

Tertiary bioreceptivity of schists from prehistoric rock art sites in the Côa Valley (Portugal) and Siega Verde (Spain) Archaeological Parks: effects of cleaning treatments

P. Sanmartín ^{1*}, E. Fuentes ¹, C. Montojo ², P. Barreiro ³, G. Paz-Bermudez ⁴, B. Prieto ¹

1. Departamento de Edafoloxía e Química Agrícola. Facultade de Farmacia. Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain
2. Escola Superior de Conservación e Restauración de Bens Culturais de Galicia, Pontevedra, Spain
3. Departamento de Física Aplicada, E.T.S.I. Industriales, Universidade de Vigo, Spain
4. Departamento ERNMA. Escola de Enxeñería Forestal. Universidade de Vigo, Spain

*Corresponding author: Patricia Sanmartín.

Departamento de Edafoloxía e Química Agrícola
Facultade de Farmacia. Pavillón A - Soto. Campus Vida
Universidade de Santiago de Compostela
15782 Santiago de Compostela (A Coruña)
Telephone: +34 881814984.
E-mail address: patricia.sanmartin@usc.es

1 **Abstract**

2 Schist weathering induced by the presence of lichen is one of the main concerns in the Côa
3 Valley (northeast Portugal) and Siega Verde (northwest Spain) Archaeological Parks. In this
4 study, different types of lichens, including mainly crustose forms (*Caloplaca pellodella*,
5 *Candelariella vitellina*, *Circinaria hoffmanniana*, *Diploschistes actinostomus* and *Lecidea*
6 *fuscoatra*) as well as some foliose forms (*Parmelina tiliacea* and *Xanthoparmelia conspersa*),
7 were removed from schistose samples of both lithotypes in different locations. The lichens
8 were removed by treatment with Biotin T[®] biocide or by laser treatment with the first (1064
9 nm, IR) and fourth (266 nm, UV) harmonics of a Nd:YAG laser. To assess the effects of the
10 treatments, a recolonization experiment was carried out with biofilm-forming phototrophic
11 microorganisms, and a bioreceptivity index (BI) was calculated for each lithotype and
12 treatment. A water-based treatment (the removal method currently used in both parks) was
13 used as a control cleaning treatment in the bioreceptivity experiment. The study findings show
14 the importance of bioreceptivity studies for evaluating the effectiveness of cleaning
15 treatments. Treatment of the schist samples with the chemical biocide significantly decreased
16 the bioreceptivity (complete inhibition of biofilm formation), whereas laser treatments
17 (especially IR laser) significantly increased the tertiary bioreceptivity. Moreover, the tertiary
18 bioreceptivity of the schists depended on both the treatment applied and the nature of the
19 substrate.

20 **Keywords:** Bioreceptivity index (BI); conservation; lichen removal procedure; non-destructive
21 techniques; colour measurements; chlorophyll fluorescence.

22 **1. Introduction**

23 Lichens play an important role in the biodeterioration and weathering of natural stone
24 (Sanders et al., 1994; Silva et al., 1999; Favero-Longo et al., 2005; Marques et al., 2016) within
25 a relatively short time-scale (Prieto et al., 1997; Seaward, 2004). This is particularly notable in
26 metamorphic rock (relative to igneous rock) because water circulates more easily through the
27 fractures in this type of rock and also because of the clay mineral constituents are of a
28 hygroscopic nature.

29 In the Côa Valley (Vila Nova Foz-Côa, Trás-os-Montes and Alto Douro, Portugal) and Siega
30 Verde (Serranillo- Villar de la Yegua, Salamanca, Spain) Archaeological Parks, lichens are the
31 most conspicuous and important colonizers of the schist rocks (medium-grade metamorphic
32 rock) (Fernandes, 2007; Marques, 2013; Marques et al., 2015) and partly obscure the open-air

33 rock art in these sites. More than two hundred saxicolous and terricolous lichen taxa have
34 been isolated from schist panels in the sites.

35 Both archaeological sites are included in the UNESCO World Heritage List and are home to very
36 large numbers of petroglyphs from the Upper Paleolithic (22,000-10,000 BC). The engravings
37 are unique and considered fine examples of the earliest and most important artistic creations
38 of humankind. Both parks have a series of schist panels on which numerous figures of animal
39 species as well as anthropomorphic representations (e.g. extinct aurochs, goats, deer and
40 mythological hybrid creatures) are engraved (Martinho Baptista, 1999; Luís, 2008). Attempts
41 were made to remove lichen from some of the panels when the archaeological parks were first
42 created in the 1990s. The cleaning processes usually involved careful washing with water and a
43 soft scrubbing brush (Fernandes 2007; Cristina Escudero, pers. comm.; Nicolás Benet, pers.
44 comm.), although in Siega Verde an unspecified chemical product was also used in the early
45 1990s (Nicolás Benet, pers. comm.).

46 In 2014, more innovative and invasive cleaning treatments were tested on schist samples from
47 both parks. The treatments included the use of Biotin T[®] biocide and laser cleaning with the
48 first (1064 nm, IR) and fourth (266 nm, UV) harmonics of a Nd:YAG laser. Biotin T[®] is a broad-
49 spectrum biocide (based on N-octyl isothiazolamine [OIT] and quaternary ammonium salts),
50 which inhibits microbial growth and metabolism and induces irreversible cell damage. It is
51 especially active against cyanobacteria (Sanmartín et al., 2011, 2015) and epilithic lichens (De
52 los Ríos et al., 2012; Vannini et al., 2018). Regarding laser treatments, recent studies have
53 reported IR (1064 nm) and UV (355 and 266 nm) laser irradiation to be particularly suitable for
54 removing lichen colonization (Osticioliet al., 2014; Sanz et al., 2015, 2017; Pena-Poza et al.,
55 2018). IR irradiation at a wavelength of 1064 nm produces ultrastructural changes and
56 metabolic damage in the mycobiont hyphae, which respectively favour the inactivation and
57 extraction of endolithic and epilithic colonizers (Speranza et al., 2013). UV irradiation (either at
58 266 and 355 nm) induces rather superficial, although effective, damage to the lichen thalli
59 (Sanz et al., 2015); the effect is more pronounced at 266 nm than at 355 nm, probably due to
60 the higher absorption at the shorter wavelength (Sanz et al., 2015, 2017).

61 Studies of the effect of cleaning treatments on the rock bioreceptivity (i.e. the potential to host
62 living organisms, Guillitte 1995; Miller et al., 2012) are very scarce, even though this may be a
63 good way of determining the effectiveness of the treatments. Very few studies have
64 determined the potential of stone to be recolonized after conservation treatment (i.e. tertiary
65 bioreceptivity studies). Ariño and Saiz-Jimenez (1996) and Pinna and Salvadori (1999) were
66 among the first authors to report this type of research, in which they studied recolonization by
67 lichen on substrates treated with polyester resin and acrylic resin respectively. Prieto et al.

68 (2014) studied the usefulness of several plastic-based products in inhibiting biological
69 colonization on the exterior walls of granite buildings, showing that these did not inhibit
70 biological activity but increased tertiary bioreceptivity. Similarly, Bracci et al. (2002)
71 demonstrated that sandstone treated with Akeogard CO (a water repellent) tended to retain
72 dust and particles, thus intensifying the establishment and growth of lichen propagules. Jurado
73 and Miller et al. (2014) found that cleaning and restoration of the walls of the church of San
74 Roque (Campeche, Mexico) accelerated the bioreceptivity and biodeterioration of the
75 materials and that new microorganisms recolonized the walls, thus indicating the
76 ineffectiveness of the method used. Coutinho et al. (2016) studied the efficacy of four biocides
77 on photosynthetic-based biofilms attached to glazed wall tiles and reported re-colonization by
78 “new” colonizers 6 months after the treatment.

79 Other studies have shown that different treatments lead to a reduction in recolonization. For
80 instance, De Leo and Urzi (2007) compared the bioreceptivity of two types of mortar treated
81 with water repellents and biocides, concluding that the combination of both products reduced
82 microbial recolonization, unlike when only water-repellent products were used. Radulovic et
83 al. (2013) and Zhang et al. (2013) tested the use of titanium dioxide (TiO₂) nanoparticles
84 improved with silane/siloxane and evaluated the resistance of the nanoparticles to algal
85 colonization, observing a reduction in the bioreceptivity of the treated substrate derived from
86 the photocatalytic degradation capacity of the nanoparticles. Graziani et al. (2013) studied the
87 biocidal effect of TiO₂ applied to bricks under weak UV radiation and found that the treatment
88 did not completely prevented adhesion of microalgae.

