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Lichens and other lithobionts on the carbonate rock surfaces of the heritage site of the tomb of Lazarus (Palestinian territories): diversity, biodeterioration, and control issues in a semi-arid environment

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1 **LICHENS AND OTHER LITHOBIANTS ON THE CARBONATE ROCK SURFACES OF**
2 **THE HERITAGE SITE OF THE TOMB OF LAZARUS (PALESTINIAN TERRITORIES):**
3 **DIVERSITY, BIODETERIORATION AND CONTROL ISSUES IN A SEMI-ARID**
4 **ENVIRONMENT**

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36 **Abstract**

137 **Purpose-** Investigations on the lithobiotic colonization of the stone cultural heritage in (semi-)arid
138 regions are needed to address conservation strategies. In this work, lithobiotic communities were
139 examined on the carbonate rock surfaces of the heritage site of the Tomb of Lazarus. We aimed to
140 evaluate their distribution and interaction with the lithic substrate, together with the efficacy of
141 biocidal treatments for their control.

142 **Methods-** Diversity and abundance of lithobionts were surveyed on the Jerusalem stone blocks of
143 three architectural elements. Observations at the lichen-rock interface were carried out by reflected
144 light and scanning electron microscopy. The efficacy against lichens of the widely used biocide
145 benzalkonium chloride (BZK) was compared for different concentrations and application methods,
146 and evaluated by epifluorescence microscopy.

147 **Results-** Chlorolichens were the dominant component of lithobiotic communities, more
148 thoroughly adapted to the semi-arid conditions of the site than mosses and black biofilms of
149 cyanobacteria and dematiaceous fungi. A different structural organization, in terms of thallus
150 thickness and depth of the hyphal penetration component, characterized epilithic and endolithic
151 lichen species, responsible for different deteriogenic activities. Biocidal assays showed that even the
152 methodologies that are usually effective in temperate conditions (as the application of BZK 1.5% by
153 poultice) may not completely devitalize lichens adapted to the stress conditions of semi-arid
154 climates, unless a pervasive biocide diffusion through metabolically active thalli is carefully
155 guaranteed.

156 **Conclusion-** Lithobionts act as biodeteriogens on the semi-arid surfaces of the investigated heritage
157 site. Their removal is thus recommendable, but it needs to be adequately supported with a careful
158 calibration of devitalization strategies.

159
160 **Keywords:** biocide, biodiversity, biofilm, didactic activities, lichens, stone conservation.

164 Lithobiontic (micro-)organisms are recognized as biodeterioration agents of the stone Cultural
 265 Heritage, causing aesthetic damage and often affecting the substrate durability (Tiano 2016).
 366 Although biodeterioration phenomena are a worldwide issue, threatening archaeological and
 567 monumental areas throughout bioclimatic and biogeographical regions, scientific literature on the
 668 interactions of lithobionts with the stone substrates, and related control strategies, mostly deal with
 869 temperate, Mediterranean and tropical areas (Caneva and Pacini 2008). In (semi-)arid regions, the
 970 diversity of lithobionts has been investigated focusing on their tolerance to harsh conditions
 1071 (Wierzchos et al. 2012), while information on biodeterioration processes and experimental support
 1272 to address control strategies are generally poor (Caneva and Pacini 2008; Sohrabi et al. 2017).

1473 Lithobionts, including bryophytes, lichens and biofilms of cyanobacteria, green algae, black yeasts
 1574 and/or meristematic fungi, variously affect rock durability (Sterflinger 2010; Albertano 2012;
 175 Seaward 2015). In some cases, they physically support disaggregation processes by penetrating the
 1876 substrate and changing their volume according to water availability (Chen et al. 2000). In other
 2077 cases, they chemically modify the mineral rock composition by secreting metabolites with acidic
 2178 and chelating functions, which leach the original mineral constituents and/or promote the
 2279 neoformation of biominerals (Adamo and Violante 2000, Crispim and Gaylarde 2005, Gadd 2017).
 2480 On the other hand, a bioprotective role of some lithobiontic communities, acting as a physical
 2581 barrier (umbrella-effect) and biomineralization agents, has sometimes been recognized on certain
 2782 substrates (Gadd and Dyer 2017). Evaluations of biodeterioration vs. bioprotection are thus far to be
 2883 generalizable, as patterns and effects of the interaction between lithobionts and lithic substrates may
 2984 be different for different (micro-)organisms, or even for the same species depending on the
 3185 colonized lithotype and the (micro-)environmental context (Salvadori and Casanova-Municchia
 3286 2016). Similarly, methodologies adopted in restoration work to devitalize lithobionts and support
 3487 effective cleaning procedures showed different efficacy depending on the target microorganism(s)
 3588 (e.g. different lichen species, microbial community), but also on the target cultural object, because
 3689 of some influences of substrate properties and/or weather conditions during treatment and in the
 3890 days after (Caneva et al. 2008; Barresi et al. 2017; Favero-Longo et al. 2017). Accordingly, policies
 3991 for the conservation of Cultural Heritage in areas poorly considered by recent advances of scientific
 4192 research on biodeterioration and its control -as (semi-)arid regions-, may benefit from widening *in*
 4293 *situ* investigations to characterize the effective diversity and impact of lithobiontic communities and
 4394 to calibrate suitable control strategies. Proper choices on the opportunity of removing or preserving
 4595 lithobiontic communities on heritage surfaces, and of (routinely) adopting a specific methodology
 4696 for restoration, need indeed to be based on fitting reference investigations, in terms of (micro-
 4897)organisms, lithologies and environmental scenarios (Caneva et al. 2008).

