International Biodeterioration & Biodegradation 163 (2021) 105276

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Effectiveness and durability of chemical- and laser-based cleanings of lichen mosaics on schists at archaeological sites

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ARTICLE INFO

Keywords:
Rock art
Archaeology
Schist
Lichen
Biocide
Laser
Raman spectroscopy
Cleaning effectiveness

ABSTRACT

Lichen mosaics on different-textured schists located in the Coa Valley (Portugal) and Siega Verde (Spain) archaeological sites were cleaned using different chemicals, namely ethanol (50% v/v in distilled water), benzalkonium chloride (3% v/v) or Biotin T® (3% v/v), and different Nd:YAG laser wavelengths (1064 nm or 266 nm). The surfaces were evaluated 24 h and 4 years after cleaning to determine its durability using colour spectrophotometry and Raman spectroscopy. Unlike lasers, chemicals achieved overall satisfactory results. Cleaning effectiveness, harmfulness and durability of chemicals were highly influenced by the orientation of the schistosity planes of the stone; in Siega Verde samples, the schistosity planes parallel to the surface contributed to a low impact of the methods on the surface colour and absence of lichen recolonization. Cleaning carried out upon the devitalization of lichens with benzalkonium chloride and Biotin T were maintained longer in both sites.

1. Introduction

Biological growth (i.e. algae, fungi, lichens, bacteria and cyanobacteria) covering rock engravings in archaeological sites can compromise the artistic and historic value of these cultural heritages, contributing to physical and chemical decay (Silva et al., 2009; Gaylarde et al., 2012). Therefore, in order to preserve the appearance of the engravings, cleaning must be performed. Traditionally, the cleaning methods can be classified as those using chemical products, mechanical procedures and laser ablation (Doehne and Price, 2010). Biocides, mainly quaternary ammonium-based products, are the most-used chemical products to devitalize biological growths before their removal from archaeological surfaces (Saiz-Jimenez et al., 2012; Pozo et al., 2013; Favero-Longo et al., 2017; Sanmartín et al., 2019). Biocidal devitalization of microorganisms makes indeed easier to remove using brushes and lancets (Speranza et al., 2012). Regarding mechanical cleaning, scalpel and projection of a mixture of air, water and a micro-grained abrasive agent at low-pressure in order to safeguard the surface are among the most used methods (Caneva et al., 2009; Pozo et al., 2013). Laser cleaning is currently being fine-tuned to adequately clean cultural heritage stones (Pozo-Antonio et al., 2016a). The advantages of this technology lie in its selectivity and graduality, and the fact that it does not come into contact with the surface to be cleaned and is environmentally friendly (Fotakis et al., 2006).

Considering the deontological code for conservation of cultural heritage, cleaning should not change the physical and chemical characteristics of the treated materials (Venice Charter, 1964). Therefore, by-effects such as chemical contamination from cleaning agents, grain extraction by mechanical procedures and melting of forming minerals by laser are not admissible. Cleaning of outcrops with archaeological value has to achieve the most significant removal of lichens while simultaneously avoiding the by-effects on the engravings (Pozo-Antonio et al., 2016a).

For cleaning of biological colonization on stones belonging to cultural heritage, removal effectiveness depends mainly on the complexity of the patina or crust; this biological growth can range from weak biofilms composed of green algae and/or cyanobacteria, to lichens, which do not only colonize stone surfaces causing epilithic impacts, but also the deeper parts of the stone materials establishing interactions with the rock forming-minerals, causing endolithic damage through physical and chemical alterations (Ascaso et al., 1998; Gaylarde et al., 2012).

In order to remove weak biofilms composed of green algae and

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https://doi.org/10.1016/j.ibiod.2021.105276
Received 19 December 2020; Received in revised form 2 June 2021; Accepted 6 June 2021
Available online 17 June 2021

cyanobacteria, satisfactory cleaning was achieved regardless of the method applied (López et al., 2010; Pozo et al., 2013; Barreiro et al., 2019, 2020). Pozo et al. (2013) cleaned granite covered by filamentous green algae and cyanobacteria with an abrasive cleaning method, chemical products in aqueous media (using acids, bleach, benzalkonium chloride, ethanol and others) combined with vigorous brushing and a laser (Nd:YVO_4 at 355 nm). The best cleaning methods were the acid-based products and laser. In both cases, biological remains were not found on the surfaces. Satisfactory results were also achieved when different laser systems working at different wavelengths (Nd: YAG at 1064 nm, 532 nm and 355 nm and Er:YAG at 2940 nm) and different pulse durations (ns and fs) were applied to extract similar biological colonizations from silicate stones, such as granite and schist (Rivas et al., 2013; Barreiro et al., 2019, 2020).

However, the results are not as encouraging when removing lichens. Although damage to the lichens (e.g. loss of the upper cortex and severe effects on the algal layer) was detected, their complete removal was not achieved (Speranza et al., 2013; Osticioli et al., 2014; Sanz et al., 2015, 2017, Rivas et al., 2018; Pozo-Antonio et al., 2016b, 2019).

In addition to cleaning approaches developed in laboratories, it is important to know how the cleaning procedures work *in situ*. Some studies that focus on cleaning effectiveness, mainly for carbonate stones, have been carried out *in situ* and different authors recommended the use of re-treatments in order to avoid biological proliferation (Nugari and Salvadori, 2003; Tretiach et al., 2007; Cuzman et al., 2008; de los Ríos et al., 2012). It is therefore important to understand the durability of the different cleaning techniques, however, the problem with these studies is the duration of the research, as the studies must be continued over many years, which is sometimes difficult to achieve due to administrative implications. As a case study, Nascimbene et al. (2009) found that 12 years (1996–2008) after cleaning the calcareous statue Musa del Parnaso located in the garden of Villa Manin (Passariano, NE Italy, Friuli-Venezia Giulia), the surface was recolonized by lichen communities different from those covering the statue before restoration. While cleaning a biofilm composed of algae and cyanobacteria on a granitic wall, Pozo-Antonio et al. (2017) found the biocides tested presented different cleaning durability levels and surfaces that initially showed satisfactory results, quickly began to be colonized. Therefore, from a practical point of view, the cleaning durability should be taken into account in the selection of suitable procedures.

The Coa Valley (Trás-os-Montes and Alto Douro, Portugal) and the Siega Verde (Serranillo - Villar de la Yegua, Salamanca, Spain) archaeological parks, included in the UNESCO World Heritage List, show relevant petroglyphs from the Upper Paleolithic (22,000–10,000 BC). The panels in both locations show figures of animals and anthropomorphic forms (e.g. extinct aurochs, goats, deer and mythological hybrid creatures) engraved in schist outcrops (Fernandes et al., 2017). The environment is humid due to the presence of the Águeda River in Siega Verde and the Coa River in the Coa Valley, and subsequently, the biological colonization of the stones is favoured. Cleaning attempts were performed in the 1990s (Fernandes, 2007) with water-based soft scrubbing at both sites. Moreover, an unidentified chemical product was applied in Siega Verde, but no analysis of the benefits and/or harms of such actions has been carried out. The biological growth on these surfaces and their historical and artistic interests require research on more effective and more durable cleaning procedures.

Present research was focused on: i) the effectiveness of cleaning with ethanol at 50% (v/v), two biocides (Benzalkonium chloride and Biotin T) and two Nd:YAG wavelengths (1064 nm and 266 nm) applied to devitalize lichens prior to their removal from schist outcrops at the Coa Valley and at Siega Verde archaeological areas and ii) the durability of the cleaning after 4 years. Cleaning actions were performed on typical saxicolous lichen mosaics growing on the schists at these two archaeological sites. Chemical cleaning was performed *in situ*. For laser cleaning, stones slabs colonized by lichens were collected at each site and after being irradiated with the different wavelengths in a laboratory setting, they were returned to their original locations. The influence of the different rock textures in the two sites, related to the schistosity orientation, on the cleaning effectiveness and the corresponding durability was also evaluated. Moreover, for comparative purposes, cleaning with distilled water was also performed *in situ* as a control sample. The cleaning effectiveness was evaluated in terms of lichen removal and by-effects caused to the schists.