89 The above-mentioned studies considered tertiary bioreceptivity by using different laboratory
90 protocols and evaluation methods. More recently, Vázquez-Nion et al. (2018) proposed a
91 bioreceptivity index (BI) for granitic rock and phototrophic organisms; in this index the biomass
92 is estimated via (1) the in vivo fluorescence of chlorophyll *a* and (2) the global colour change
93 that the lithic surface undergoes due to colonization. The experimental protocol used by these
94 authors can be used with other types of granitic rocks and lithic and/or construction materials,
95 and is of interest in the present study.

96 The aim of the present study was to carry out bioreceptivity studies to assess the effect of
97 Biotin T[®] biocide and both the first (1064 nm, IR) and fourth (266 nm, UV) harmonics of a
98 Nd:YAG laser to remove lichen colonization from archaeological schist substrates in the Iberian
99 Peninsula.

100 **2. Materials and methods**

101 **2.1. Schistose samples colonised by lichens**

102 Sample blocks of schists profusely colonized by lichens were obtained from natural outcrops in
103 the Côa Valley (Portugal) and the Siega Verde (Spain) Archaeological Parks. Samples from both
104 sites were petrographically characterized via observation of thin sections (<30 µm thick) under
105 a Carl Zeiss polarized light optical microscope. The characterisation revealed that the rock from
106 the Foz-Côa Park is quartz-mica schist with chlorite mainly composed by quartz (53%), biotite
107 (7%), muscovite-sericite (22%) and chlorites (17%). The rock from the Siega Verde Park is
108 quartz-mica schist with biotite mainly compose by quartz (60%), biotite (10%), muscovite-
109 sericite (21%) and chlorites (8%).

110 The lichen specimens were characterised by traditional methods for lichenized fungi based on
111 examination of morphological features and colour responses to chemical reactions (Smith et
112 al, 2009; Wirth et al., 2013). For morphological characterisation, the thalli and ascomata were
113 examined under a stereomicroscope (Nikon SMZ800), and hand-cut sections mounted in water
114 were also examined in a Nikon Eclipse 50i microscope. The sample blocks were colonized by
115 lichen species belonging to a typical community found on these rocks (Marques, 2013; Paz-
116 Bermúdez et al, 2018), dominated by crustose taxa, mainly *Caloplaca pellodella* (Nyl.) Hasse,
117 *Candelariella vitellina* (Hoffm.) Müll. Arg., *Circinaria hoffmanniana* (S. Ekman & Fröberg) A.
118 Nordin, *Diploschistes actinostomus* (Ach.) Zahlbr., *Lecidea fuscoatra* (L.) Ach., and some foliose
119 species, such as *Parmelina tiliacea* (Hoffm.) Ach., *Xanthoparmelia conspersa* (Ehrh.) Hale and *X.*
120 *gr. pulla* (Ach.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch.

121 **2.2. Removal of lichens from schists**

122 The experience of the professional restorers working in the archaeological parks was taken
123 into account in removing lichens from the schist samples in the laboratory as there is no
124 standardized cleaning protocol. The restorers use methods based on their own experience.

125 The treatments tested included mechanical removal of lichens plus application of (1) Biotin T®
126 biocide in water (3% v/v) (following the recommendations of the commercial supplier) or (2)
127 distilled water and laser cleaning by UV irradiation (wavelength, 266 nm) or IR irradiation
128 (wavelength, 1064 nm). All cleaning treatments were also applied to surfaces not colonized by
129 lichens.

130 To assess the results of the treatments, samples were analysed by optical microscopy (NIKON
131 SMZ800 stereoscope) and PAM fluorometry following the protocol described in section 2.3.3.
132 No lichen remains were observed on any of the samples, and the maximum values of quantum

133 yield (F_V/F_M photosynthetic parameter) were lower than 0.1, representative of dead lichen
134 material (Nayaka et al., 2009).

135 In the bioreceptivity experiments (Section 2.3), water-based cleaning was used as the control
136 treatment, as in previous studies of lichen removal (e.g. Favero-Longo et al., 2017 and Vannini
137 et al., 2018), because water is used to dissolve the biocide and (as mentioned in the
138 introduction) because it is the cleaning method mainly used to date in both parks.

139 Schist samples from each archaeological park were cut into small sample blocks of ca. 4x4x1
140 cm (FiguresS1 and 1.a.) for cleaning and later use in bioreceptivity experiments.

141 2.2.1. Chemical cleaning

142 Mechanical removal combined with biocide application is the most common way of controlling
143 lichens on stone cultural heritage (Favero-Longo et al., 2017). Sometimes, and according to the
144 manufacturer's instructions of the chemical product or/and the restorer/researcher's
145 experience, the biological cover is mechanically removed before the chemical compound is
146 applied (e.g. Caneva et al., 1991; Cámara et al., 2011). In the case of lichens colonising rocks,
147 prior mechanical removal ensures deeper penetration of the biocide to the areas reached by
148 the fungal hyphae and where phototrophic cells occupy endolithic positions. Therefore, before
149 chemical cleaning, the lichen was carefully removed from the stone surface with the aid of a
150 soft brush and, in the case of fruticose thallus (i.e. lichen species belonging to the genus
151 *Ramalina* or *Usnea*) with the aid of a scalpel. Water or Biotin T[®] was subsequently applied with
152 a paintbrush until the entire surface of the stone was thoroughly saturated. Cotton poultices
153 impregnated with biocide or water were then securely attached to the surfaces. The sample
154 blocks were held in trays and covered with dark plastic film, to exclude light and to help retain
155 the humidity at the stone surface. Once the solution had completely evaporated (after six
156 days), the cover and poultices were removed and samples were gently washed with a mixture
157 of water and alcohol (1:1) and brushed with a soft brush.

158 A second complete application of each treatment was required for complete removal lichen
159 thalli (Figure 1.b.).

160 2.2.2. Physical cleaning

161 Laser cleaning of the sample blocks was carried out with a Q-switched Nd: YAG laser (Quanta-
162 Ray INDI-series, Spectra Physics) using the fundamental wavelength of 1064 nm and its fourth
163 harmonic at 266 nm, with a repetition rate of 10 Hz and a pulse width of 6-10 ns. The laser

164 beam was focused using a spherical plane-convex lens with a focal length of 250 mm
165 (NewPort).

166 Infrared (IR) laser cleaning was carried out by irradiation of the samples at a wavelength of
167 1064 nm. Fluence was established in previous experiments that explored the threshold for
168 complete elimination of the lichen. The fluence values varied depending on the type of lichen
169 and the thickness of the biological layer, which were different in the selected sample blocks
170 from both locations. Thus, blocks from Siega Verde were irradiated at a fluence of 23 Jcm⁻² and
171 those from Foz-Côa at a fluence of 49 Jcm⁻² (Figure 1.c.).

172 Ultraviolet (UV) laser cleaning was applied by irradiation of the samples at a wavelength of 266
173 nm. The sample blocks from both locations (Siega Verde and Foz-Côa) were irradiated at a
174 fluence of 0.66 Jcm⁻². This value was established in previous experiments exploring the
175 threshold from which ablation of the rock took place, and the fluence immediately below that
176 threshold value was chosen. In this case, lichens underwent morphological and physiological
177 changes (colour changes and decrease of the maximum quantum yield), but they were not
178 detached from the substrate. For complete lichen removal, the UV laser-radiated stone blocks
179 were also gently brushed (Figure 1.c.).

180 **2.3. Bioreceptivity experiments**

181 In order to analyse the effect of the cleaning treatments on the susceptibility of the rock to be
182 recolonized, five sample blocks from each park and cleaned using each treatment (5 samples x
183 4 treatment: 20 Foz-Côa samples; and 5 samples x 4 treatment: 20 Siega Verde samples) were
184 inoculated with a multispecies phototrophic culture. Biofilm development on the sample
185 blocks was then monitored every 14 days for 42 days by using non-destructive techniques.
186 Finally, the bioreceptivity index (BI) of each sample was calculated following the method
187 proposed by Vázquez-Nion et al. (2018).

188 **2.3.1. Inoculation**

189 Cleaned samples were inoculated with a multispecies phototrophic culture derived from a
190 biofilm growing on the San Martín Pinario Monastery in Santiago de Compostela (NW Spain).
191 The culture was grown in BG-11 liquid medium (Rippka et al., 1979). The use of a complex
192 multispecies culture ensures simulation of the natural competition and synergies existing
193 between the different microorganisms (Foster and Bell, 2012). The culture mainly comprised
194 chlorophytes (*Bracteacoccus minor* (Schmidle ex Chodat) Petrová, *Stichococcus bacillaris* Nägeli
195 and *Chlorella* sp.) and cyanobacteria (*Aphanocapsa* sp., *Leptolyngbya cebennensis* (Gomont)

196 and *Isochrysis* sp.). This culture has previously been described in detail (culture C5)(Vázquez-
197 Nion et al., 2016).