5098 In the case of the carbonate rocks generally named Jerusalem stone, widely used since ancient times
 5199 in buildings in and around Jerusalem, biodeterioration phenomena on heritage surfaces have been
 5299 investigated since the 1980s: different weathering patterns were related to present (and past)
 54101 biodeteriogenic lithobionts and (micro-)climatic conditions (Danin et al. 1982, Danin 1985, Danin
 55102 and Caneva 1990). Environmental factors driving colonization patterns by epilithic and endolithic
 57103 lithobionts, together with their adaptation strategies and biogeomorphological effects, have been
 58104 deeply characterized in the Negev desert (Garty 1999, Kidron and Temina 2008, 2013, Kidron et al.
 60105 2016). However, on both natural and heritage surfaces, the attribution of weathering patterns to
 61106 different lithobionts has been only marginally associated to the microscopical evaluation of their

107 structural organization on and/or within the lithic substrate, which may account for their actual
108 biodeteriorative or bioprotective impact. Moreover, at the best of our knowledge, strategies to
109 control biodeterioration on heritage buildings in (semi-)arid bioclimatic areas still need
110 experimental calibration. In particular the efficacy of biocide application, variously carried out in
111 temperate and Mediterranean regions to devitalize lithobionts during restoration works (Caneva et
112 al. 2008), should need *in situ* evaluation against target microorganisms adapted to harsh conditions.

113 In this work, we investigated lithobiontic communities on the Jerusalem stone blocks of the heritage
114 site of the Lazarus Tomb, located in the East side of the Jerusalem's metropolitan area. Lithobiontic
115 communities, responsible for deterioration phenomena worth to be considered in planning and
116 performing conservation activities, were characterized, with a particular focus on lichens. We
117 examined if and how the structural organization of lithobiontic microorganisms and biofilms, and
118 their interaction with the lithic substrate, may account for different weathering patterns. Moreover,
119 we aimed to verify the biocidal efficacy of benzalkonium chloride, a quaternary ammonium salt
120 widely used in restoration work to devitalize biodeteriogens (Caneva et al. 2008), against a
121 dominant lithobiont in the site (the epilithic lichen *Variospora aurantia*), by using concentrations
122 usually recommended and widely practiced application methods.

123 This research work was developed as a side activity of an interdisciplinary course on Conservation
124 of Cultural Heritage held at the Lazarus Tomb site, consisting of fourteen weekly training modules
125 to provide basic skills about heritage sciences (chemistry, geology and biology), archaeology, as
126 well as practical knowledge dealing with the conservation of stone, wall paintings and wood. The
127 aim was to introduce a multidisciplinary approach to heritage conservation practice and to develop
128 the critical awareness of young local people, involved with various roles in the ongoing restoration
129 of the heritage site (12 students, both women and men), in relation to the most important
130 conservation issues. The didactic possibility and opportunity of running *in situ* investigations to
131 directly support teaching on biodeterioration topics in countries still lacking of scientific education
132 in restoration is finally discussed.

133 **Material and methods**

134 *Study site and lithobiontic diversity survey*

135 The site of the Tomb of Lazarus (UTM 36R 713635E 3517340N; 670 m a.s.l.) is located in El-
136 Eizariya municipality (ancient Bethany). The climate is transitional between the hot-summer
137 Mediterranean and arid types (Kottek et al. 2006), with 18°C and 264 mm of mean annual
138 temperature and rainfall, respectively, and approx. 60% of mean annual air humidity reported for
139 El-Eizariya (ARIJ 2012).