2. Materials and methods

2.1. Sites and cleaning procedures

In 2014, in order to perform *in situ* cleanings, a vertical oriented surface composed of quartz-mica schists was selected at each archaeological site (Coa valley and Siega Verde sites) to evaluate different chemical cleaning procedures (SM-Fig. 1). In Siega Verde, the cleaning area faced the SW, and in the Coa Valley the selected wall faced SE-S. Macroscopically, depending on the site, the schist showed differences in terms of colour and texture. At Siega Verde, the colour of the stone was greenish-blue, while at Coa, a more brownish tone was present. Moreover, at Siega Verde, schistosity planes were parallel to the surface (SM, Fig. 2); at Coa, they were sub-perpendicular to the surface (SM, Fig. 3). Mineralogically both stone surfaces showed similar composition: Coa schist is composed of quartz (53%), biotite (7%), muscovite-sericite (22%) and chlorite (17%) and Siega Verde schist is composed of quartz (60%), biotite (10%), muscovite-sericite (21%) and chlorite (8%) (Sanmartín et al., 2019).

Regarding the chemical cleanings, the following products were selected:

- Ethanol at 50% (v/v in distilled water).
- Benzalkonium chloride at 3% (v/v in distilled water) supplied by CTS Srl (Italy). Benzalkonium chloride is an organic salt classified as a quaternary ammonium compound (Pereira and Tagkopoulos, 2019). The quaternary ammonium group is approved for conservation of cultural heritage monuments by the European Biocide Directive (EC 2032/2003).
- Biotin T® at 3% (v/v in distilled water) supplied by CTS Srl (Italy), which is a water-soluble n-octyl-isothiazolone-based product and a quaternary ammonium salt (a cationic surfactant) following its technical sheet. Therefore, Biotin T® exhibits a broad-spectrum activity against phototrophs and is often used to clean façades of buildings (Gladis et al., 2010; Sanmartín et al., 2011a). It interacts with thiol-groups of amino acids, degrading proteins that play an important role in protecting the cells of organisms (Collier et al., 1990).

At each site, each product was applied by brush in one area of 14 × 10 cm (SM-Figs. 1–3). The *in situ* cleanings were performed in January 2014. At that time, air temperature was low (ranging between 1 °C to 6 °C) with high humidity due to constant rain and the presence of the rivers at both sites. Due to these conditions, the thalli were completely humid with metabolic activity. Therefore, the biocides are effective as soon as they are applied (Tretiach et al., 2007). After application, each treated area was covered by a black plastic sheet for 3 h to avoid the evaporation of the products. Next, surfaces were rinsed with distilled water and cleaned with a plastic barbed brush. A wooden rod was then used to mechanically remove the remnants with firmer adhesion to the surface. For the surfaces cleaned with Biotin T and benzalkonium chloride, once clean, they were neutralized using a 50% ethanol (v/v in distilled water) spray solution to avoid chemical contamination from chlorides salts. In addition to the three cleaned areas, a fourth area was also cleaned with only distilled water for comparative purposes. After 24 h, cleaning procedures were repeated. It should be noted that during cleaning, an important extraction of soil from the fractures associated to the schistosity planes was observed in the Coa samples.

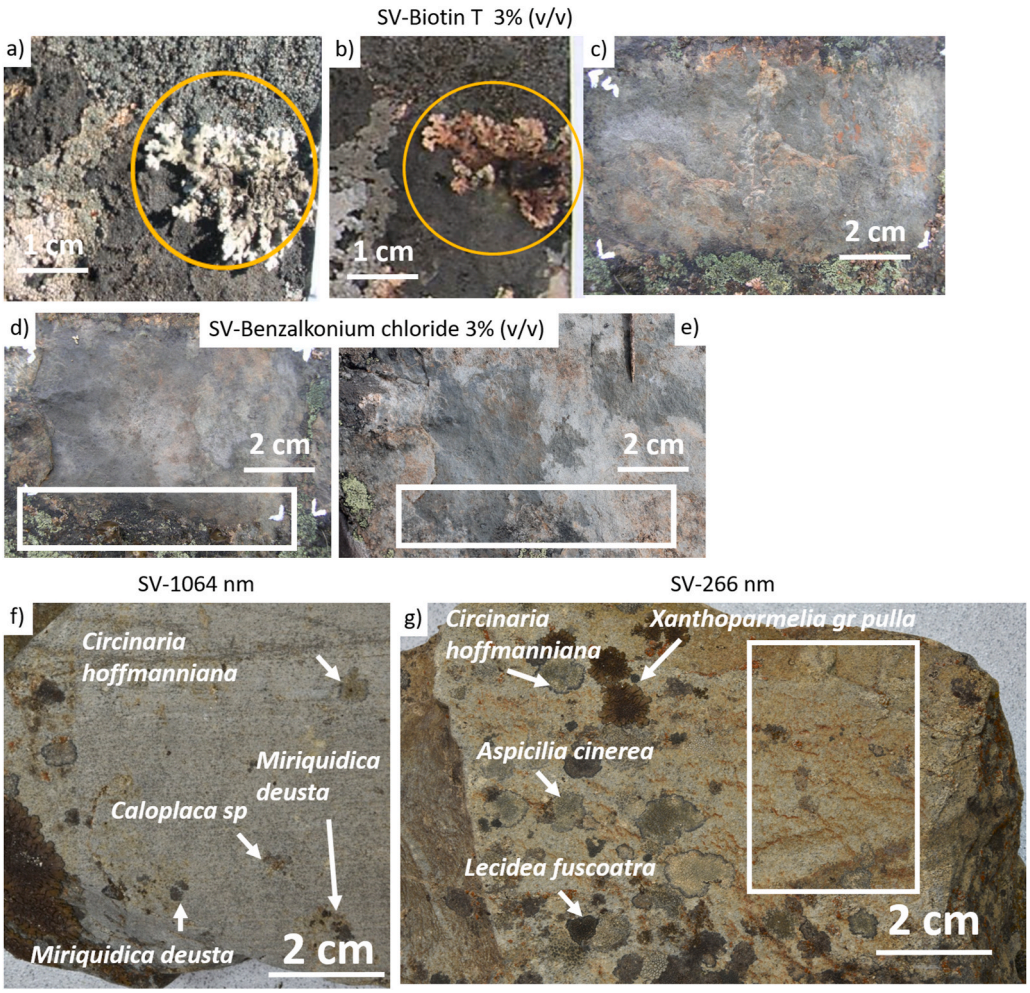

Fig. 1. Digital photographs of Siega Verde samples. Surface before being cleaned with Biotin T (a), during Biotin T application (b) and after cleaning with Biotin T (c). Benzalkonium chloride-cleaned surface 24 h after cleaning (d) and 4 years after cleaning (e). 1064 nm-cleaned surface after 4 years (f) and 266 nm-cleaned surface after 4 years (g). SV: Siega Verde samples. FC: Coa samples.

Moreover, two samples with a representative lichen mosaic of each site were collected to be cleaned in a laboratory setting with a nanosecond pulse duration Nd:YAG laser. The equipment was a Q-switched Nd:YAG laser (Quanta Ray INDI-series, Spectra Physics) with a repetition rate of 10 Hz and a 6 ns-pulse duration capable of delivering the fundamental 1064 nm wavelength (IR radiation) and the 4th harmonic (266 nm – UV radiation). Therefore, for each site, one sample was subjected to 1064 nm and the other one to 266 nm. The laser beam reached the surface using a spherical plane-convex lens (NewPort) with a focal length of 250 mm. As reported in Sanmartín et al. (2019), after previous tests, the fluence selected for each wavelength and each site depends on the nature of the lichen and the thickness of the biological colonization. After performing several tests working with different fluences ranging from 5 J cm^{-2} to 30 J cm^{-2}, the evaluation of the irradiated surfaces under stereomicroscopy allowed for the identification of the fluence that achieved the highest lichen removal with one scan with minimal damage to the substrates. For the 1064 nm cleaning, in the Coa sample, 25 J cm^{-2} was used, while for the Siega Verde sample, 23 J cm^{-2} was used. For the 266 nm cleaning, for both locations, 16 J cm^{-2} was used. Once laser cleaning was performed in the laboratory, samples were returned to their original positions at each site.