198 The optical density (OD) of cells during the exponential growth phase was measured at a
199 wavelength of 750 nm (OD₇₅₀) in a spectrophotometer (UV-Vis Varian Cary 100). The biomass
200 of the inoculant was determined (see e.g. Lu et al., 2017; Sanmartín et al., 2017; Gambino and
201 Sanmartín et al., 2019) by constructing a calibration curve of OD against known quantities of
202 dry weight (g L⁻¹), which yielded the following relationship:

203 $\text{Biomass (g L}^{-1}\text{)} = 0.3521 * \text{OD}_{750} - 0.0063, R^2 = 0.99 \text{ (OD}_{750} < 1 \text{ AU)}$

204 Biofilms were developed by inoculating 1 mL of the culture (equivalent to depositing a dry
205 weight biomass of 25.2 µg cm⁻²) on to the upper surface of each cleaned stone sample block.
206 The inoculated samples were maintained permanently in contact with water by placing them
207 in dishes periodically filled with sterilized water. To promote biofilm development (Vázquez-
208 Nion et al., 2017), the sample blocks were held in an INCUDIGIT (Group Selecta) incubator for 6
209 weeks (42 days) at a temperature ranging between 22°C and 27°C, relative humidity of 95%
210 and with 12h light (~25 µmol photon m⁻² s⁻¹)/12h darkness cycles.

211 2.3.2. Monitoring biofilm growth by colour measurements

212 Colour measurement has been demonstrated to be a reliable non-destructive method for
213 monitoring biofilm growth and also for estimating the biomass of phototrophic
214 microorganisms on lithic surfaces (see e.g. Prieto et al., 2004; Sanmartín et al., 2012).

215 A portable spectrophotometer (Konica Minolta CM-700d) equipped with CM-S100w software
216 (Spectra Magic TM NX) was used for instrumental colour measurements, under the following
217 conditions: D65 illuminant, 2° observer and a target area of 8 mm diameter. Fourteen
218 measurements were made at random on the humid surface of each sample, according to the
219 protocols developed by Prieto and colleagues (Prieto et al., 2010a, 2010b).

220 The data were analysed using the CIELAB colour system (CIE 1986), in which each colour is
221 defined by three Cartesian or scalar coordinates: the L* parameter represents the lightness,
222 ranging from 0 (absolute black) to 100 (absolute white); a* represents the chromatic variations
223 from red to green (negative values indicate green while positive values indicate red); and b*
224 represents the chromatic variations from yellow to blue (negative values indicate blue while
225 positive values indicate yellow). To analyse the colour variations over time, the total colour
226 difference (ΔE^*_{ab}) was calculated as follows:

227
$$\Delta E^*_{ab} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$$

228 where

229 $\Delta L^* = L^*_i - L^*_0$; $\Delta a^* = a^*_i - a^*_0$; $\Delta b^* = b^*_i - b^*_0$, the subscript *i* denotes the colour parameter at
230 14, 28 and 42 days, and the subscript 0 denotes the colour parameter at the beginning of
231 experiment, before inoculation.

232 2.3.3. Monitoring biofilm growth by PAM fluorometry

233 Pulse amplitude modulation (PAM) fluorometry was used to evaluate biofilm growth on the
234 sample blocks throughout the 42-day incubation period, complementing the colour
235 measurements (Section 2.3.2). Fluorescence signals were measured every two weeks (14th-,
236 28th- and 42nd-day) using a Phyto-PAM system (Heinz Walz GmbH) equipped with a Phyto-
237 EDF fiber optics emitter-detector unit, which allows measurement on surfaces via a perspex
238 rod (length 50 mm and diameter 4 mm) (Schrieber et al., 2002; Vázquez et al., 2018). The
239 system measures *in vivo* fluorescence at four wavelengths (470 nm, 520 nm, 645 nm and 665
240 nm).

241 The schist sample blocks with the developing biofilms were maintained in darkness for 20-30
242 minutes and hydrated prior to the measurements. A total of 9 readings were taken at
243 randomly selected zones on each inoculated surface of each block, by placing the tip of the
244 measurement rod directly in contact with the surface (Vázquez et al., 2018). The following
245 fluorescence parameters were recorded at 665 nm: F_0 , the minimal fluorescence signal of dark-
246 adapted cells, and F_m , the maximal fluorescence signal after a saturating light pulse in dark-
247 adapted cells. These parameters were used to estimate the maximum quantum yield $Y =$
248 $F_v/F_m = (F_m - F_0)/F_m$, an indicator of the overall viability of the photosynthetic organisms. F_0 is
249 related to the amount of chlorophyll *a*, and F_0 values were expressed as $\mu\text{g chlorophyll } a \text{ cm}^{-2}$
250 by use of the calibration curve developed by Vázquez-Nion et al. (2018) for culture C5 (Section
251 2.3.1).

252 The signal at 470 nm is also related to chlorophyll *b* and the signal at 645 nm to
253 phycobiliprotein allophycocyanin; therefore, the F_0 470 nm/ F_0 645 nm ratio is used as an
254 indicator of the dominance of green algae (giving rise high values) or cyanobacteria (giving rise
255 to low values) in microbial ecology (Schrieber et al., 2002; Vázquez et al., 2018).

256 2.3.4. Relative bioreceptivity index

257 To compare the effect of the cleaning treatments on the bioreceptivity, a bioreceptivity index
258 (BI) was assigned to each cleaned rock sample following the method developed by Vazquez-
259 Nion et al. (2018). Thus, the BI was calculated as $BI = (2BI_{\text{growth}} + BI_{\text{colour}}) / 3$, where

260 $BI_{\text{growth}} = 10 (\text{chl } a \text{ } \mu\text{g.cm}^{-2} / \text{max chl } a \text{ } \mu\text{g.cm}^{-2})$

261 $BI_{\text{colour}} = 10 (\Delta E^*_{\text{ab}} / \text{max } \Delta E^*_{\text{ab}})$

262 Max chl a and max ΔE^*_{ab} are maximum values of chlorophyll a and total colour difference for
263 each lithotype. Thus, the BI obtained is a relative BI that only allows comparison between
264 treatments for each lithotype in the context of the present study.

265 **2.4. Statistical analysis**

266 The chlorophyll fluorescence data (F_0 and maximum quantum yield (Y) at 665 nm) obtained at
267 the end of the experiment were analysed using SPSS software statistics, version 22.0, to
268 compare the effectiveness of each cleaning treatment on biofilm growth. Analysis of variance
269 (ANOVA) was performed separately for each lithotype, and a post hoc Tukey b test was
270 applied. Differences were considered significant at $p \leq 0.05$.

271 **3. Results and discussion**

272 All cleaning treatments successfully eliminated lichen patina, as indicated by the complete
273 removal of the lichen thalli (Figure 1). However, gentle brushing was needed after treatment
274 application to completely remove all of the lichen material. Because of the tendency of schist
275 to split along a plane parallel to the arrangement of the constituent minerals, mechanical
276 brushing sometimes led to loss of material. Moreover, IR laser treatment caused substantial
277 damage to the schistose surfaces, in the form of visible streaks that increased the surface
278 roughness (Figure 1.c.). These aspects should be taken into account when planning removal of
279 biological crusts from cultural heritage monuments made from schist.

280 The bioreceptivity experiment revealed differences in the growth of the biofilm in relation to
281 the treatment applied. Forty-two days after inoculation, biofilm growth was greatly reduced in
282 the biocide-treated samples, while a well-developed green biofilm was observed on the laser-
283 treated samples (especially those treated by IR laser); biofilm growth was intermediate in the
284 control sample, cleaned with water (Figure 2).

285 Figure 3.a. shows the variations in colour of the treated surfaces 14, 28 and 42 days after
286 inoculation with the culture, expressed as variations in ΔE^*_{ab} (total colour difference) and Δa^*
287 (associated with changes in greenness). The total colour variation, ΔE^*_{ab} , has been widely used

288 for monitoring biofilm-forming microorganisms as the quantity of phototrophic organisms on a
289 surface is directly related to the colour generated (Prieto et al., 2002).

290 In relation to total colour variation, for each lithotype, samples cleaned with biocide yielded
291 the lowest values, while samples cleaned with IR laser yielded the highest values. The ΔE^*_{ab}
292 values were similar for water and UV laser treatments.

