A' (E2)
WD1:1											
T0	58.49 ± 1.16 a	-0.95 ± 0.03 a	3.36 ± 0.14 a	3.49 ± 0.13 a	105.94 ± 1.06 a						
After WD	54.24 ± 0.38 b	-0.44 ± 0.01 b	5.00 ± 0.10 b	5.02 ± 0.10 b	95.10 ±0.31 b	-4.25 ± 0.86 a, B	0.51 ± 0.01 a, B	1.64 ± 0.14 a, B	1.52 ± 0.14 a, B	-10.78 ± 0.91 a, B	4.58 ± 0.78 a, B (E4)
After WW	56.00 ± 0.13 c	-0.60 ± 0.01 c	3.78 ± 0.06 c	3.83 ± 0.06 c	99.04 ± 0.34 c	-2.51 ± 1.08 b, A'	0.35 ± 0.01 b, B'	0.47 ± 0.11 b, A'	0.33 ± 0.10 b, A'	-6.89 ± 0.80 b, B'	2.58 ± 1.01 b, A' (E3)
WD1:10											
T0	61.61 ± 2.33 a	-0.97 ± 0.08 a	2.65 ± 0.45 a	2.78 ±0.32 a	110.55 ±4.24 a						
After WD	59.26 ± 2.00 a	-0.52 ± 0.07 b	5.18 ± 0.28 b	5.21 ± 0.27 b	95.70 ± 0.84 b	-2.35 ± 0.97 a, B	0.45 ± 0.08 a, B	2.53 ± 0.50 a, C	2.42 ± 0.39 a, C	-14.82 ± 3.65 a, C	3.51 ± 0.89 a, B (E4)
After WW	58.81 ± 1.55 b	-0.54 ±0.04 b	3.35 ± 0.3 c	3.40 ± 0.30 c	99.31 ± 1.29 c	-2.79 ± 1.06 a, A'	0.42 ± 0.11 a, B'	0.70 ± 0.29 b, A'	0.61 ± 0.15 b, B'	-11.24 ± 3.95 a, B'	2.93 ± 0.95 a, A' (E3)

Figure captions

Fig. 1. Lichen devitalization assays in sites A (a-d) and B (e-h). (a) Bricks delimiting a flowerbed in the Botanical Garden of the University of Siena (Italy); (b-e) thallus of *Protoparmeliopsis muralis* (b) before, (c) during and (d) after the WD poultice application; (e) sandstone balustrade of a monumental stairway in the park of Pratolino at Vaglia (Florence, Italy); (f-h) parcels colonized by targeted lichen species (f) before, (g) during and (h) after the poultice application of WD and traditional biocides.

Fig. 2. Dose-response curves of WD at different concentrations (0%; 0.5%; 0.75%; 1%; 5%; 10%) in terms of variation in *P. muralis* maximum quantum efficiency of Photosystem II photochemistry (F_V/F_M) after 4 hours (a) and after 16 hours (b) the treatment. See Table S1 for estimates, and the corresponding estimated standard errors, and possibly lower and upper confidence intervals to find the effective dose (ED) values.