Moreover, in order to identify the effect of the cleaning methods on uncolonized surfaces, the same cleaning procedures (ethanol, benzalkonium chloride, Biotin T, water, 1064 nm and 266 nm) were applied in the laboratory on uncolonized samples taken from the sites. Therefore, six uncolonized slabs were collected from each site.

2.2. Analytical techniques

2.2.1. Characterization of biological colonization

Previous lichen characterization included *in situ* identification using a hand lens with 10× magnification, together with basic reagents (potassium hydroxide and bleach). Identification followed standard methods for lichenized fungi (Smith et al., 2009). Moreover, lichens on the samples collected to be exposed to laser irradiation (two from each archaeological site) were also identified in the laboratory. Thallus morphology was examined under stereomicroscope (SMZ25 NIKON®). The structure of the lichen thalli and ascomata was studied from hand-cut sections mounted in water and observed under a microscope (Eclipse 80i NIKON®). Micrographs were taken with digital cameras (1100D EOS CANON® and DS-2Mv NIKON®), which were mounted on the aforementioned stereomicroscope.

2.2.2. Cleaning effectiveness and durability

The cleaned surfaces were evaluated 24 h after and 4 years after cleaning by direct observation using a hand lens with 10× magnification, together with basic reagents (potassium hydroxide and bleach), following Smith et al. (2009), to evaluate the presence of lichen remnants or recolonization phenomena.

Spectrophotometry was used to characterize the colour of the

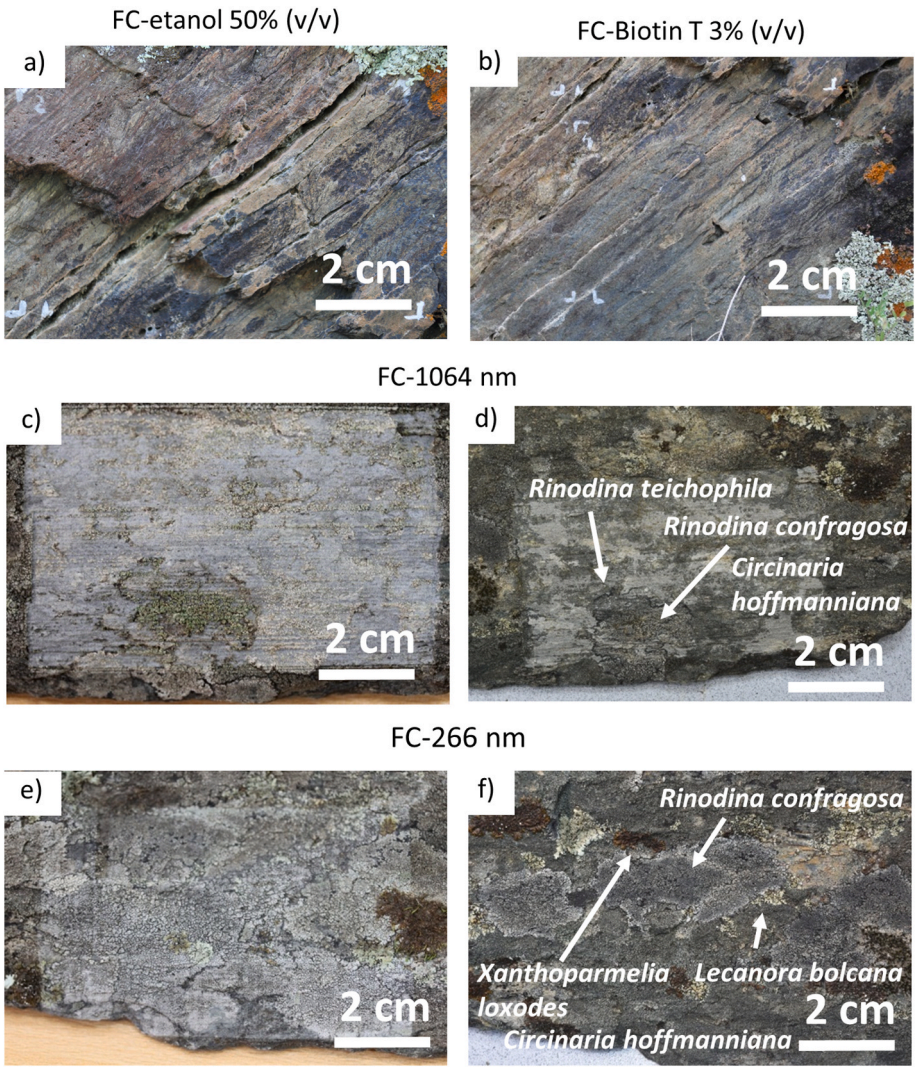

Fig. 2. Digital photographs of Coa samples. Surface cleaned with ethanol (a), and Biotin T (b) 4 years after application. Cleaned surfaces with laser at 1064 nm after 24 h (c) and after 4 years (d). Cleaned surfaces with laser at 266 nm after 24 h (e) and after 4 years (f). SV: Siega Verde samples. FC: Coa samples.

surfaces 24 h after cleaning and also after 4 years of exposure to natural environmental conditions. Moreover, the colour of the uncolonized surfaces subjected to cleaning methods was also measured in order to identify the effect of the methods on both schists. The colour from the uncolonized surfaces before treatment was also measured as a reference. Colour was characterized in CIELAB and CIELCH colour spaces (CIE S 014-4/E, 2007) by means of a Minolta CM-700d spectrophotometer. The coordinate L* represents the lightness in values ranging from 0 (black) to 100 (white) and coordinates a* and b* express the colour wheel, taking values ranging from +a* (red) to - a* (green) and from +b* (yellow) to - b* (blue). It was computed the parameter C^*_{ab}, the chroma or colour saturation, which expresses the relative strength of a colour, and its value corresponds to $C^*_{ab} = [(a^*)^2+(b^*)^2]^{1/2}$ and the hue, $h_{ab} = \tan [1 - (a^*/b^*)]$. Measurements taken included specular reflection (SCI), with a spot diameter of 8 mm, illuminant D65 and an observation angle of 10°. Twenty colour measurements per sample were randomly taken, in order to obtain statistically representative results (Prieto et al., 2010).

Considering colour measurements of the uncolonized surfaces before treatment as reference, colour difference after cleaning (24 h after cleaning and 4 years later) was computed as follows:

$$\Delta L^* = L^*_x - L^*_i$$

$$\Delta a^* = a^*_x - a^*_i$$

$$\Delta b^* = b^*_x - b^*_i$$

$$\Delta C^*_{ab} = C^*_{ab},\ x - C^*_{ab,i}$$

$$\Delta h = h^*_x - h^*_i$$

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

The subscript $_x$ denotes the colour parameter of a cleaned area at different moments *x*, specifically just 24 h after cleaning (step 0) and 4 years later (step 1), and the subscript $_i$ denotes the colour parameter of the uncolonized surface. Therefore, a lower ΔE^*_{ab} would indicate a low modification of the colour surface for uncolonized samples exposed to cleaning methods or a greater effectiveness of biological colonization removal for the cleaned samples.

Raman spectroscopy was used to detect organic remnants on surfaces after laser treatment. The measurements were made using a portable i-Raman – BWS415-785S equipment, which delivers an excitation beam at 785 nm provided by a narrow bandwidth CleanLaze model laser, with 300 mw of maximum power adjustable from 0 to 100% and coupled to an optical head. Individual areas of measurement were controlled with a light-emitting diode and a high-resolution colour camera. The scattered radiation was collected through the objective lens, passed through an edge filter that cut off Rayleigh scattering, and focused into an optical