293 There was a large difference in ΔE^*_{ab} values between lithotypes. In the cleaned samples from
294 the Siega Verde Park, except those cleaned with biocide, the ΔE^*_{ab} was higher than 3 CIELAB
295 units (units of the CIELAB colour space, see e.g. CIE, 1986), considered the upper limit of
296 rigorous colour tolerance or perceptible change in colour (Wyszecki and Stiles, 1982; Prieto et
297 al., 2010a, 2010b). By contrast, the values for cleaned samples from Foz-Côa, except those
298 cleaned with IR laser, were all below this threshold. Nevertheless, the variation in total colour
299 (ΔE^*_{ab}) at the end of the incubation period was not higher than 7 CIELAB units in either case:
300 this is not very high relative to those obtained in previous bioreceptivity experiments in Foz-
301 Côa schists. In a study addressing the primary bioreceptivity (initial potential of biological
302 colonization of sound stone) and secondary bioreceptivity (potential of biological colonization
303 of weathered stone) of schists from the Portuguese archaeological park, Marques et al. (2015)
304 obtained ΔE^*_{ab} values of between 9.87 and 12.50 CIELAB units 30 days after inoculation of the
305 rocks.

306 The Δa^* parameter is associated with changes in redness/greenness and is closely related to
307 concentrations of phototrophic pigments, including chlorophyll *a*, phycocyanin and carotenoid
308 (Sanmartín et al, 2010). Greening occurred in all samples except those cleaned with biocide
309 and those from Siega Verde cleaned with UV, as indicated by the lower a^* value 42 days after
310 inoculation relative to the value prior to inoculation (i.e. negatives values of Δa^*) (Figure
311 3.b.).The difference between Δa^* values at 14 days and Δa^* values at 42 days was very small,
312 and in all cases the Δa^* value was lowest after 14 days. Thus, no further greening occurred
313 after 14 days. Similar results were obtained by Marques et al. (2015) in the above-mentioned
314 bioreceptivity study of Foz-Côa schist. These authors reported lower a^* values in samples after
315 inoculation than before inoculation but very similar values for parameter a^* 30, 60, 90 and 120
316 days after inoculation of the rocks.

317 Although the a^* parameter indicated that in both cases (Foz-Côa and Siega Verde) the biofilm
318 was established and developed quickly, the Δa^* values were not as negative as reported in
319 other similar studies evaluating the effects of biocides. In studies characterizing the variations
320 in the Δa^* parameter representing the growth of *Chlorella vulgaris* on limestone samples

321 treated with different biocide products, Eyssautier-Chuine et al. (2015, 2016) reported
322 variations in Δa^* of between 1.1 and 10.6 CIELAB units, depending on the treatment, four
323 weeks after inoculation of the rock samples with the culture.

324 Results of monitoring the fluorescence parameters F_0 and Yield (F_v/F_m) at 665 nm throughout
325 the experiment are shown in Figure 4.

326 The minimal fluorescence signal (F_0) of dark-adapted cells can be used as a biomass estimator
327 for the culture used as inoculum in this study, as it is correlated with the chlorophyll *a* content
328 (Vázquez-Nion et al., 2018). The signal was similar in both lithotypes. No signal was detected in
329 samples cleaned with biocides, while the strongest signal was obtained in samples cleaned by
330 IR laser treatment. F_0 values for water and IR laser were similar in Foz-Côa samples, but a very
331 weak signal was obtained in Siega Verde samples cleaned by UV laser treatment. As in ΔE^*_{ab}
332 and Δa^* , there was little difference between the values obtained on days 14 and 42 after
333 inoculation with the culture.

334 The maximum quantum yield (Y), which provides a measure of the maximum photochemical
335 efficiency of photosystem II, can be used as indicator of the overall viability of photosynthetic
336 organisms. The observed trends in Y were similar to those observed for the other parameters
337 monitored: the largest values were obtained for IR laser cleaning and the lowest (zero in this
338 case) for biocide application. Differences between lithotypes were only observe in samples
339 cleaned with UV laser. However, the value of this parameter increased from 14 to 42 days in all
340 Foz-Côa samples and in Siega Verde samples cleaned only with water.

341 Considering the water-based treatment as the minimal cleaning treatment that can be used to
342 remove microbial colonization, statistical comparison of F_0 and Yield values at the end of the
343 experiment (Table 1) showed significant differences in biomass (F_0 values) between samples
344 from both Foz-Côa and Siega Verde cleaned with water and those cleaned by the other three
345 treatments. However, while biocide cleaning caused a decrease in the amount of biomass,
346 relative to the water-based treatment, the IR laser treatment led to an increase in the same
347 parameter. Nevertheless, the amount of biomass on the samples cleaned with UV laser
348 depended on the lithotype and was higher in the Foz-Côa than in Siega Verde samples.

349 The overall viability of the already developed biofilms was higher in the Foz-Côa samples
350 treated with both types of laser than in the samples treated only with water. However, for
351 Siega Verde samples, the viability of the biofilms on the blocks treated with IR laser and those

352 cleaned with water were similar, while that of biofilms on sample blocks treated with UV was
353 lower.

354 The $F_0(470\text{nm})/F_0(645\text{nm})$ ratio was very similar in all samples except those from Siega Verde
355 cleaned with UV laser, in which the lower index indicated greater development of
356 cyanobacteria.

357 The bioreceptivity index was calculated for each lithotype and cleaning treatment (Figure 5).
358 Although BI values are not absolute values, they can be used in the context of this study for
359 purposes of comparison. The BI values were higher in the Siega Verde samples than in the Foz-
360 Côa samples, independently of the cleaning method. Thus, the primary bioreceptivity of the
361 Siega Verde lithotype is either higher than that of the Foz-Côa lithotype, or the Siega Verde
362 lithotype is more affected by the manipulation (i.e. more negatively affected by cleaning
363 treatments) than the Foz-Côa lithotype; however, both options are possible.

364 Relative to the control water-based treatment, which is theoretically the most innocuous of
365 the treatments tested, the application of Biotin T[®] greatly reduced the bioreceptivity by
366 preventing further biofilm formation. The effect of Biotin T[®] on recolonization was also
367 evaluated by Vannini et al. (2018), who observed no physiological recovery of the lichen
368 *Xanthoria parietina* 90 days after application of Preventol RI80 and Biotin T[®].

369 The increased bioreceptivity observed in the laser-treated samples may be due to the physical
370 damage (streaks) generated by the laser on the stone surfaces. IR laser irradiation causes
371 greater thermal interaction between the laser and the stone, while the action of UV is of a
372 photochemical nature, so that less of the surface is affected than with the IR laser treatment
373 (Gemedá et al., 2018). The laser treatment created ridges and grooves in the stone (Figure
374 1.c.). This may be the main cause of the increase in bioreceptivity in the laser cleaned samples
375 as the treatment creates a rougher surface, which facilitates adherence of microorganisms.

376 **4. Conclusions**

377 The study findings showed the importance of bioreceptivity studies for evaluating the
378 effectiveness of cleaning treatments. Treatment with the chemical biocide significantly
379 decreased the bioreceptivity (complete inhibition of biofilm formation), whereas laser
380 treatments significantly increased the tertiary bioreceptivity, especially the IR laser treatment,
381 which caused substantial damage to the schistose surface in the form of streaks and a
382 consequent increase in surface roughness. If the effectiveness of the cleaning treatment is only
383 evaluated by considering the degree of cleaning on the surface, all of the treatments tested

384 would be valid, except the IR irradiation at 1064 nm. However, the findings of this
385 bioreceptivity study demonstrated that cleaning with the biocide was the most effective
386 treatment. Moreover, the tertiary bioreceptivity of schists was found to depend on both the
387 treatment applied and the nature of the substrate. In addition, the bioreceptivity index (BI),
388 previously only used with granite rocks, was successfully applied to schistose rocks.

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396 **References**

397

398 Ariño, X., Saiz-Jimenez, C., 1996. Biological diversity and cultural heritage. *Aerobiologia* 12(4),
399 279-282.

400

401 Bracci, S., Melo, M.J., Tiano, P., 2002. Comparative study on durability of different treatments
402 on sandstone after exposure in natural environment. *The Silicates in Conservative Treatments.*
403 *Tests, Improvements and Evaluation of Consolidating Performance. Proceedings of the*
404 *International Congress. Torino: Fondazione per le Biotecnologie and Associazione Villa*
405 *dell'Arte, pp. 129-35.*

406

407 Cámara, B., de los Ríos, A., Urizal, M., Alvarez de Buergo, M., Varas, M.J., Fort, R., Ascaso, C.
408 2011. Characterizing the microbial colonization of a dolostone quarry: implications for stone
409 biodeterioration and response to biocide treatments. *Microbial Ecology* 62: 299-313.

410

411 Caneva, G., Nugri, M.P., Salvadori, O. 1991. *Biology in the Conservation of Works of Art.*
412 *Rome:ICCROM.*

413

414 CIE, 1986. Publication 15-2: Colorimetry. CIE Central Bureau, Vienna.

415

416 Coutinho, M.L., Miller, A.Z., Rogerio-Candelera, M.A., Mirao, J., Cerqueira Alves, L., Veiga, J.P.,
417 Aguas, H., Pereira, S., Lyubchik, A., Macedo, M.F., 2016. An integrated approach for assessing
418 the bioreceptivity of glazed tiles to phototrophic microorganisms. *Biofouling* 32, 243-259.