140 On the side of a steep hill, above the hypogean tomb recognized as that mentioned in the Gospel
141 since at least the 4th century A.D., remains of several ancient religious buildings lie between and
142 around the Mosque of al-Uzair (XVI century) and the modern Catholic Church of Saint Lazarus
143 (XX century) (Caleri 2014, Vella 2017; Fig. 1a). Lithobiontic communities were surveyed in the
144 period October-November 2017 on the architectural elements on which they were cause of aesthetic
145 damage. In particular, 20 relevés 50×50 cm were preferentially distributed, in a number
146 proportional to the commonness of biodeterioration phenomena, on horizontal and vertical surfaces

148 of the Jerusalem stone blocks of: (i) pillars of the superimposed Byzantine and Crusaders' antique
149 churches in the lower "Plaza" (n= 10; Fig. 1b) and (ii) walls of the Crusaders' Monastery in the
150 upper part of the site (n= 6; Fig. 1c), uncovered by archaeological excavations in 1949-1953, and
151 (iii) the facade of the modern Saint Lazarus church (n= 4; Fig. 1b), built in 1952-1955. The
152 Jerusalem stone *s.l.* is a bio-micritic dolomitic limestone of Cretaceous Age, belonging to the
153 Menhua and Mishash Formations (Rabinovich et al. 2014; Avnimelech, 1966). It has been quarried
154 around Jerusalem, hence its name, since the 2nd millennium BC and constitutes the main building
155 material of the city and its surroundings (Calvo and Regueiro, 2010).

156 Each relevé (plot) was surveyed using a square grid divided into 25 quadrats (10×10 cm), where the
157 presence of different lithobionts (bryophytes, lichens, cyanobacteria- and/or black-fungi dominated
158 biofilms) and, in more detail, lichen species was visually estimated. Provisional identifications
159 during fieldwork were subsequently checked in the laboratory by microscopical observations on
160 small samples of biofilms and lichen thalli, collected by means of micro-invasive techniques. The
161 frequency of lithobionts and lichen species within each plot was calculated as the sum of their
162 occurrences within the grid quadrats (Giordani et al. 2014). Lichen species were identified using
163 Clauzade and Roux (1985) and monographic descriptions. Nomenclature follows Nimis (2016).
164 Sample vouchers were deposited at the Cryptogamic Herbarium of the University of Torino (HB-
165 TO Cryptogamia).

166 *Reflected light and scanning electron microscopy at lichen-rock interface*

167 Carbonate rock (Jerusalem stone *s.l.*) fragments colonized by lichens, already partially detached
168 from blocks of a wall of the Monastery that underwent rebuilding, were collected with the aid of a
169 lancet and used to investigate lichen-rock interactions. After including fragments in a polyester
170 resin, polished cross sections (approx. 25-40×10×5 mm) were obtained for the epilithic *Variospora*
171 *aurantia* (Pers.) Arup, Frödén & Söchting (n=3) and *Verrucaria ochrostoma* (Leight.) Trevis. (n=1)
172 and the endolithic *Bagliettoa baldensis* (A. Massal.) Vězda (n=1) and *Pyrenodesmia erodens*
173 (Tretiach, Pinna & Grube) Söchting, Arup & Frödén (n=1). Sections were stained using the periodic
174 acid – Schiff (PAS) to visualize the biological component within the lithic substrate (Favero-Longo
175 et al. 2005). Reflected light microscopy (RLM) observations were carried out using an Olympus
176 SZH10 to measure thallus thickness and the depth of hyphal penetration component (*sensu* Favero-
177 Longo et al. 2005) of *V. aurantia*, *V. ochrostoma* and *B. baldensis*. Average values of massive
178 penetration, i.e. depth at which hyphal penetration is continuous beneath the thallus, and maximum
179 penetration, i.e. depth at which hyphal penetration is occasionally observed, were quantified by
180 measuring intervals established at every 800 µm from the thallus margin (Favero-Longo et al.
181 2011).

182 Polished cross sections were also observed with a JEOL JSM IT300LV (High Vacuum - Low
183 Vacuum 10/650 Pa - 0.3–30 kV) scanning electron microscope in back-scattered (BSE) modes
184 (BED-C and BED-S) to visualize the ratio between (bio-)clasts (white to light grey-coloured in
185 BSE) and voids including clast boundaries, pores and cracks (porosity *sensu lato*, *s.l.*, black
186 coloured in BSE) (Sardini et al., 2006; Favero-Longo et al., 2009; Morando et al. 2017). On two
187 cross sections, BED-C images were acquired (at 150× magnification) of: a 300 µm layer beneath
188 the surface showing the epilithic *V. aurantia*, the endolithic *P. erodens*, and absence of lichens
189 (section 1); a 300 µm layer beneath the surface showing the endolithic *B. baldensis* and absence of
190 lichens (section 2); 300 µm layers at more than 2 mm from the surface (sections 1 and 2). BED-C

191 images of sectioned fresh fragments (n=2) sampled from the interior of blocks were also acquired as
192 control. Images (at least three per each different case study) were processed by a pixel-based grey
193 scale classification using the software WinCAM Pro 2007d (Regent's Instrument, Canada) to
194 quantify total percentage porosity s.l. BED-S images on the same areas were examined to
195 characterize hyphal penetration patterns with respect to the rock microstructure.