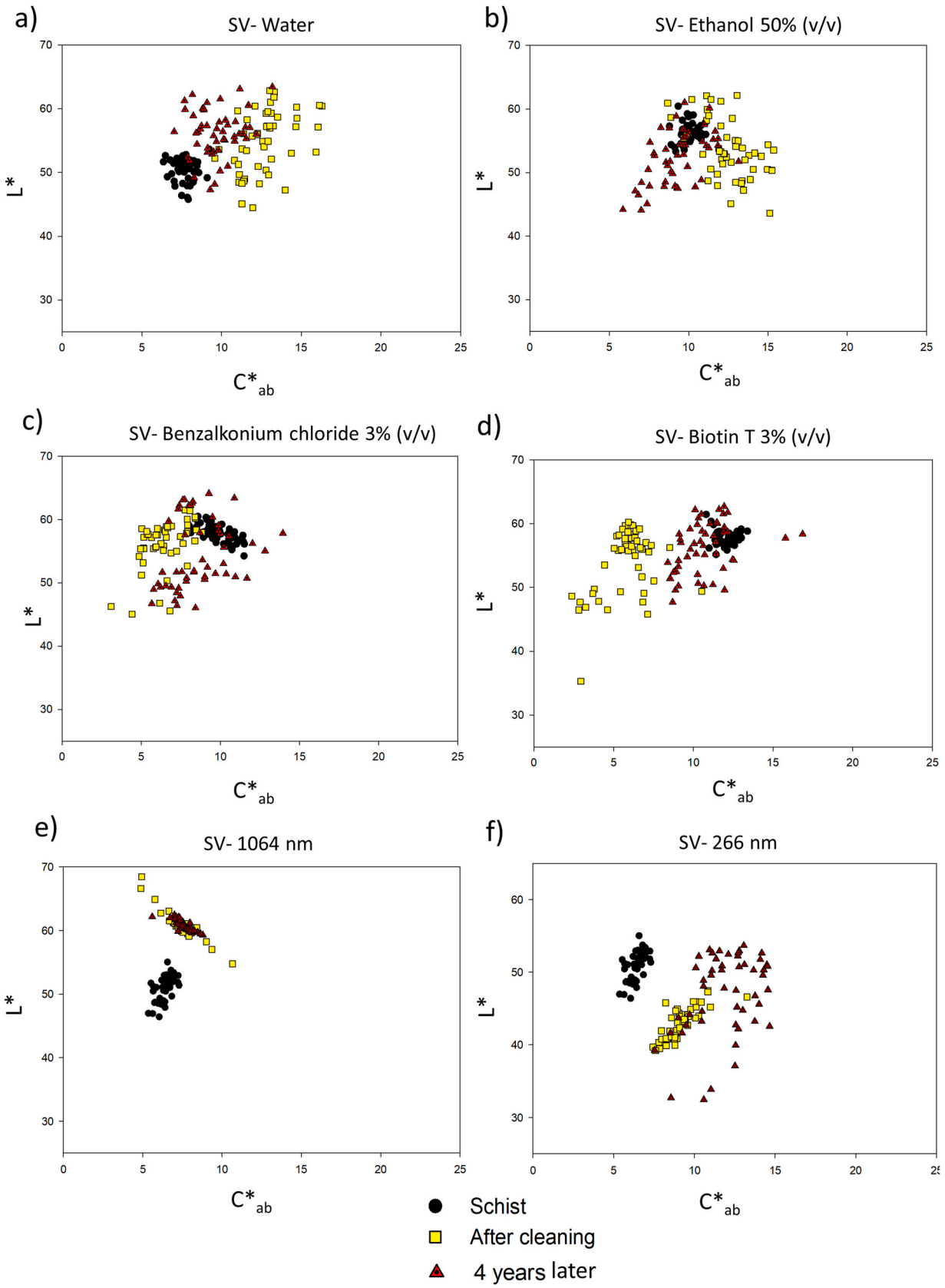

Fig. 3. L*-C*$_{ab}$ scatter plots of Siega Verde surfaces cleaned with water (a), ethanol at 50% (v/v) (b), benzalkonium chloride at 3% (v/v) (c), Biotin T at 3% (v/v) (d), a Nd:YAG working at 1064 nm (e) and 266 nm (f). SV: Siega Verde samples. FC: Coa samples.

fibre that was fed into a compact spectrograph, equipped with a concave grating, providing spectral coverage in the 175–3200 cm^{-1} range at a spectral resolution of approximately 3–7 cm^{-1}. The detector, a Synapse ™ CCD (2048 pixels), was Peltier-cooled and featured high sensitivity with low dark counts. During the analysis, the power delivered by the laser beam on the sample surface was adjusted from 9 to 300 mW and an exposure time range between 1000 and 15000 ms in order to obtain the optimal spectrum in each measurement. Spectra corresponded to an average of 5 consecutive scans on the same point. Raman spectroscopy was applied on the uncolonized schist, on the lichens and the cleaned surfaces 24 h after cleaning and 4 years later.

3. Results

3.1. Characterization of biological colonization

Identification of lichens colonizing each area before the cleaning by the different procedures (ethanol, biocides and water) was done in both archaeological sites (Table 1). The lichens found were already reported in previous works (Marques et al., 2014; Pozo-Antonio et al., 2019; Paz-Bermúdez et al., 2018).

3.2. Cleaning effectiveness and durability

3.2.1. In situ observations

Regarding the performance of the cleaning with chemicals combined with mechanical removal, 24 h after the treatment it was possible to identify by the naked-eye that in the selected walls from both outcrops, satisfactory results were achieved, even for the surface cleaned with distilled water (Siega Verde: SM-Fig. 2 and Coa: SM-Fig. 3). It was observed that Biotin T induced a colour change towards the pinkish-orange of the foliose lichen–*Dermatocarpon miniatum* (Fig. 1a and b). An orange-whitish coloration was identified in Siega Verde, regardless of the biocide (Fig. 1c and d; SM-Fig. 2). Contrary to the high cleaning levels obtained by the chemicals and the water, the results were not as encouraging for both laser wavelengths (SM-Figs. 2 and 3). With laser cleaning, the lichen must be removed while safeguarding stone integrity, and therefore, total removal of the lichen was not achieved (Table 2), with the 266 nm being less effective in comparison to the 1064 nm (SM-Figs. 2 and 3). It is important to highlight that in addition to the unsatisfactory cleaning, 1064 nm induced grooves on the surfaces at both locations, with an intense paling effect (SM-Figs. 2 and 3).

After 4 years, in general terms, the cleaning level achieved by chemical treatments was maintained over time (Table 1, Figs. 1e and 2a, b, SM-Figs. 2 and 3) due to scarce recolonization. In some cases, the small thalli left that surrounded the cleaning areas had completely disappeared (highlighted with a rectangle in Fig. 1d and e). With further details (Table 1), for Siega Verde samples, on ethanol-cleaned surfaces, no recolonizing lichen species were detected, except for some minute, unidentifiable thalli. In the areas treated with either benzalkonium chloride or Biotin T, the surfaces appeared to be cleaner after 4 years as the few organic remnants detected 24 h after the cleaning were no longer present (Fig. 1d and e). Regarding Coa samples, in the areas treated with ethanol, benzalkonium chloride and Biotin T (Fig. 2a and b), only small thalli of *Aspicilia/Circinaria* were identified filling fissures and voids. Regarding the control cleanings with water, some isolated small areolas of *Aspicilia/Circinaria* sp, *Miriquidica deusta* and *Rhizocarpon geographicum,* were found in the Siega Verde sample, while in the Coa sample, minute thalli of *Aspicilia/Circinaria* were detectable. Moreover, in the chemical- and water-cleaned samples from Coa, deep cavities were identified associated with subperpendicular schistosity planes (Fig. 2a and b) as a result of soil/sediment dragging between these planes due to the mechanical effect of the brushing. Regarding laser cleaning, the low effectiveness registered 24 h after cleaning was also detected on the surfaces after 4 years. For Siega Verde samples subjected to 1064 nm (Fig. 1f), *Circinaria hoffmanniana* remnants, which

Table 1
Lichens identified by the authors on the cleaned areas at Siega Verde and Coa Valley archaeological sites, before and four years after cleaning.