419

420 De los Ríos, A., Pérez-Ortega, S., Wierzchos, J., Ascaso, C., 2012. Differential effects of biocide
421 treatments on saxicolous communities: Case study of the Segovia cathedral cloister (Spain).
422 *International Biodeterioration and Biodegradation* 67, 64-72.

423

424 De Leo, F., Urzi C., 2007. Evaluation of the efficiency of water-repellent and biocide
425 compounds against microbial colonization of mortars. *International Biodeterioration and*
426 *Biodegradation*, 60, 25-34.

427

428 Eyssautier-Chuine, S., Vaillant-Gaveau, N., Gommeaux, M., Thomachot-Schneider, C., Pleck, J.,
429 Fronteau, G., 2015. Efficacy of different chemical mixtures against green algal growth on
430 limestone: a case study with *Chlorella vulgaris*. *International Biodeterioration and*
431 *Biodegradation* 103, 59-68.

432

433 Eyssautier-Chuine, S., Vaillant-Gaveau, N., Gommeaux, M., Thomachot-Schneider, C., Pleck, J.,
434 Fronteau, G., 2016. Chlorophyll Fluorescence and Colorimetric Analysis for Monitoring the
435 Algal Development on Biocide-Treated Stone. *Open Conference Proceedings Journal* 7(1), 55-
436 64.

437

438 Favero-Longo, S.E., Castelli, D., Salvadori, O., Belluso, E., Piervittori, R., 2005. Pedogenetic
439 action of the lichens *Lecidea atrobrunnea*, *Rhizocarpon geographicum* gr. and *Sporastatia*
440 *testudinea* on serpentinized ultramafic rocks in an alpine environment. International
441 Biodeterioration and Biodegradation 56(1), 17-27.

442

443 Favero-Longo, S.E., Benesperi, R., Bertuzzi, S., Bianchi, E., Buffa, G., Giordani, P., Loppi, S.,
444 Malaspina, P., Matteucci, E., Paoli, L., Ravera, S., Roccardi, A., Segimiro, A., Vannini, A. 2017.
445 Species- and site-specific efficacy of commercial biocides and application solvents against
446 lichens. International Biodeterioration and Biodegradation 123, 127-137.

447

448 Fernandes, A.M.B., 2007. The Conservation Programme of the Côa Valley Archaeological Park:
449 Philosophy, Objectives and Action.

450

451 Foster, K.R., Bell, T., 2012. Competition, not cooperation, dominates interactions among
452 culturable microbial species. Current Biology 22: 1845-1850.

453

454 Gambino, M., Sanmartín, P., Longoni, M., Villa, F., Mitchell, R., Cappitelli, F., 2019. Surface
455 colour: an overlooked aspect in the study of cyanobacterial biofilm formation. Science of the
456 Total Environment 659: 342-353.

457

458 Gameda, B.T., Lahoz, R., Caldeira, A.T., Schiavon, N., 2018. Efficacy of laser cleaning in the
459 removal of biological patina on the volcanic scoria of the rock-hewn churches of Lalibela,
460 Ethiopia. Environmental Earth Sciences 77(2), 36.

461

462 Graziani, L., Quagliarini, E., Osimani, A., Aquilanti, L., Clementi, F., Yéprémian, C., Lariccia, V.,
463 Amoroso, S., D'Orazio, M., 2013. Evaluation of inhibitory effect of TiO₂ nanocoatings against

464 microalgal growth on clay brick façades under weak UV exposure conditions. *Building and*
465 *Environment* 64, 38-45.

466

467 Guillitte, O., 1995. Bioreceptivity: a new concept for building ecology studies. *Science of the*
468 *Total Environment* 167(1-3), 215-220.

469

470 Jurado, V., Miller, A., Cuezva, S., Fernandez-Cortes, A., Benavente, D., Rogerio-Candelera, M.,
471 Reyes, J., Cañaveras, J., Sanchez-Moral, S., Saiz-Jimenez, C., 2014. Recolonization of mortars by
472 endolithic organisms on the walls of San Roque church in Campeche (Mexico): A case of
473 tertiary bioreceptivity. *Construction and Building Materials*, 53, 348-359.

474

475 Lu, L., Yang, G., Zhu, B., Pan, K., 2017. A comparative study on three quantitating methods of
476 microalgal biomass. *Indian Journal of Geo-Marine Sciences* 46(11), 2265-2272.

477

478 Luís, L., 2008. Em busca dos cavaleiros com cabeça de pássaro: Perspectivas de investigação da
479 Proto-história no Vale do Côa (In Portuguese). In: Balbín Behrmann R. (ed.) *Arte al aire libre en*
480 *el Sur de Europa*. Junta de Castilla y León, Salamanca, pp. 415-38.

481

482 Marques, J., 2013. A framework for assessing the vulnerability of exposed schist surfaces to
483 lichen induced weathering in the Upper Douro region (NE Portugal). *Doctoral Thesis*

484

485 Marques, J., Vázquez-Nion, D., Paz-Bermúdez, G., Prieto, B., 2015. The susceptibility of
486 weathered versus unweathered schist to biological colonization in the Côa Valley
487 Archaeological Park (north-east Portugal). *Environmental Microbiology* 17, 1805-1816.

488

489 Marques, J., Gonçalves, J., Oliveira, C., Favero-Longo, S.E., Paz-Bermúdez, G., Almeida, R.,
490 Prieto, B., 2016. On the dual nature of lichen-induced rock surface weathering in contrasting
491 micro-environments. *Ecology* 97(10), 2844–2857.
492
493 Martinho Baptista, A., 1999. No tempo sem tempo. A arte dos caçadores paleolíticos do Vale
494 do Côa(In Portuguese). Parque Arqueológico Vale do Côa (ed.).
495
496 Miller, A.Z., Sanmartín, P., Pereira-Pardo, L., Saiz-Jimenez, C., Dionísio, A., Macedo, M.F.,
497 Prieto, B., 2012. Bioreceptivity of building stones: a review. *Science of the Total Environment*
498 426, 1–12.
499
500 Nayaka, S., Ranjan, S., Saxena, P., Pathre, U.V., Upreti, D.K. and Singh, R. (2009). Assesing the
501 vitality of Himalayan lichens by measuring their photosyntethic performances using chlorophyll
502 fluorescence technique. *Current Science* 97 (4): 538-545.
503
504 Osticioli, I., Mascalchi, M., Pinna, D., Siano, S., 2014. Removal of *Verrucaria nigrescens* from
505 Carrara marble artefacts using Nd:YAG lasers: comparison among different pulse durations and
506 wavelengths. *Applied Physics A. Materials Science & Processing* 118, 1517-1526.
507
508 Paz-Bermúdez, G., Carballal, R., Marques, J., López de Silanes M.E., 2018. Catálogo de los
509 líquenes saxícolas (*Ascomycota*) del área arqueológica de Siega Verde (Salamanca, España).
510 *Anales del Jardín Botánico de Madrid* 75 (2): e076 (In Spanish).
511
512 Pena-Poza, J., Ascaso, C., Sanz, M., Pérez-Ortega, S., Oujja, M., Wierzchos, J., Souza-Egipsy, V.,
513 Cañamares, M.V., Urizal, M., Castillejo, M., García-Heras, M., 2018. Effect of biological

514 colonization on ceramic roofing tiles by lichens and a combined laser and biocide procedure
515 for its removal. *International Biodeterioration and Biodegradation* 126, 86–94.

516

517 Pinna, D., Salvadori, O., 1999. Biological Growth on Italian Monuments Restored with Organic
518 or Carbonatic Compounds. In: *Proceedings of the International Conference on Microbiology
519 and Conservation ICMC' 99; Florence, Italy*, pp. 149-54.

520

521 Prieto, B., Silva, B., Rivas, T., Wierzchos, J., Ascaso, C., 1997. Mineralogical transformation and
522 neoformation in granite caused by the Lichens *Tephromela atra* and *Ochrolechia parella*.
523 *International Biodeterioration and Biodegradation* 40, 191-199.

524

525 Prieto, B., Rivas, T., Silva, B., 2002. Rapid quantification of phototrophic microorganisms and
526 their physiological state through their colour. *Biofouling* 18, 229-236.

527

528 Prieto, B., Silva, B., Lantes, O., 2004. Biofilm quantification on stone surfaces: comparison of
529 various methods. *Science of the Total Environment* 333, 1-7.

530

531 Prieto, B., Sanmartín, P., Silva, B., Martínez-Verdú, F., 2010a. Measuring de the color of granite
532 rocks. A proposed procedure. *Color Research and Application* 35:368–375.

533

534 Prieto, B., Sanmartín, P., Aira, N., Silva, B., 2010b. Color of cyanobacteria: some methodological
535 aspects. *Applied Optics* 49, 2022–2029.