196 The cross sections were prepared in the Laboratory of Lichenology (ISO 9001:2015) and are
197 conserved in the Lichen-Petrographic Collection of the Herbarium of the University of Torino
198 (Gazzano et al. 2007). Petrographic thin sections, prepared from the fresh fragments of the interior
199 of rock blocks and used for a petrographic analysis of the lithotype, are conserved in the Rocks and
200 Thin Sections Collection of the Earth Science Department of the University of Torino.

201 *Biocide assays*

202 Biocide assays were performed, with commercial products available on site, on two distinct
203 carbonate rock surfaces (block-1, block-2) colonized by *V. aurantia* and, subordinately, other lichen
204 species. The foliose epilithic *V. aurantia* was chosen as target species because of its extreme
205 commonness on natural and man-made lithic surfaces in urban and rural areas of the middle East,
206 due to its tolerance to harsh environmental conditions, including air pollution (Garty 1999), and
207 because of the possibility of sampling its thalli more easily than those of epilithic crustose and
208 endolithic lichens. The commercial product BAC50 (distributed by Monum, Jerusalem), a water
209 solution of benzalkonium chloride (BZC 50% v/v), was applied at two different final concentrations
210 of the active substance (BZC 0.25% and 1.5%), the highest one defined according to manufacturers'
211 instructions for analogous products (e.g. BAC50 distributed by Sinopia, Torino, Italy). Tap water
212 was used for dilutions and for control assays, as it was the water available in the restoration yard.
213 On block-1, after having pre-wetted the rock surface and the thalli with sprayed tap water, the
214 biocide was applied on distinct plots (approx. 10×10 cm), (i) using a paint brush and (ii) with a
215 cellulose-sepiolite poultice applied on Japanese paper. The treated plots were kept covered for 3 h
216 with aluminium foils. Thereafter, the poultice was gently removed and all the treated surfaces were
217 rinsed with water and a nylon brush. On block-2, after having moistened the rock surface and the
218 thalli with sprayed tap water, the biocide was applied on distinct plots, using (i) a paint brush and
219 (ii) a nebulizer. The plots were not kept covered after the treatment and, after 3 h, were rinsed with
220 tap water or left unrinsed.

221 Fragments of *V. aurantia* thalli were collected from plots on blocks-1 and -2, at the end of the
222 biocide treatment and after 40 days, to compare the biocide effects when applied by brush vs. with
223 poultice and on rinsed vs. unrinsed plots, at a short and long term. The sampled thalline fragments
224 were conserved in Falcon tubes for 48 h and then hand-made cross sectioned to carry out
225 epifluorescence observations under a Nikon Eclipse 300 microscope. Quality and quantity of the
226 fluorescence emitted by photobiont cells, spatially informative on the vitality of the photobiont
227 layer (e.g. Tretiach et al., 2012), were evaluated, and the data interpreted using an ordinal scale on
228 the relative abundance of viable (red coloured) and devitalized (appearing white) cells (Favero-
229 Longo et al. 2017).

230 **Results**

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233 The field survey and subsequent microscopical observations indicated chlorolichens, bryophytes
 234 and black biofilms of different compositions as biodeteriogens responsible for phenomena of
 235 aesthetic damage in the site of the Tomb of Lazarus (Figs. 1d-f and 2). On the carbonate rock blocks
 236 of the antique churches and the Monastery, chlorolichens were the dominant component in terms of
 237 both frequency per plot (median >90% of quadrats) and surface cover (median >20%). Black
 238 biofilms and, subordinately, bryophytes were common on the pillars of the antique churches, on
 239 which they were responsible for remarkable covers (av. > 10%), while their presence was rather
 240 negligible on the Monastery walls. On the walls of the modern church, chlorolichens and black
 241 biofilms displayed high frequency values (av. > 30%), but remarkable covers only sparsely occurred
 242 (median < 5%) and were mostly located in the upper parts of the façade (Fig. 1b), not accessed for
 243 the relevés.

244 A total of 17 chlorolichen *taxa* were listed in the site, most of which displayed crustose epilithic
 245 (65%) and crustose endolithic (24%) growth form (Table 1). A similar number of species was
 246 detected on walls of the antique churches (n=14) and the Monastery (n=13), while only few species
 247 (n=6) characterized the façade of the modern church. The crustose endolithic *Verrucaria*
 248 *ochrostoma* and the crustose placodiomorph *Variospora aurantia* were the most widespread species
 249 through the overall surveyed quadrats (frequency of 33% and 24%, respectively), and determined
 250 remarkable covers on the surfaces of both the antique churches and the Monastery (max. values >
 251 10%). The endolithic *Bagliettoa baldensis* and the epilithic *Lepraria* sp. were also locally
 252 responsible for remarkable covers (20%) on the walls of the antique churches, while other species,
 253 even when characterized by high frequency values, never displayed cover values \geq 20%.