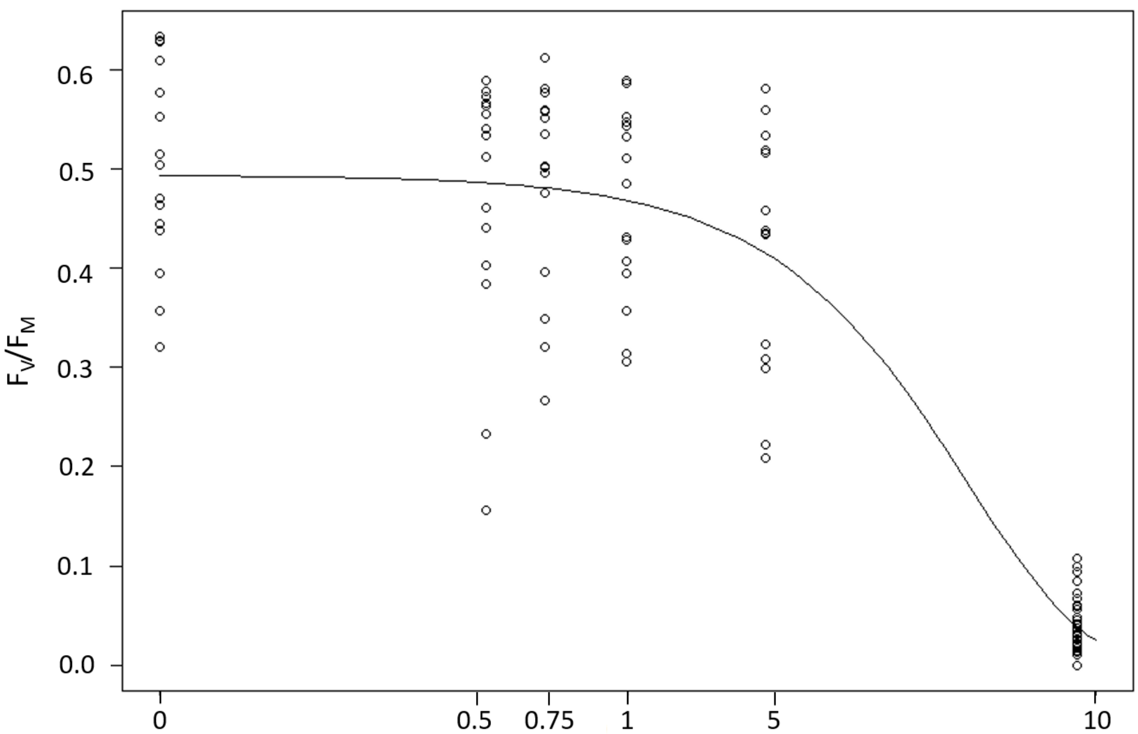
Fig. 3. Maximum quantum efficiency of Photosystem II photochemistry (F_V/F_M) variation in thalli of *B. crenularia*, *C. hoffmanniana*, *P. muralis* and *V. nigrescens* in site B measured 1-day (T1) and 15 (T2) days after the biocides application (Control: blotted water; OIT-DDAC; BAC and WD). See Table S2a for rANOVA results. For each species: lower case letters denote significant differences between biocides and control during each time; individual treatment differences over time were marked *.

Fig. 4. F_0 variations (scaled to the maximum relative to each species) in thalli of *B. crenularia*, *C. hoffmanniana*, *P. muralis* and *V. nigrescens* measured after 1-day (T1) and 15 days (T2) the biocides application in site B (Control: blotted water; OIT-DDAC; BAC and WD). See Table S2b for rANOVA results. For each species: lower case letters denote significant differences between biocides and control during each time; individual treatment differences over time were marked *.

Fig. 5. Photomicrographs in cathodoluminescence (CL) of sandstone (Pietra Serena), with orange luminescence marking the presence and abundance of calcite cement and granules, distinguishable from other mineral phases (feldspars, blue; apatite, green; dolomite, red; quartz, no/poor luminescence). (a-c) Surface of slabs incubated (a) in water (negative control, left) and stirred WD (at concentration 1:10, right); (b) in static WD (at concentration 1:10) and (c) treated with WD (at concentration 1:10) with a cellulose poultice application; (d-g) cross-sectioned profiles of slabs incubated (d) in water, (e) stirred and (f) static WD (at concentration 1:10) and (g) treated with WD (at concentration 1:10) with a cellulose poultice application. Scale bars: 1 mm (a-c), 0.5 mm (d-g).