Archaeological site	Treatment	Before cleaning	4 years later
Siega Verde	Distilled water	*Caloplaca* sp, *Dermatocarpon miniatum, Lasallia pustulata, Lecidea fuscoatra, Rhizocarpon geographicum* and *Xanthoparmelia* gr *pulla.*	Some isolated small areolas of: *Aspicilia/Circinaria* sp., *Miriquidica deusta* and *Rhizocarpon geographicum,*
	Ethanol at 50% (v/v in distilled water).	*Dermatocarpon miniatum, Lasallia pustulata, Rhizocarpon geographicum* and *Xanthoparmelia conspersa.*	No recolonizing lichen species were detected, except for some tiny, unidentifiable thalli.
	Benzalkonium chloride at 3% (v/v in distilled water).	*Caloplaca* sp, *Circinaria hoffmanniana, Dermatocarpon miniatum, Lasallia pustulata* and *Rhizocarpon geographicum*	No recolonizing lichens.
	Biotin T at 3% (v/v in distilled water).	*Caloplaca* sp, *Circinaria hoffmanniana, Dermatocarpon miniatum, Lasallia pustulata, Rhizocarpon geographicum* and *Xanthoparmelia* gr *pulla.*	No recolonizing lichens.
	1064 nm	*Aspicilia* gr. *contorta, Caloplaca* sp, *C. pellodela, C. variabilis Circinaria. hoffmaniana, Miriquidica deust* and *Xanthoparmelia* gr. *pulla*	*Caloplaca* sp, *Circinaria hoffmanniana, Miriquidica deusta* and *Xanthoparmelia gr pulla*
	266 nm	*Aspicilia* gr. *contorta, Caloplaca* sp, *C. pellodela, C. variabilis. Circinaria. Hoffmaniana, Miriquidica deusta* and *Xanthoparmelia* gr. *pulla*	*Aspicilia cinerea, Circinaria hoffmaniana* and *Lecidea fuscotra*
Coa	Distilled water	*Xanthoparmelia conspersa* and *X.* gr *pulla*	Small thalli of *Aspicilia/Circinaria* sp
	Ethanol at 50% (v/v in distilled water).	*Caloplaca subsoluta, Lecanora pseudistera, Peltula euploca, Xanthoparmelia conspersa* and *X.* gr *pulla*	Small thalli of *Aspicilia/Circinaria* sp
	Benzalkonium chloride at 3% (v/v in distilled water).	*Circinaria hoffmanniana, Xanthoparmelia conspersa* and *X.* gr *pulla.*	Small thalli of *Aspicilia/Circinaria* sp
	Biotin T at 3% (v/v in distilled water).	*Caloplaca subsoluta, Peltula euploca* and *Xanthoparmelia conspersa*	Small thalli of *Aspicilia/Circinaria* sp
	1064 nm		

(*continued on next page*)

Table 1 (*continued*)

Archaeological site	Treatment	Before cleaning	4 years later
		Candelariella vitellina, Circinaria hoffmanniana, Lecanora bolcana, Rinodina confragosa, R. teichophila and *Xanthoparmelia loxodes.*	*Circinaria hoffmanniana, Rinodina confragosa* and *R. teichophila*
	266 nm	*Candelariella vitellina, Circinaria hoffmanniana, Lecanora bolcana, Rinodina confragosa, R. teichophila* and *Xanthoparmelia loxodes.*	*Candelariella vitellina, Circinaria hoffmanniana, Lecanora bolcana, Rinodina confragosa, R. teichophila and Xanthoparmelia loxodes*

were detected after cleaning, were maintained over time. Burnt thalli belonging to *Xanthoparmelia* gr *pulla* were detected after the cleaning, and 4 years later they had disappeared. Moreover, lichens such as *Miriquidica deusta* and *Caloplaca* sp., which were not detected after cleaning, were identified on the surface 4 years later (Fig. 1f). On the 266 nm-cleaned surface, the lichens *Aspicilia cinerea*, *Circinaria hoffmaniana* and *Lecidea fuscotra* were found (Fig. 1g) 4 years after the cleaning, while some *Caloplaca* sp. and thalli of *Xanthoparmelia* gr *pulla* disappeared during these years. In Fig. 1g, a rectangle points out the area where these two lichens were found 24 h after cleaning. In a Coa sample cleaned with 1064 nm, the lichens *Rinodina teichophila* and *Rinodina confragosa* damaged by the laser presented an enhanced vital state with time and the thalli belonging to *Circinaria hoffmanniana* showed recovery of the upper cortex (Fig. 2c and d). For the 266 nm-cleaned surface, as just after cleaning, lichens were slightly damaged as reflected by a greyish colour due to burning (Fig. 2e). After 4 years, the original colour appeared again due to lichen re-growth (Fig. 2f). *Xanthoparmelia loxodes* thalli were observed to be detached in only some areas.

Therefore, for both sites, areas cleaned with ethanol, Biotin T and benzalkonium chloride showed similar conditions, and the last two chemicals showed to be the most efficient and durable biocides, as a more intense recolonization was identified in the ethanol-cleaned surface. Also, Coa samples showed slightly higher recolonizations than those of the Siega Verde samples. Despite the cleaning level being satisfactory, areas cleaned with water showed a slight recolonization. Among both unsatisfactory wavelengths, the 1064 nm radiation achieved the highest removal level, but visible grooves were also detected on the surfaces. Both laser-cleaned surfaces were notably recolonized after 4 years.

3.2.2. *Colour spectrophotometry*

Considering colour evaluation of the cleaned surfaces, the L^*-C^*_{ab} scatter plots (Siega Verde samples: Fig. 3 and Coa samples: Fig. 4) showed the colour of the uncolonized schists, the cleaned areas 24 h

Table 2
Colorimetric differences (ΔL^*, Δa^*, Δb^*, ΔC^*_{ab} and ΔH^*) and the global colour change (ΔE^*_{ab}) considering the colour of the uncolonized schist as reference. In addition to the data for the areas cleaned of lichens, data from the uncolonized surfaces subjected to the cleaning methods was also provided (depicted with *No lichen*). 0: corresponds to the treated surface 24 h after cleaning and 1: corresponds to the surface 4 years after cleaning.

Method	Time	ΔL^*	Δa^*	Δb^*	ΔC^*_{ab}	ΔH^*	ΔE^*_{ab}
SIEGA VERDE							
Water	No lichen	0.80	−0.04	−0.59	−0.76	0.34	0.99
	0	1.52	−2.59	−5.29	−5.73	1.15	6.08
	1	−1.56	0.47	−0.15	−0.14	−0.41	1.64
Ethanol	No lichen	1.66	−0.06	−0.94	−0.94	0.07	1.91
	0	0.21	−2.84	−5.41	−5.91	1.54	6.11
	1	−5.42	−0.11	−0.57	−0.53	0.22	5.45
Biotin T	No lichen	0.48	−0.56	−2.17	−2.17	0.57	2.29
	0	3.68	0.04	−1.96	−1.90	0.46	4.17
	1	−1.77	0.56	0.80	0.83	−0.50	2.02
Benzalkonium chloride	No lichen	0.05	0.28	−1.00	−0.93	−0.06	1.04
	0	5.66	0.17	−1.25	−1.24	0.11	5.80
	1	−3.40	−0.05	−1.30	−1.25	0.27	3.64
1064 nm	No lichen	9.88	−0.12	1.15	1.16	−0.02	9.95
	0	10.58	0.91	−0.34	−0.41	−0.94	10.63
	1	10.33	0.12	−0.05	−0.07	−0.12	10.33
266 nm	No lichen	0.61	0.03	0.60	0.59	−0.10	0.85
	0	−7.54	1.75	1.52	1.46	−1.81	7.88
	1	−3.55	3.23	4.10	4.23	−3.10	6.32
COA							
Water	No lichen	2.68	−0.95	−1.88	−2.03	0.55	3.41
	0	3.18	−1.01	−2.68	−2.83	0.45	4.28
	1	−8.67	−2.52	−6.24	−6.68	0.26	10.98
Ethanol	No lichen	3.59	−0.37	1.09	0.92	0.70	3.77
	0	8.39	−3.57	−0.90	−1.88	3.21	9.16
	1	−14.86	−2.05	−7.84	−7.98	−1.02	16.92
Biotin T	No lichen	0.94	0.71	0.53	0.76	−0.48	1.29
	0	4.75	−3.76	−2.25	−3.24	3.00	6.47
	1	−13.09	−4.44	−9.57	−10.44	1.49	16.81
Benzalkonium chloride	No lichen	6.59	−4.40	−4.85	−5.91	2.89	9.29
	0	6.29	−3.56	−2.21	−3.16	2.80	7.56
	1	−9.03	−2.60	−5.09	−5.60	0.92	10.69
1064 nm	No lichen	4.27	−2.54	−4.58	−5.02	1.57	6.75
	0	5.75	−0.68	−2.72	−2.78	0.36	6.40
	1	−11.44	0.15	−0.66	−0.65	1.40	11.46
266 nm	No lichen	2.04	−0.37	0.51	0.39	0.50	2.14
	0	−3.40	−0.55	−2.01	−2.06	0.37	3.99
	1	−14.96	−1.57	−6.99	−7.07	0.98	16.58