3254 Coccoid and, rarely, filamentous cyanobacteria, together with black yeasts and meristematic fungi,
 3255 were the main components of the black biofilms, which developed directly on the carbonate
 3256 substrate and -more often- on senescent thalli of chlorolichens (Fig. 3). Black fungi were
 3257 particularly abundant on the walls of the modern church, while their detection on the antique church
 3258 and Monastery blocks was sporadic.

260 *Lichen interactions with the carbonate rocks*

4261 RLM observations at the lichen-substrate interface showed a different structural organization of the
 4262 epilithic *V. aurantia* and endolithic species (Fig. 4). The former displayed a thicker thalline
 4263 component (av. 0.2 mm), but its hyphal penetration component (HPC sensu Favero-Longo et al.
 4264 2005) was scarce, characterized by an av. depth of massive penetration of 0.03 mm and av. values
 5265 of maximum penetration of 0.2 mm (Fig. 4b-c). In some cases, local staining of biological
 5266 structures within the substrate beneath the *V. aurantia* thalli corresponded to free colonies of
 5267 dematiaceous fungi, already observed for their coloured appearance before the PAS treatment, and
 5268 not to mycobiont penetrating hyphae (Fig. 4a). By contrast, only a thin lithocortex and
 5269 discontinuous thalline structures (av. thickness \leq 0.1 mm) were visible on surfaces colonized by the
 5270 endolithic *B. baldensis* and *V. ochrostoma*, respectively. Both the species showed a remarkable
 5271 hyphal penetration component, massively penetrating the substrate down to av. depths of 0.8 mm
 6272 and with maximum penetration av. values of 2.1 and 1.7 mm, respectively (Fig. 4d-e). Moreover,
 6273 their perithecia endolithically developed down to 0.3-0.4 mm, their growth appearing the potential

274 responsible for pitting phenomena sparsely observed on the carbonate blocks through the site.
275 Massive hyphal growth through the carbonate matrix, rather than limited to intragranular porosities
276 or rock fractures (as sometimes observed for *V. aurantia*) characterized both endolithic species. It is
277 worth noting that strong penetration of endolithic thalli (like those of *Pyrenodesmia erodens* in Fig.
278 4c) growing adjacently to *V. aurantia*, which shows negligible hyphal penetration component,
279 indicates that the different species, rather than variations in substrate properties, are responsible for
280 the heterogeneous structural organizations of the different *taxa* above and beneath the rock surface.
281 SEM-BSE observations of the petrographic thin sections showed the bioclastic structure of the
282 Jerusalem stone and a remarkable porosity internal to bioclats (Fig. 5a). The polished cross
283 sections of the same fresh fragments highlighted that the microfossils are tightly embedded within a
284 fine-grained calcite matrix (Fig. 5f). The massive hyphal penetration by endolithic lichens affects
285 the total porosity of the upper rock layers (Fig. 5b). The 300 μ m layer penetrated by *P. erodens*
286 (Fig. 5e) and *B. baldensis* (Fig. 5g-h) beneath the rock surface showed significantly higher values of
287 total porosity than those covered by *V. aurantia* (Fig. 5d) or not colonized by lichen thalli (Fig. 5c).
288 Such endolithic effect was observed on both section 1, which in absence of *P. erodens* showed a
289 low total porosity similar to control sections, and section 2, showing a total porosity higher than
290 control sections even at depths lower than 2 mm from the surface. BED-S observations confirmed
291 the hyphal penetration through the rock matrix (Fig. 5g) and showed the hyphal invasion of the
292 bioclast internal porosity (Fig. 5h).

293 *Biocide efficacy*

294 Fluorescence microscopy showed that any but one of the assayed biocide treatments significantly
295 affected the vitality of *V. aurantia*. In thalline fragments collected from block-1 at the end of the
296 biocide treatments, the photobionts of thalli exposed to 0.25% and 1.5% BZC applied by brush or to
297 0.25% BZC applied with poultice still widely preserved their chlorophyll integrity (indicated by the
298 red photobiont fluorescence), particularly in the lower part of the algal layer (Fig. 6b). Only the
299 1.5% BZC applied with poultice remarkably killed most of the treated thalli: a residual occurrence
300 of viable photobiont cells was only rarely observed in the lower part of the algal layer in few of the
301 thalline fragments (Fig. 6a). Nevertheless, also this treatment failed to devitalize the whole
302 photobiont populations in all thalli, and some recovery of viability was observed after 40 days. On
303 block-2, neither the rinsed nor the non-rinsed thalli treated with BZC 0.25% and 1.5%, by brush or
304 nebulizer, displayed the devitalization of the whole photobiont layer, with wide amounts of viable
305 cells persisting in its lower part (Fig. 6c). Moreover, a remarkable recovery of viability was
306 observed after 40 days, with many viable cells appearing through the photobiont layer (Fig. 6d).