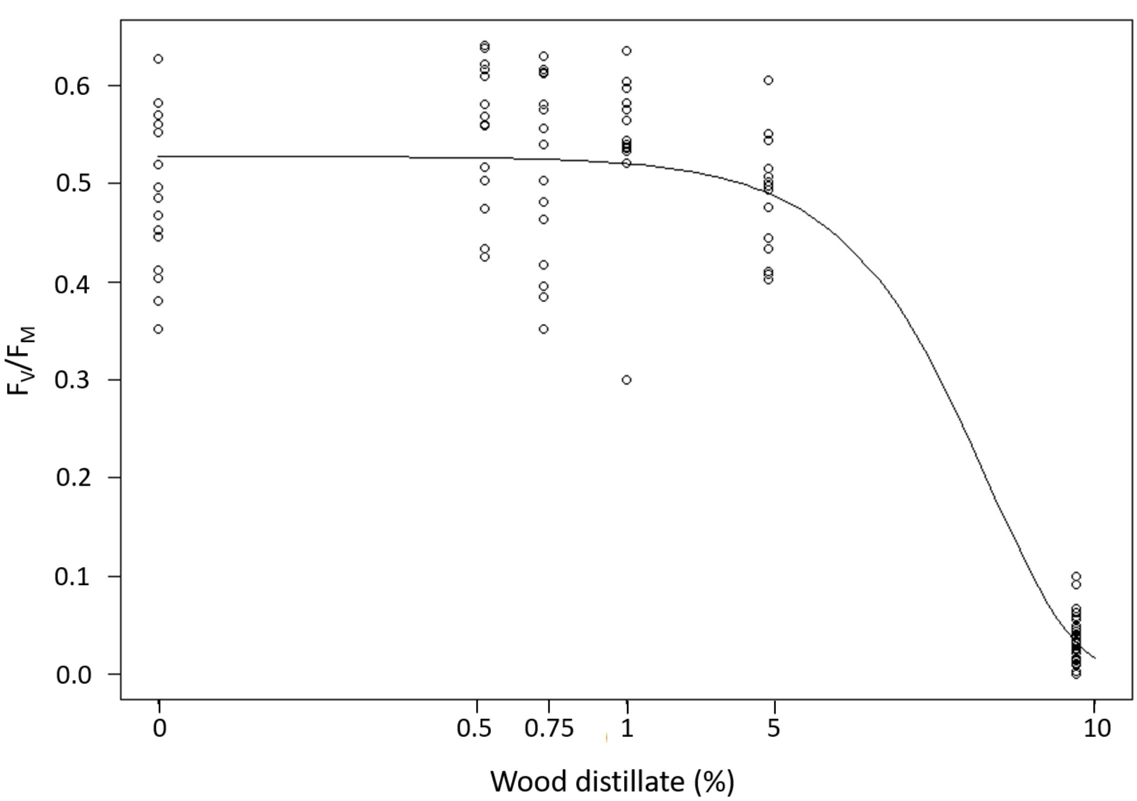

a)
b)
c)
d)
e)
f)
g)
h)

a)