536

537 Prieto, B., Sanmartín, P., Silva, C., Vázquez-Nion, D., Silva, B., 2014. Deleterious effect plastic-
538 based biocides on back-ventilated granite facades. *International Biodeterioration and
539 Biodegradation* 86, 19-24.

540

541 Radulovic, J., MacMullen, J., Zhang, Z., Dhakal, H., Hannant, S., Daniels, L., Elford, J.,
542 Herodotou, C., Totomis, M. y Bennett, N., 2013. Biofouling resistance and practical constraints
543 of titanium dioxide nanoparticulatesilane/siloxane exterior facade treatments. Building and
544 Environment, 68, 150-158.

545

546 Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., Stanier, R.Y., 1979. Generic assign-
547 ments, strain histories and properties of pure cultures of cyanobacteria. Journal of General
548 Microbiology 111, 1-61.

549

550 Sanders W.B., Ascaso C., Wierzchos J., 1994. Physical interactions of two rhizomorph-forming
551 lichens with their rock substrate. Botanica Acta 107(6), 432-439.

552

553 Sanmartín, P., Aira, N., Devesa-Rey, R., Silva, B., Prieto, B., 2010. Relationship between color
554 and pigment production in two stone biofilm-forming cyanobacteria (*Nostoc* sp PCC 9104 and
555 *Nostoc* sp PCC 9025). Biofouling 26, 499-509.

556

557 Sanmartín, P., Villa, F., Silva, B., Cappitelli, F, Prieto, B., 2011. Color measurements as a reliable
558 method for estimating chlorophyll degradation to phaeopigments. Biodegradation 22, 763-
559 771.

560

561 Sanmartin, P., Vazquez-Nion, D., Silva, B., Prieto, B., 2012. Spectrophotometric color
562 measurement for early detection and monitoring of greening on granite buildings. Biofouling
563 28, 329-338.

564

565 Sanmartín, P., Villa, F., Polo, A., Silva, B., Prieto, B., Cappitelli, F., 2015. Rapid evaluation of
566 three biocide treatments against the cyanobacterium *Nostoc* sp. PCC 9104 by color changes
567 *Annals of Microbiology* 65, 1153-1158.
568

569 Sanmartín, P., Vázquez-Nion, D., Arines, J., Cabo-Domínguez, L., Prieto, B., 2017. Controlling
570 growth and colour of phototrophs by using simple and inexpensive coloured lighting: A
571 preliminary study in the Light4Heritage project towards future strategies for outdoor
572 illumination. *International Biodeterioration & Biodegradation* 122: 107-115.
573

574 Sanz, M., Oujja, M., Ascaso, C., de los Ríos, A., Pérez-Ortega, S., Souza-Egipsy, V., Wierzchos, J.,
575 Speranza, M., Cañamares, M.V., Castillejo, M., 2015. Infrared and ultraviolet laser removal of
576 crustose lichens on dolomite heritage stone. *Applied Surface Science* 346, 248–255.
577

578 Sanz, M., Oujja, M., Ascaso, C., Pérez-Ortega, S., Souza-Egipsy, V., Fort, R., de los Ríos, A.,
579 Wierzchos, J., Cañamares, M.V., Castillejo, M., 2017. Influence of wavelength on the laser
580 removal of lichens colonizing heritage stone. *Applied Surface Science* 399, 758–768.
581

582 Schreiber, U., Gademann, R., Bird, P., Ralph, P.J., Larkum, A.W.D., Kuhl, M., 2002. Apparent
583 light requirement for activation of photosynthesis upon rehydration of desiccated beachrock
584 microbial mats. *Journal of Phycology* 38, 125–134.
585

586 Seaward, M.R.D., 2004. Lichens as subversive agents of biodeterioration. L.L. St. Clair, M.R.D.
587 Seaward (Eds.), *Lichens and biofilms as weathering agents of rocks and cultural heritage*,
588 *Biodeterioration of stone surfaces*, Kluwer Academic Press, Dordrecht, pp. 9-18.
589

590 Silva B, Rivas T, Prieto B., 1999. Effects of lichens on the geochemical weathering of granitic
591 rocks. *Chemosphere* 39, 379–388.
592

593 Smith, C. W., Aptroot, A., Coppins, B.J., Fletcher, A., Gilbert, O.L., James, P.W., Wolseley, P.A.,
594 (Eds.) 2009. *The lichens of Great Britain and Ireland*. –London: British Lichen Society. British
595 Lichen Society; 2nd New edition edition.
596

597 Speranza, M., Sanz, M., Oujia, M., de los Rios, A., Wiezchos, J., Pérez-Ortega, S., Catillejo, M.,
598 Ascaso, C., 2013. Nd-YAG laser irradiation damages to *Verrucaria nigrescens*. *International*
599 *Biodeterioration and Biodegradation* 84, 281-290.
600

601 Vannini, A., Contardo, T., Paoli, L., Scattoni, M., Favero-Longo, S.E., Loppi, S., 2018. Application
602 of commercial biocides to lichens: Does a physiological recovery occur over time? *International*
603 *Biodeterioration and Biodegradation* 129, 189-194.
604

605 Vázquez-Nion, D., Rodríguez-Castro, J., López-Rodríguez, M.C., Fernández-Silva, I., Prieto, B.,
606 2016. Subaerial biofilms on granitic historic buildings: microbial diversity and development of
607 phototrophic multi-species cultures. *Biofouling* 32, 657–669.
608

609 Vázquez-Nion, D., Silva, B., Troiano, F., Prieto, B., 2017. Laboratory grown subaerial biofilms on
610 granite: application to the study of bioreceptivity. *Biofouling* 33, 24–35.
611

612 Vázquez-Nion, D., Silva, B., Prieto, B., 2018. Bioreceptivity index for granitic rocks used as
613 construction material. *Science of the Total Environment* 633, 112-121.
614

615 Wirth, V., Hauck, M., Schultz, M., 2013. Die Flechten Deutschlands. Band 1 und Band 2. Ed
616 Ulmer, Stuttgart, Germany.
617
618 Wyszecki, G., Stiles, W.S., 1982. Color science. Concepts and Methods, Quantitative Data and
619 Formulae. John Wiley and Sons, New York.
620
621 Zhang, Z., MacMullen, J., Dhakal, H., Radulovic, J., Herodotou, C., Totomis, M., Bennett, N.,
622 2013. Biofouling resistance of titanium dioxide and zinc oxide nanoparticulate silane/siloxane
623 exterior facade treatments. Building and Environment 59, 47-55.

624 **Figure legends**

625 Figure 1. Schist samples (a) before cleaning treatment, (b) after chemical cleaning with water
626 and with Biotin T[®], and (c) after laser treatment by (left) UV irradiation at 266 nm and (right) IR
627 irradiation at 1064 nm. Scale bars = 1 cm.

628 Figure 2. Macroscopic appearance of the samples studied at the end of the bioreceptivity
629 experiment. Scale bars = 1 cm.

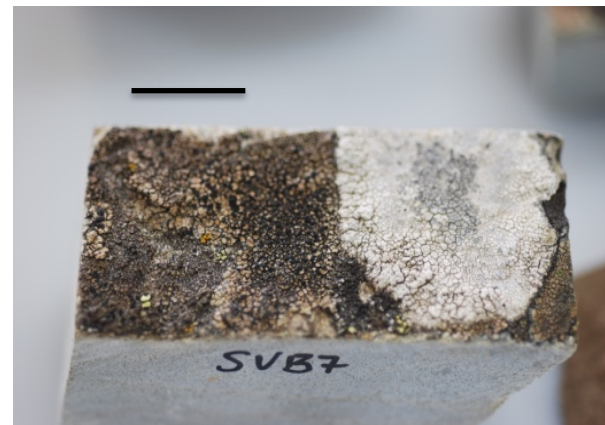
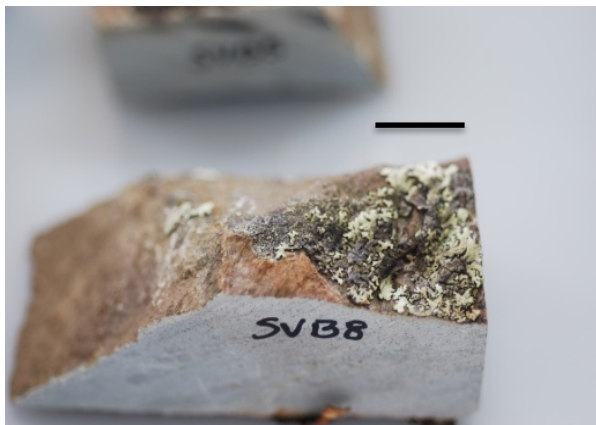
630 Figure 3. Variations in (a) total colour (ΔE^*_{ab}) and (b) the chromatic parameter a^* throughout
631 the experiment for Foz-Côa and Siega Verde lithotypes.