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308 **Discussion**

309 *Biodiversity of lithobionts*

310 Cyanobacteria, black fungi and/or lichens were indicated as specialized biodeteriogenic
311 microorganisms on archaeological and monumental surfaces in (semi-)arid areas of the
312 Mediterranean basin, mostly because of their high resistance to desiccation and high solar
313 irradiation (Warscheid 2003; Mihajlovski et al. 2015). However, a low number of studies is so far
314 available on biodeterioration of cultural heritage in semi-arid regions (Caneva and Pacini 2008;

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315 Sohrabi et al. 2017), and they poorly considered interactions between lithobionts and their substrate,
316 and potential control strategies. In the heritage site of the Tomb of Lazarus, we found chlorolichens
317 as the dominant component of lithobiontic communities on the surveyed architectural elements,
318 where cyanobacteria and black fungi were instead only locally abundant. Such subordinate
319 occurrence of cyanobacteria and the absence of cyanolichens, reported as dominant lithobiontic
320 components on the walls of historic buildings in Jerusalem, at few kilometres and on the same
321 carbonate substrate (Danin and Caneva 1990), likely follows the strong aridity gradient from the
322 center of the metropolitan area of Jerusalem (500-600 mm of annual rainfall) to the Eastern suburbs,
323 including El-Eizariya (<300 mm). Although cyanobacteria and cyanolichens may longer retain
324 water by their gelatinous sheats, chlorolichens can better adapt to the scarcity of liquid water and
325 the availability of humid air typical of (semi-)arid areas (e.g. 60% mean annual air humidity in El-
326 Eizariya; ARIJ 2012), as their photobionts are able to photosynthesize in the partially hydrated state
327 (Honegger et al. 2012). Regional climate variability is thus the primary factor to drive lithobiontic
328 diversity and different deterioration threats, (Caneva and Pacini 2008).

329 Differences in the relative abundance of lithobiontic components on the surveyed architectural
330 elements highlight the parallel remarkable role of local topography and building geometry to
331 determine micro-environmental conditions and shape different micro-niches at the scale of a single
332 heritage site (Nimis et al. 1998; Tonon et al. 2019). Similarly, the distribution pattern of lichen
333 species is characterized by higher species turnover (beta-diversity) between than within each
334 architectural element (SDR analysis, following Podani and Schmera 2011, not shown). High
335 frequency and cover by coccoid cyanobacterial biofilms only characterized the pillars of the antique
336 churches, located in the lower and wind-sheltered part of the site, where the co-presence of
337 abundant bryophyte mats also indicates the periodical availability of liquid water, necessary for
338 their photosynthetic activity and survival (Lakatos 2011). By contrast, chlorolichens are also
339 adapted to the more xeric conditions of the walls of the Monastery, located in the wind-exposed
340 upper part of the site, where rock surfaces are widely covered by dust (Fig. 1e). It is worth noting
341 that nitrophytic lichen species, tolerant of (and even favoured by) nitrogen deposition, including
342 ammonia pollution, prevail throughout the site (nine out of the 17 species have maximum
343 eutrophication index ≥ 4 ; Table 1), as generally reported for monuments in urban (Aptroot and
344 James 2002, Seaward 2015) and agricultural areas (Nascimbene and Salvadori 2008, Sohrabi et al.
345 2017). Both the dominant species *Variospora aurantia* and *Verrucaria ochrostoma* are nitrophytic
346 species (Nimis 2016). The former, displaying highest frequency and cover values on the Monastery
347 walls, was previously reported as highly tolerant to harsh conditions of sun irradiation, aridity and
348 air pollution (Garty 1999). Only the nitrophytic *Calogaya pusilla* determined remarkable lichen
349 covers on the walls of the modern church, made of the same lithotype, but characterized by a poor
350 colonization with respect to the surrounding antique surfaces. Abiotic and biotic weathering forces
351 may have not so far modified the scarce intrinsic bioreceptivity of the strong and durable Jerusalem
352 stone (Ghadban and Ashhab 2011) towards a remarkable secondary (i.e. weathering induced)
353 bioreceptivity (Guillitte 1995, Miller et al. 2009). A prominent occurrence of species of *Caloplaca*
354 s.l. (six out of the 17 species in the investigated site) also characterized carbonate surfaces of the
355 Pasargadae UNESCO world heritage site (Iran), where frequent events of wind-storms contribute to
356 nitrogen and dust deposition (Sohrabi et al. 2017). In such species, adapted to alkaline substrates
357 (Ariño et al. 1995), a role of dust deposits on the thalline surface in protecting photobionts by
358 excessive sun irradiation, together with biosynthesized calcium oxalate and parietin deposits (Smith
359 et al. 2009), may be worth to be considered.