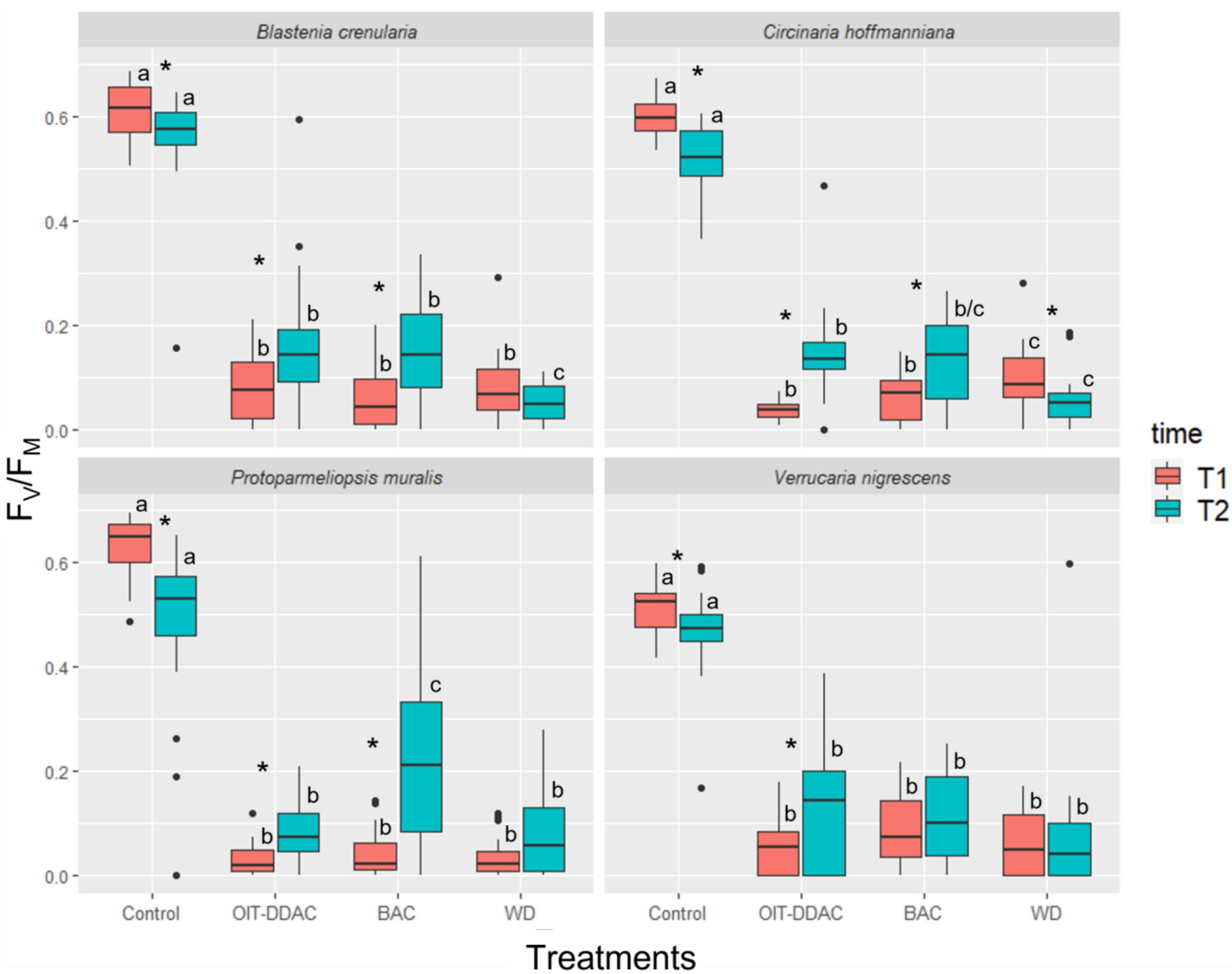


0.6
0.5
0.4
0.3
0.2
0.1
0.0
F_V/F_M
0
0.5
0.75
1
5
10

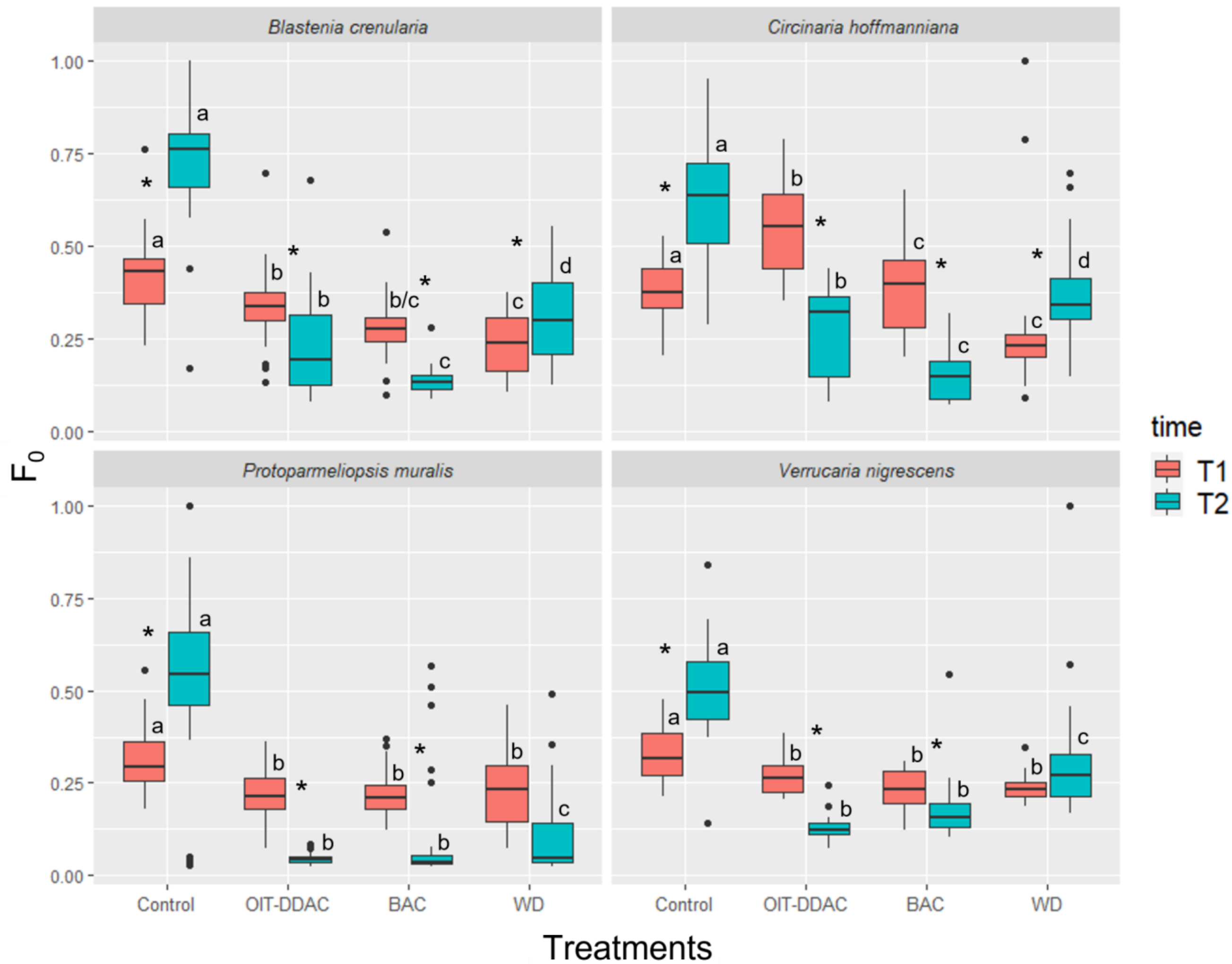
b)