632 Figure 4. (a) F_0 (665 nm) values and (b) Yield (665 nm) values throughout the experiment for
633 Foz-Côa and Siega Verde lithotypes.

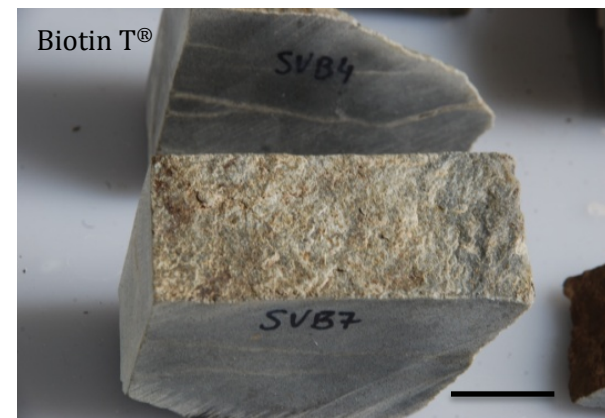
634 Figure 5. Foz-Côa and Siega Verde bioreceptivity index (BI).

635 Figure S1. Example of a schist sample block (c.a. 4x4x1 cm) before cleaning treatment.

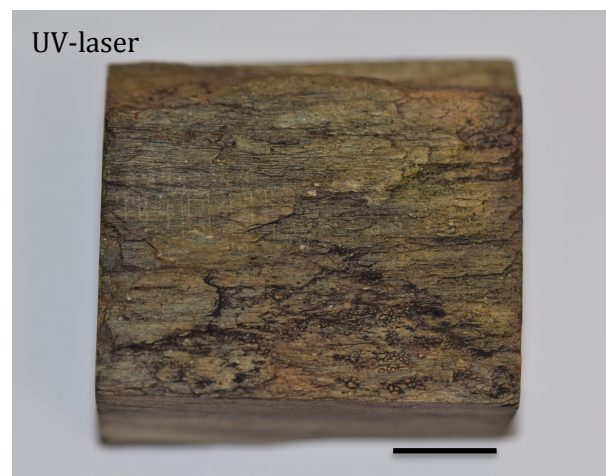
a)



b)



c)



FOZ-CÔA

SIEGA VERDE

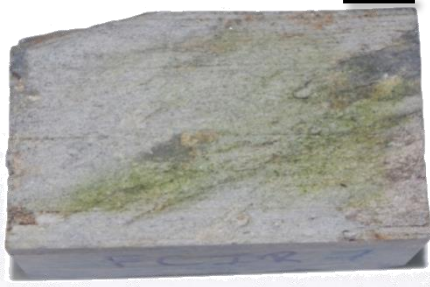
WATER



BIOCIDE



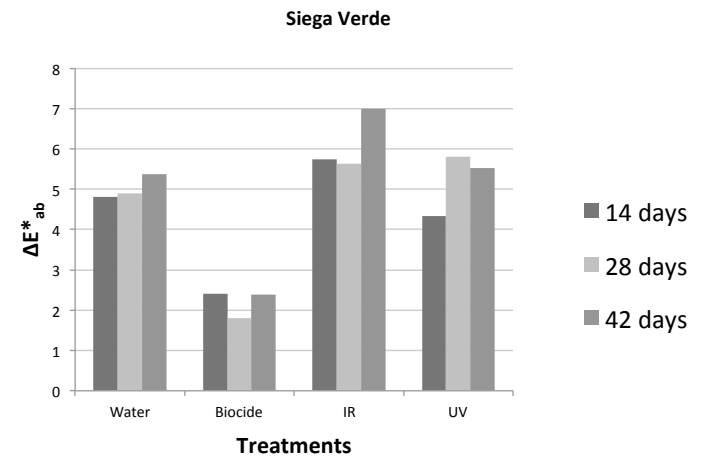
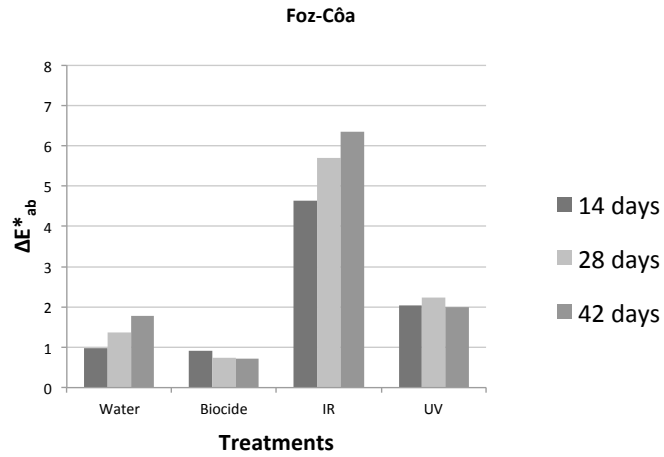
INFRARED



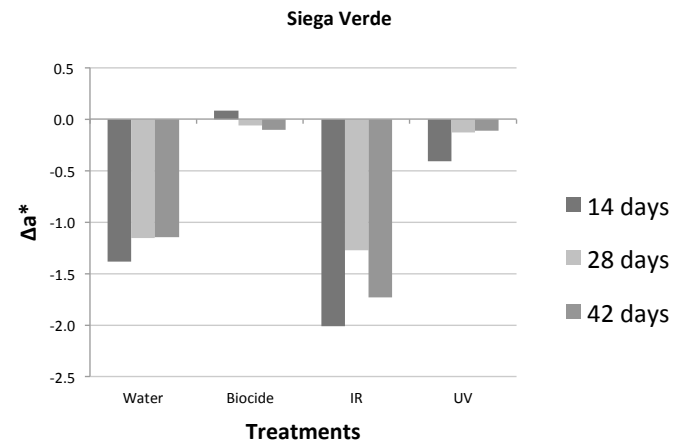
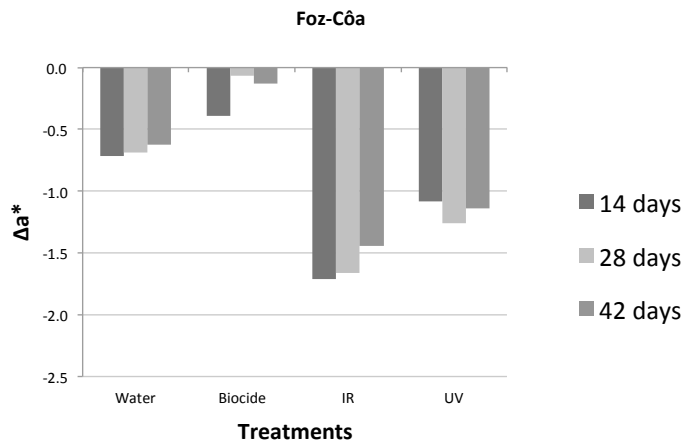
ULTRAVIOLET



a)

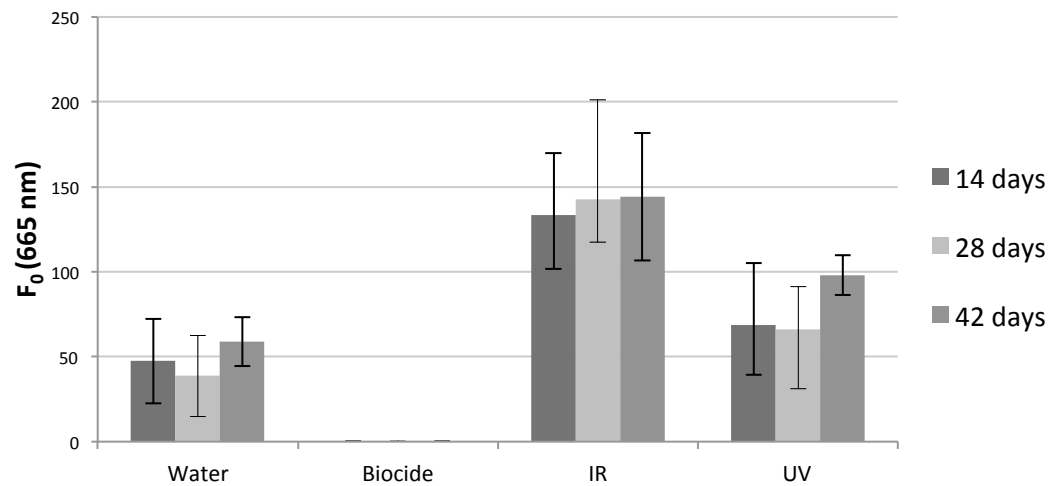


b)