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361 The endolithic growth, characterizing *V. ochrostoma* and other species in the site, represents a
362 strategy to protect photobionts from harsh environmental conditions in hot and cold arid
363 environments (Wierzechos et al. 2012). Physiology and environmental adaptation of euendolithic
364 (i.e. actively boring) lichens in (semi-)arid environments has been widely investigated on natural
365 outcrops (Kidron et al. 2016). Such adaptive pattern has consequences in terms of biodeteriorative
366 effects, as widely documented by pitting phenomena on carbonate rocks of (semi-)arid natural
367 environments (Danin 1992; Bungartz et al. 2004; Kidron and Temina 2008), but also, remarkably,
368 on monuments of temperate and Mediterranean areas (Salvadori and Casanova Municchia 2016;
369 Pinheiro et al. 2018). Endolithic lichens also characterized the Pasargadae iranian site (Sohrabi et al.
370 2017), but the presence of discocarpous rather than pyrenocarpous species determined the absence
371 of remarkable biopitting phenomena (Pinna et al. 1998). On the contrary, such pitting phenomena
372 characterize the investigated site, where both the pyrenocarpous *V. ochrostoma* and *B. baldensis*,
373 producing large perithecia, widely occur on and penetrate the carbonate rock blocks, determining
374 physical and chemical deterioration processes. Owing to conservative reasons, microscopy
375 observations were limited to one single cross sectioned fragment for each endolithic species.
376 Nevertheless, it is worth noting that the depths of massive and maximum hyphal penetration
377 (approx. 1 and 2 mm respectively) were the same quantified for *B. baldensis* in fine grained
378 limestones from the temperate N-Italy (Favero-Longo et al. 2009). The pattern of endolithic growth
379 within a certain substrate thus appears as a functional trait poorly influenced by the climate context.
380 On the other hand, as previously observed for the epilithic *Calogaya biatorina* (A. Massal.) Arup,
381 Fröden & Sötching in Pasargadae (Sohrabi et al. 2017), *V. aurantia* did not show a conspicuous
382 hyphal penetration, which seems to exclude mechanical disaggregation activity or active substrate
383 dissolution. Accordingly, no weathering patterns were observed in (semi-)arid environments on the
384 surface of cobbles colonized by other epilithic species of *Caloplaca* s.l., and a potential
385 bioprotective role was even suggested (Kidron et al. 2016). Our SEM-BSE observations,
386 characterizing the lichen effect on porosity, i.e. a physical property relevant for surface durability,
387 did not show bioprotection by *V. aurantia*, but a remarkable deterioration impact by endolithic
388 lichens. In particular, hyphal penetration seems to promote the connection between the surface and
389 the internal porosity of bioclasts, finally threatening the endolithically colonized rock volumes more
390 than the others.

391 Moreover, the observed occurrence of additional lithobionts at the thallus-rock interface, in
392 particular beneath *V. aurantia*, confirms a role of lichens in creating suitable microhabitats for the
393 colonization of rock substrata and thus supporting biodeterioration dynamics (de los Ríos et al.
394 2009). The abundance of senescent thalli intermingled in the black cyanobacterial and fungal
395 biofilms also suggests a role of lichens in promoting the lithobiontic colonization on the rock
396 surfaces in semi-arid environments. In this sense, both endolithic and epilithic lichen species behave
397 as biodeterioration agents on carbonate stone materials in semi-arid environments, physically and
398 chemically affecting the durability of their surface layers, at a millimeter scale, and/or determining
399 surface aesthetic damage with the growth of the thalline component and/or favouring a lithobiontic
400 succession. Biodeterioration processes particularly threaten the surface stone conservation in the
401 case of the antique architectural elements, more colonized by lichens and already exposed to a long
402 history of abiotic and biotic weathering forces. Poor lichen colonization on the approx. 60 years old
403 surface of the modern church, mostly due to epilithic species of *Caloplaca* s.l., suggests that very

404 long times (likely centuries) may be necessary before fresh surfaces of the Jerusalem stone
405 significantly suffer lichen-driven physical disaggregation or chemical leaching, while the aesthetic
406 damage is a more immediate concern. However, the contribution by endolithic lichens in increasing
407 open porosity may likely speed up the process.