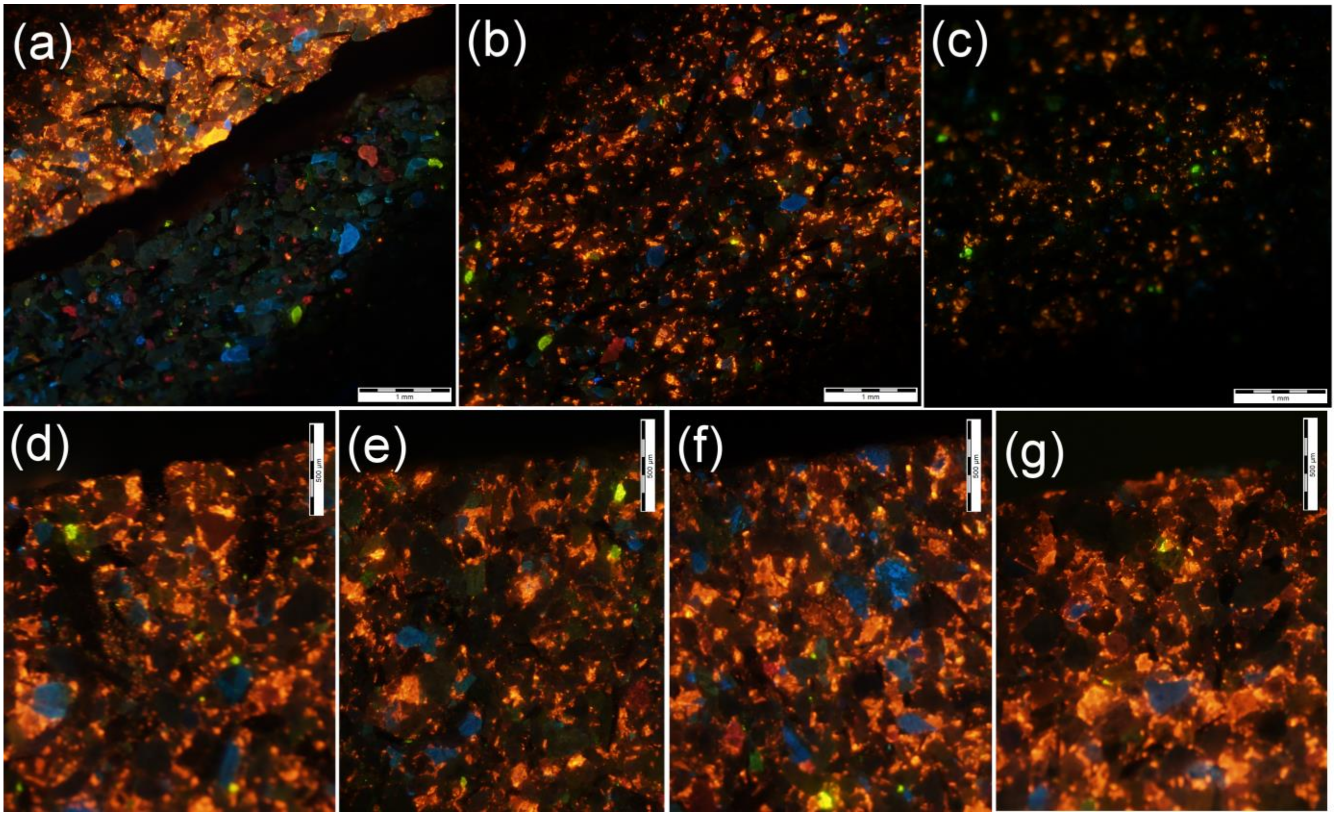


0.6
0.5
0.4
0.3
0.2
0.1
0.0
F_V/F_M
0
0.5
0.75
1
5
10
Wood distillate (%)



Blastenia crenularia
Circinaria hoffmanniana
Protoparmeliopsis muralis
Verrucaria nigrescens
F_V/F_M
0.0
0.2
0.4
0.6
Control
OIT-DDAC
BAC
WD
Treatments
time
T1
T2


Blastenia crenularia
Circinaria hoffmanniana
Protoparmeliopsis muralis
Verrucaria nigrescens
F0
Treatments
Control
OIT-DDAC
BAC
WD
time
T1
T2
0.00
0.25
0.50
0.75
1.00


(a)
(b)
(c)
(d)
(e)
(f)
(g)
1 mm
1 mm
1 mm
500 μm
500 μm
500 μm
500 μm