after cleaning and the same areas 4 years later. The graphs show that despite the fact that in some areas no lichen recolonization was detected in the *in situ* evaluation, the complete recovery of colour was not obtained after any of the cleaning procedures, regardless of time. It is important to highlight that more colour dispersion was detected in the samples from Coa: the colours corresponding to each state were further away from each other than those detected in Siega Verde samples. L*-C^*_{ab} scatter plots showed different colour evolution depending on the site:

i) For Siega Verde cleanings (Fig. 3), the colour of the surfaces cleaned with chemicals and water, 4 years after cleaning, was closer to the uncolonized schist than the colour of the areas 24 h after cleaning (Fig. 3a–d). For laser-cleaned areas, the colour of cleaned surfaces regardless of time, was similar because colour for both time lapses (24 h and 4 years) was overlapped and different than the colour of the uncolonized schist (Fig. 3e and f), mainly for the 1064 nm-cleaned surface (Fig. 3e). Similar to the uncolonized surface, benzalkonium chloride- (Fig. 3c) and Biotin T- (Fig. 3d) cleaned areas showed C^*_{ab} decreases (the colour was paler) for both time lapses, while the surfaces cleaned with water (Fig. 3a), ethanol (Figs. 3b) and 266 nm (Fig. 3f) showed C^*_{ab} increases (the colour was more vivid). Of the latter, an L* decrease (a darkening) was also detected (Fig. 3f). On the surfaces cleaned with 1064 nm, no C^*_{ab} change was detected while the L* showed increases for both time lapses (Fig. 3e).

ii) For Coa cleanings (Fig. 4), the colour of the cleaned surfaces 24 h after cleaning was closer to that of the uncolonized schist than the colour observed 4 years later. In comparison to the uncolonized surface, on the surfaces 24 h after cleaning, C^*_{ab} decreased and L* slightly increased, with exception of the surface cleaned with 266 nm, where a slight L* decrease was detected (Figure 7f). After 4 years, both L* and C^*_{ab} decreased.

Considering the colorimetric differences of the cleaned areas (Table 2), L* was the parameter most affected, with the exception of the Siega Verde areas cleaned with water and ethanol, 24 h after cleaning, where b* was the most affected parameter (b* decreases show a reduction of yellow coloration), and cleaned with 266 nm, 4 years later, where b* showed increases (intensifying the yellow colour). Despite L* being the most affected parameter in most cases, it showed different trends: i) for the chemicals- and water-cleaned areas, L* showed increases 24 h after cleaning and decreases after 4 years, ii) for the areas cleaned with 266 nm, from both sites, a darkening was detected, regardless of the time lapse and iii) for the areas cleaned with 1064 nm, in Siega Verde, L* showed increases regardless of the time lapse, while in Coa, L* increased 24 h after cleaning and decreased after 4 years. The parameter a* also showed changes: i) for Siega Verde samples, a* increased with exception of that measured on the ethanol-cleaned surface regardless of the time lapses and the benzalkonium chloride-cleaned surface after 4 years, where a* decrease, i.e. a greenish colour was observed. However, those a* changes are negligible as they were lower than 3 CIELAB units. For the Coa samples, all the cleaned areas, regardless of the time lapses, showed reductions of a* (a greenish effect).

As a result, C^*_{ab} and h_{ab} also have shown changes. C^*_{ab} showed mainly decreases suggesting surfaces paler than the uncolonized surfaces, with the exception of the surface from Siega Verde cleaned with 266 nm. The h_{ab} changes were lower than 3 CIELAB units.

Regarding the ΔE^*_{ab}, for Siega Verde cleanings, a reduction of this parameter was detected after 4 years while in Coa, ΔE^*_{ab} increased. For Siega Verde, 24 h after cleaning, all areas showed visible colour changes because ΔE^*_{ab} was greater than 3.5 CIELAB units (Mokrzycki and Tatol, 2011) while after 4 years, the water- and Biotin T-cleaned areas did not show visible changes. It is important to note that, considering the cleanings performed in the uncolonized surfaces, with exception of the 1064 nm, the rest of the procedures did not induce visible colour changes. For the cleanings performed in Coa stones, regardless of the time, all the ΔE^*_{ab} were higher than 3.5 CIELAB; therefore, all the colour changes would be detected by an unexperienced observer. All the cleaning methods applied to the uncolonized surfaces also induced visible colour changes.

3.2.3. *Raman spectroscopy*

Raman spectroscopy was used to identify the peaks assigned to the components of the stone and organic remnants on the schists. Fig. 5 shows the Raman spectra of the uncolonized schist, the most representative lichen on each surface and the surfaces cleaned with each cleaning method, 24 h and 4 years after cleaning. Considering that lichens were identified by the intense fluorescence they presented as was found in Pozo-Antonio et al. (2019) and Barreiro et al. (2019), when the fluorescence intensity is higher, the cleaning effectiveness is worse.

In the Raman spectrum of the schist from Siega Verde (Fig. 5a and b), peaks were identified according to the different forming minerals (Lima et al., 2001; Wang et al., 2002; Pan et al., 2010): a peak at 200 cm^{-1} associated with biotite, a peak at 264 cm^{-1} associated with muscovite, and a peak at 464 cm^{-1} associated with the main mineral component quartz, due to the Si-O-Si symmetric stretching of the six-membered rings of SiO_4. There were also low intensity peaks at 1222, 1257 and 1400 cm^{-1} associated with graphitic carbon (Sadezky et al., 2005; Beyssac and Lazzeri, 2012). Graphitic carbon is often associated with middle-grade metamorphic rocks, such as marble, schist and paragneiss (Walter, 2004; Selverstone, 2005; Lyu et al., 2020). The crystallinity of carbonaceous compounds is mainly controlled by thermal metamorphism from an amorphous stage, through a process called graphitization (Lünsdorf et al., 2017). For the Coa stone (Fig. 5c and d), the peaks associated with muscovite (264 cm^{-1}) and those at 1222, 1257 and 1400 cm^{-1} associated with graphitic carbon (Sadezky et al., 2005) were more intense than those for the Siega Verde samples. Moreover, a shoulder at 1300 cm^{-1} and peaks at 1494 and 1600 cm^{-1} which were not detected in the Raman spectra of the samples from Siega Verde, were also identified. These peaks were also associated with graphitic carbon (Sadezky et al., 2005).

In the Raman spectra of the cleaned surfaces (Fig. 5) peaks associated with the forming minerals and graphitic carbon were identified. The intensity of Raman peaks varied due to the heterogeneity in composition of these polymineralic stones. The lichens remains on the surfaces 24 h after cleaning and the recolonization of the surfaces 4 years later were identified through the fluorescence appeared in some of the areas: for Siega Verde, surfaces cleaned with water (24 h after cleaning- Fig. 5a), ethanol (regardless of the time lapse- Fig. 5a), benzalkonium chloride (24 h after cleaning- Fig. 5a), Biotin T (24 h after cleaning-Fig. 5a) and laser, mainly at 266 nm (regardless of the time lapse-Fig. 5b); for Coa, Raman spectra from the cleaned surfaces showed fluorescence with exception of those cleaned by Biotin T after 4 years, 1064 nm after 4 years and 266 nm after 24 h (Fig. 5c and d). Despite these three surfaces did not show Raman spectra with fluorescence, broad bands at 1300 cm^{-1} appeared. This band may be possibly attributed to organic matter (Edwards et al., 2003; De Oliveira et al., 2009; Jorge-Villar et al., 2011).