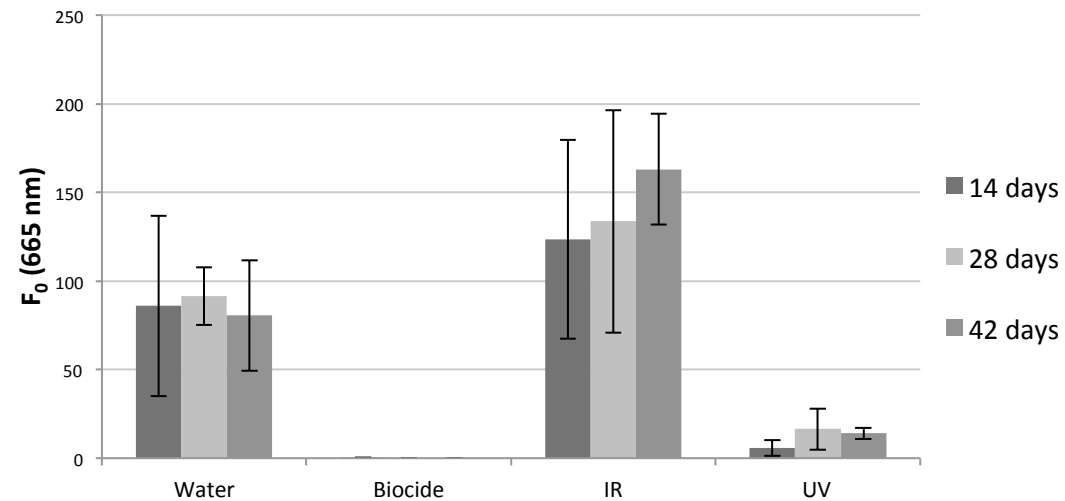


a)

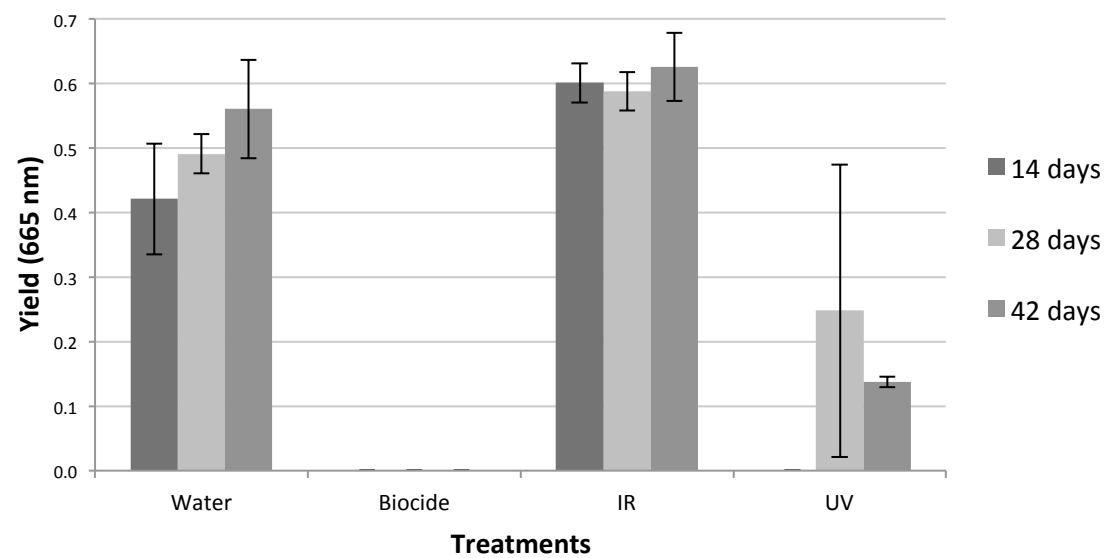
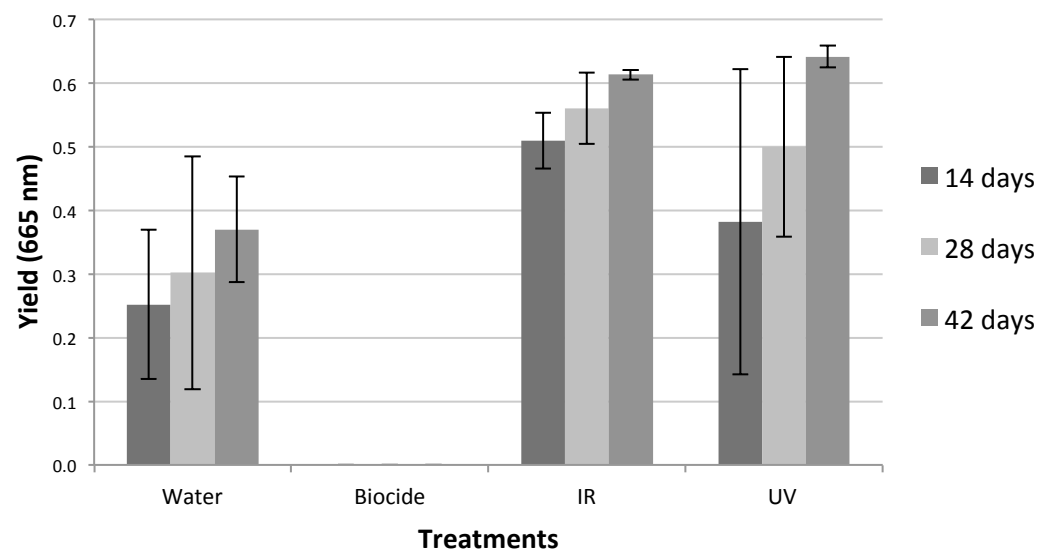
Foz-Côa



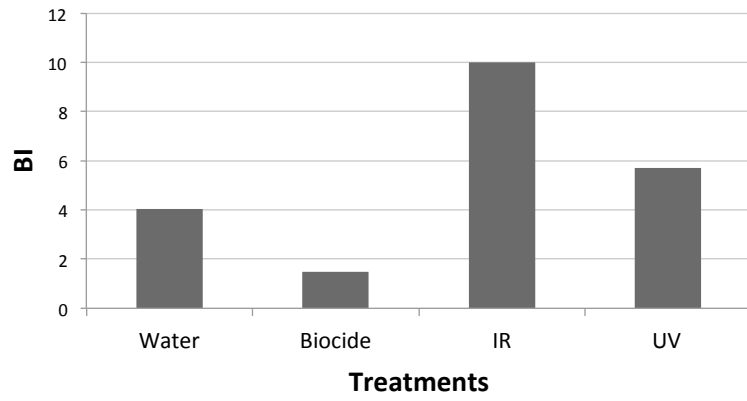
Siega Verde



b)



Foz-Côa



Siega Verde

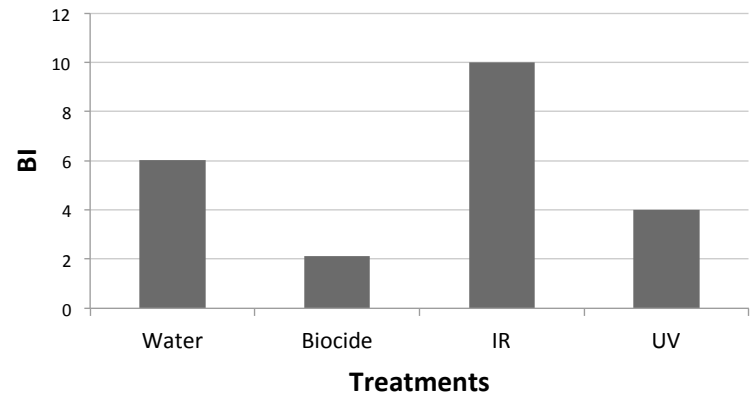


Table 1. Values of F_0 (665nm), yield (665nm) and F_0 (470nm)/ F_0 (645nm) ratio at the end of the bioreceptivity experiment.

		F_0 (665 nm)	Yield (665nm)	F_0 (470nm)/ F_0 (645nm) ratio
Foz-Côa	Water	0.370±0.083 (b)	58.800±14.516 (b)	0.365 ± 0.025
	Biocide	0.000±0,000 (a)	0,000±0,000 (a)	-
	IR	0.613±0.008 (c)	144.133±37.724 (c)	0.376 ± 0.052
	UV	0.642±0.017 (bc)	98.067±11.724 (c)	0.352 ± 0.024
Siega Verde	Water	0.561±0.076 (b)	80.600±31.248 (c)	0.364 ± 0.016
	Biocide	0.000±0.000 (a)	0.000±0.000 (a)	-
	IR	0.626±0.053 (c)	163.133±31.482 (c)	0.386 ± 0.017
	UV	0.137±0.008 (a)	14.067±3.215 (b)	0.286 ± 0.097

Different superscript letters indicate significant differences ($p < 0.05$) between the means of four independent samples for F_0 (665nm) and yield (665nm).