4. Discussion

Regardless of the site, the *in situ* evaluation, 24 h after cleaning, allowed the identification of a greater lichen removal on the chemical-cleaned surfaces than those subjected to both laser wavelengths (1064 nm or 266 nm). Comparing between both sites, for the chemical cleanings in Siega Verde stones, an orange coloration was detected, which was also found by Rivas et al. (2018) removing *Pertusaria amara* and *Pertusaria pseudocorallina* with a Nd:YVO_4 (355 nm) laser on granite. This coloration was associated with lichen thalli remnants, specifically, the algal layer and the medulla. The presence of these remnants on Siega Verde samples induced colorimetric changes, which were visible by an inexpert eye because ΔE^*_{ab} was higher than 3.5 CIELAB units (Mokrzycki and Tatol, 2011). For Coa samples, although organic remnants were not identified by the *in situ* evaluation, the ΔE^*_{ab} was higher than the threshold for a visible change (Mokrzycki and Tatol, 2011) and the

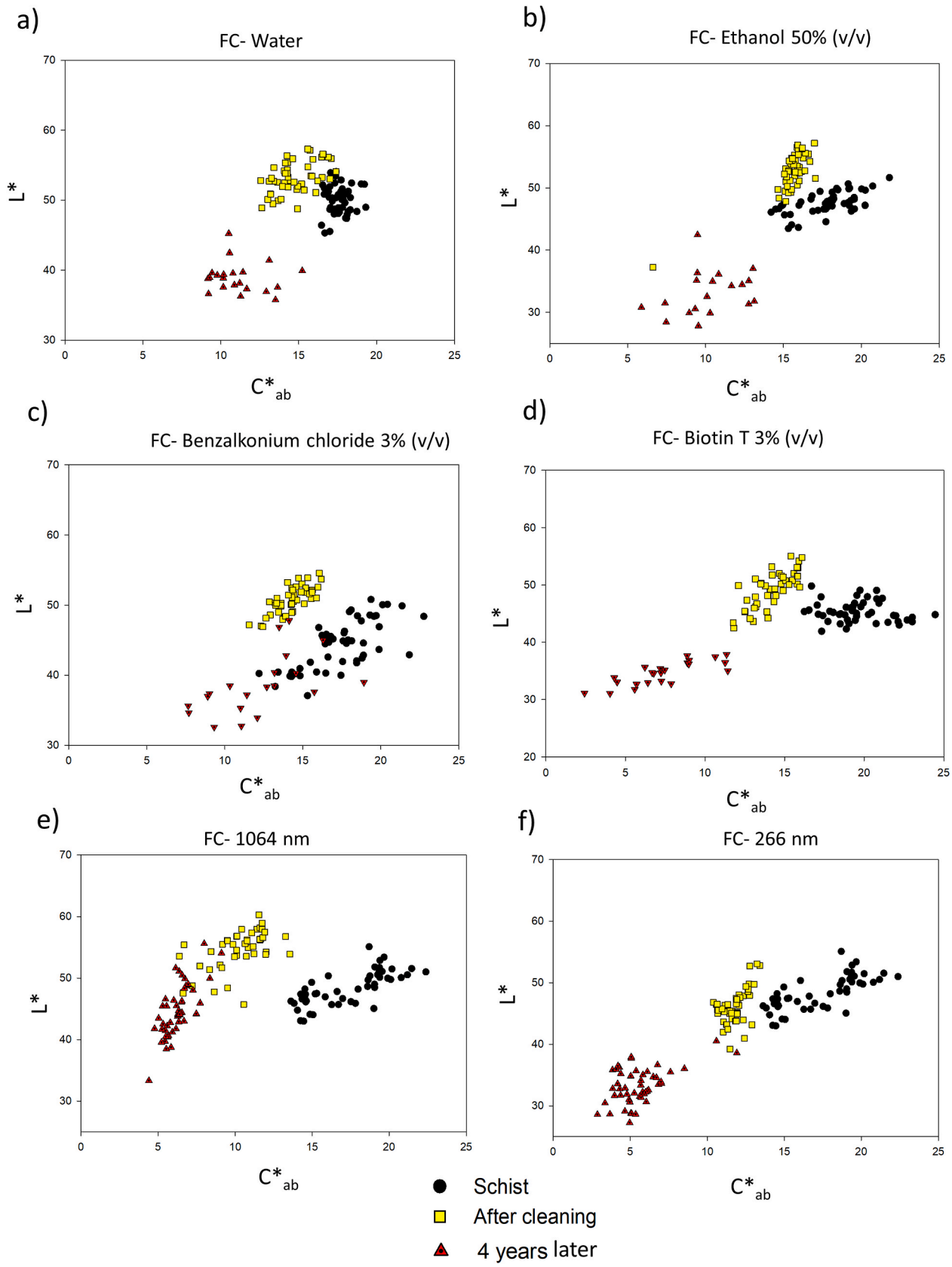

Fig. 4. L*-C*$_{ab}$ scatter plot of the surfaces cleaned of Coa site with water (a), ethanol at 50% (v/v) (b), benzalkonium chloride 3% (v/v), Biotin T 3% (v/v), 266 nm (e) and 1064 nm (f). SV: Siega Verde samples. FC: Coa samples.

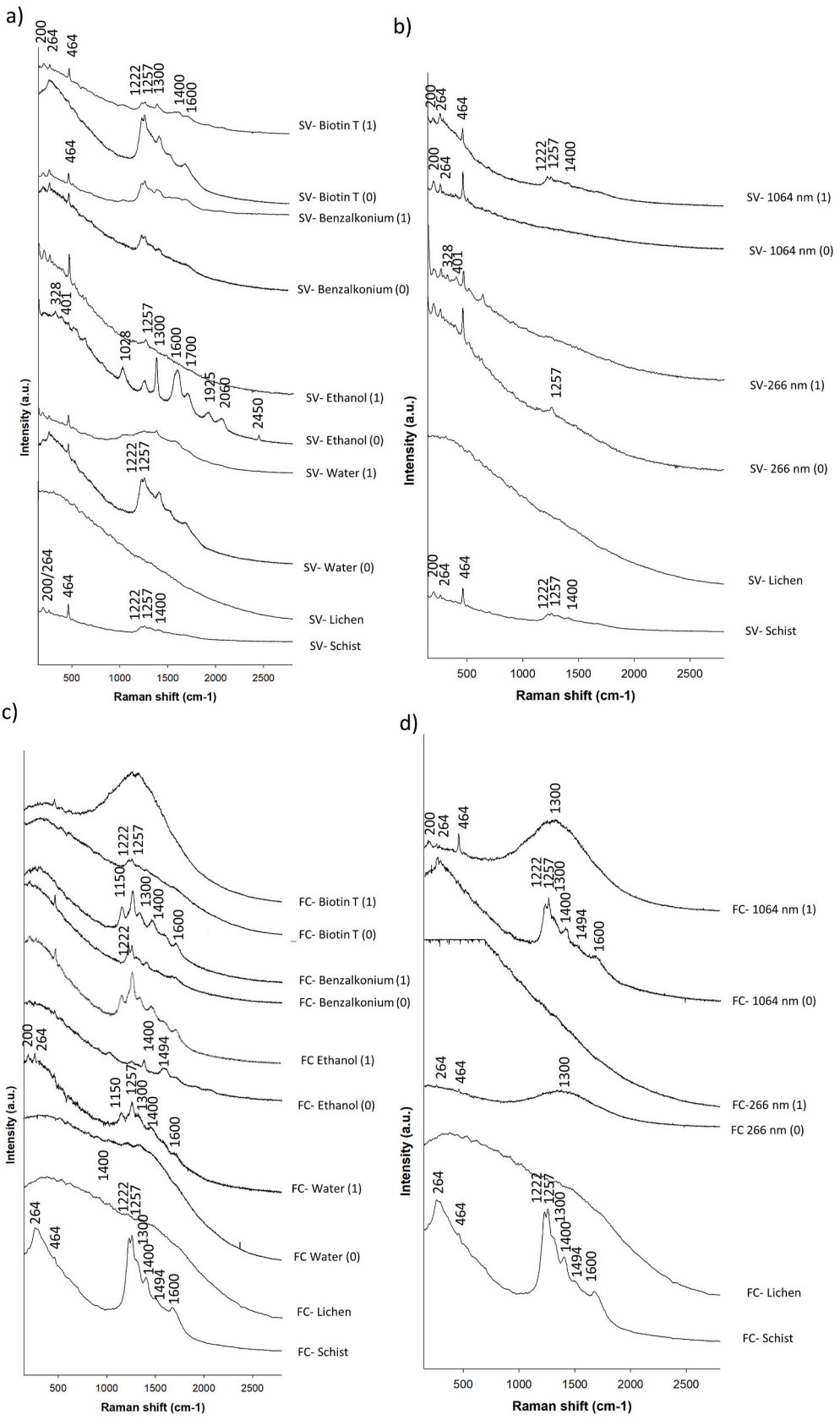

Fig. 5. Raman spectra of the cleaned surfaces from Siega Verde (a and b) and Coa (c and d). SV: Siega Verde samples. FC: Coa samples. 0: 24 h after cleaning. 1: 4 years after cleaning.

a* parameter (redness/greenness) showed lower value than that for the uncolonized schist showing the greenish effect due to the presence of an unnoticed biological colonization as was stated by Sanmartín et al. (2010). Raman spectroscopy also allowed for the identification of biological colonization on the surfaces cleaned with most of the biocides, mainly for Coa samples. The identification of this biological colonization was performed by the presence of the typical fluorescence in the Raman spectra of the lichens. Regarding the surfaces 24 h after cleaning, for the Foz Coa surfaces, and particularly that cleaned by 266 nm, the biological colonization was determined through the broad band at 1300 cm^{-1} which was attributed to organic matter (Edwards et al., 2003; De Oliveira et al., 2009; Jorge-Villar et al., 2011). This band at 1300 cm^{-1} was also detected in the Raman spectra of the surfaces treated with Biotin T and 1064 nm, 4 years after cleaning. However, it is important to consider that the spectra of the uncolonized schist showed also a shoulder at 1300 cm^{-1} and peaks at 1494 and 1600 cm^{-1} which were associated with graphitic carbon (Sadezky et al., 2005). In fact, ΔE^*_{ab} of the surfaces from Coa were higher than those for their counterparts from Siega Verde, despite the fact that in the latter, orange organic matter was found. The ΔE^*_{ab} computation also took into account the effect of the cleaning method on the surface colour, which was identified in the Coa samples as the soil extraction from the fissures associated with the schistosity planes sub-perpendicular to the surface. In Siega Verde, these planes were horizontal and therefore, earth was not dragged from the fissures. This effect was also detected by colour spectrophotometry on the uncolonized samples exposed to the different chemicals. That said, the uncolonized Coa sample cleaned with water showed visible colour changes as a result of the earth extraction.

Regarding laser performance, the cleaning was unsatisfactory mainly for the 266 nm, as complete lichen structures were recognized and the typical fluorescence of the lichen or the broad band at 1300 cm^{-1} were identified in the Raman spectra. Consequently, the ΔE^*_{ab} were higher than the threshold for a visible change (Mokrzycki and Tatol, 2011). The 266 nm did not induce visible colorimetric changes in both uncolonized stones, while 1064 nm already caused visible colorimetric changes on uncolonized surfaces. This colorimetric change, mainly on the 1064 nm-cleaned surfaces, is due to the grooves causing an intense pale effect. These grooves appeared from the thermal and mechanical effects due to the laser-stone interaction (Ramil et al., 2017). These effects depend on the melting point (as the biotite has the lowest melting point, this mineral is the most sensitive) and the respective linear thermal expansion as was reported by Ramil et al. (2017) working with graffiti cleaning on a Spanish granite. Biotite is the first mineral to melt due to its high optical absorption along with its relatively low melting point of 650 °C while other silicates also present in the schist need higher temperatures, e.g. 1710 °C for quartz (Kliem and Lehmann, 1979). In addition to the melting of minerals, mechanical effects such as fissures and grain extraction due to the linear thermal expansion coefficient cause the grooves. Therefore, although the quartz has the highest melting point, this silicate shows the higher average linear thermal expansion coefficient, 16.67 (10^{-6}/ºC), in comparison with the muscovite-biotite with 12.13 (10^{-6}/ºC) (Skinner, 1966; Fei, 1995). The higher linear thermal expansion for quartz in comparison with biotite explains why quartz shows a deterioration pattern with cleavage fracturing, inducing the existence of grooves. Quartz is the main forming mineral in both schists (53% in Coa and 60% in Siega Verde). Gemeda et al. (2018), working with laser cleaning of basalts, found intense thermal effects (melting of minerals) in areas cleaned with 1064 nm while ultraviolet radiation was more protective, minimizing the thermal effects on the stones. Ultraviolet Nd:YAG wavelength (355 nm) was also detected as the less aggressive radiation on granite to extract a sub-aerial biofilm (Barreiro et al., 2020). The L* increases were associated with the increased roughness due to the laser; the rougher the surface the lighter the colour, as was reported by other authors working with other substrates (Simonot and Elias, 2003; Yonehara et al., 2004; Sanmartín et al., 2011b).

Regarding the cleaning durability, after 4 years, the Coa samples cleaned using chemicals showed higher recolonization levels in comparison with Siega Verde samples, because small thalli of *Aspicilia/Circinaria* had grown into the cavities between schistosity planes, which were revealed after earth dragging due to the brushing performed during the cleaning. The higher recolonization on the Coa samples was confirmed by spectrophotometry as a reduction of the colour a* parameter was registered in all the surfaces cleaned with chemicals; as was stated in Sanmartín et al. (2019), these Δa* are related to concentrations of phototrophic pigments, including chlorophyll *a*, phycocyanin and carotenoid. Absence of lichen recolonization was detected on Siega Verde samples cleaned with Biotin T and benzalkonium chloride. Sanmartín et al. (2019) found, after 42 days of inoculation, a reduction of the biofilm growth on schists cleaned with Biotin T. Moreover, Pozo-Antonio et al. (2017), who evaluated the cleaning durability of a granitic wall after 2 years of exposure to a temperate humid climate, reported that benzalkonium chloride was one of the biocides with the best results. In that study, ethanol at 50% was also used and after 2 years the cleaned area showed considerable recolonization. In the current research, on the surfaces cleaned with ethanol, some tiny, unidentifiable thalli were found in Siega Verde and small thalli of *Aspicilia/Circinaria* were identified in Coa. Moreover, areas cleaned with water after 4 years showed a slight recolonization, depending on the site. The higher recolonization found in Coa samples was attributed to the irregularities associated with the schistosity planes that contribute to the adherence of microorganisms. The low bioreceptivity found in Siega Verde samples does not coincide with findings by Sanmartín et al. (2019), who reported higher bioreceptivity index values for these samples regardless of the cleaning method. This difference is related to the orientation of the schistosity planes of the sample, because as observed in micrographs by Sanmartín et al. (2019), these planes were parallel to the surfaces, which is not the real orientation of the planes in the outcrops as shown in Fig. 2. It is important to highlight that for the Siega Verde site, 4 years after cleaning with water, the visual inspection allowed us to identify some small areolas of: *Aspicilia/Circinaria* sp., *Miriquidica deusta* and *Rhizocarpon geographicum*. However, Raman spectroscopy did not register the typical fluorescence or the broad band at 1300 cm^{-1} assigned to biological colonization. This mismatching can be assigned to the size of the area covered by the different techniques. *In situ*, the visual inspection was performed using the entire surface, while Raman spectroscopy allows punctual analyses. Therefore, since the lichens showed a scattered distribution on the surface, the Raman spectra cannot be used as an unequivocal analytical technique to determine the presence of organic matter. The results of this analytical technique must be accompanied by a visual inspection and a chromatic study.

Regarding laser cleaned surfaces, unsatisfactory cleaning was recorded at both sites, and they also showed intense recolonization during these 4 years. Sanmartín et al. (2019) found that laser cleaned schist surfaces showed a well-developed biofilm after 42 days of inoculation due to the cleaning ineffectiveness and also due to the grooves created during the laser application.

5. Conclusions

The harmfulness and durability of cleaning on schist are influenced by the orientation of schistosity planes, mainly for chemicals applied using a brush. Earth dragging from the fissures among schistosity planes once they are subperpendicular to the surfaces affects the colour and the resulting cavities enhance lichen recolonization. The most effective and durable effects were obtained with the biocidal treatments with Biotin T and benzalkonium chloride.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Acknowledgements

The authors are grateful to the Coa Valley and Siega Verde Archaeological Parks for permission to collect rock samples. The study was partially financed by the European Regional Development Fund (ERDF) through the Spanish Ministry of Science and Innovation under project CGL2011-22789. J.S. Pozo-Antonio is grateful to the Spanish Ministry of Economy and Competitiveness (MINECO) for Juan de la Cierva-Incorporación (IJCI-2017-3277).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibiod.2021.105276.

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