408 *Control of biodeteriogens*

409 Findings about biodeterioration activity support the opportunity of removing lichens and other
410 lithobionts from heritage surfaces in semi-arid environments. In this context, the preliminary
411 devitalization of lithobionts is a crucial step, as the residual occurrence of viable cells on the
412 cleaned surface or their dispersion during the mechanical removal of biofilms and thalli may be a
413 source for a short-term reoccurring of colonization (Jurado et al. 2014, Pinna 2017). The necessity
414 of species- and site-specific calibrations to assess the efficacy of biocide treatments against lichens
415 has been recently experimentally remarked (Favero-Longo et al. 2017). The assays carried out in
416 the site of the Tomb of Lazarus indicate that a single treatment with BZC, used in a low (0.25%) but
417 also in the maximum (1.5%) concentration recommended for a series of BZC commercial products
418 of analogous formulation, applied by brush or with poultice, does not guarantee a good efficacy in
419 the devitalization of *V. aurantia*. A scarce efficacy of brush applications, already reported for
420 several biocide products (Favero-Longo et al. 2017), was here further confirmed. The general
421 efficacy previously documented for poultice applications, and correlated to an increased contact
422 time between biocide and the hydrated thalli (Favero-Longo et al. 2017), was not recorded in this
423 case. Hydration of thalli is a crucial step for the efficacy of the biocide treatments, as lichens and
424 the other poikilohydric lithobionts (i.e. organisms with the water status depending on the
425 environment) are strongly stress tolerant when dry, but sensitive when wet and metabolically active
426 (Tretiach et al. 2012, Pinna 2017). The layering of killed and viable cells in the upper and lower
427 parts of the photobiont layer, respectively, suggests that (i) pre-wetting of the rock surfaces and
428 thalli with sprayed water, (ii) brush, nebulizer and, even, poultice applications of biocide and (iii)
429 the final rinsing step were not sufficient to hydrate the thalli and allow the biocide penetration. The
430 efficacy of the adopted BZC at 1.5% of concentration on the target organism is indicated by the
431 successful devitalization of part of the photobiont layer (despite the use of tap water and the
432 abundance of carbonate deposits on the thallus surface), while the critical crux seems related to the
433 biocide diffusion. Biocide applications were performed in a cloudy day (temperature at midday
434 approx. 30°C) and, in the case of the poultice application, the thalline surfaces, covered with
435 aluminium foils, still appeared humid at poultice removal. The reached level of hydration, however,
436 did not likely allow the metabolic activation of the whole photobiont layer, and its lower part was
437 locally unaffected. Similarly, in the case of block-2, hydration of thalli by environmental humidity
438 during the weeks following the treatment was not sufficient to increase the biocidal effect, and
439 recovery signals were even observed after 40 days. Such resilience to biocide treatments confirms
440 the wide-spectrum stress tolerance reported for *V. aurantia* (Garty 1999) and indicates, in particular
441 for lichens adapted to (semi-)arid climates, the need of carefully evaluating what application
442 method may provide a pervasive diffusion of biocides through metabolically active thalli. In this
443 sense, results by brush and nebulizer applications could be hardly improved, while modulation of
444 the duration of poultice applications and the possibility of repeated poultice hydration may possibly
445 make the treatment effective. On the other hand, the adoption of devitalization techniques
446 alternative to biocide applications is generally recommended to limit the spread of toxic compounds
447 (Pinna 2017). Difficulties encountered in biocide treatments make the heat shock treatments of

448 lithobionts (Tretiach et al. 2012) particularly worth of consideration to control biodeterioration in
449 semi-arid environments, taking advantage of -rather than suffering- hot climate conditions.

450 *Didactic value of in situ investigations*

451 A new scientific and critical concept of conservation, based on a multidisciplinary approach
452 involving scientific, historical and artistic knowledge, was early promoted in Italy, since the end of
453 the 1930s, by Argan (1938) and Brandi (1963), contributing to the development of the profession of
454 scientific conservator-restorer. Knowledge in human and natural sciences, combined with a strong
455 awareness in conservation issues, practical intervention techniques and skills in planning activities
456 and studies specifically tailored on artwork needs, acquired through specific educational pathways,
457 is now required to be qualified as professional conservators and restorers in several countries (e.g.
458 in Italy; MiBACT 2014). In some areas of the world, however, such kind of professional training
459 can be hardly accessed, and it is difficult to involve in restoration projects experts which are
460 scientifically aware of all the conservation issues.

461 In the site of the Tomb of Lazarus, theoretical and practical activities dealing with biology applied
462 to cultural heritage (n= 2 weekly modules) made the students -including those having a coordinative
463 and an operative role in the restoration work- aware of the occurrence of lithobionts (previously
464 generally perceived as “dirt”) and of their potential role in stone biodeterioration. Moreover, they
465 were taught about the necessity of devitalizing biodeteriogens before their mechanical removal,
466 which may otherwise risk of scattering rather than control their occurrence, as experienced in other
467 restoration projects documented for the (semi-)arid regions (Sohrabi et al. 2017). Such taking-home
468 messages is an essential part of the stock of knowledge that everyone working in conservation of
469 the stone cultural heritage throughout the world should have.

471 **Conclusions**

472 In conclusion, lithobiontic communities colonize antique and modern carbonate rock surfaces in the
473 heritage site of the Tomb of Lazarus. Epilithic and endolithic chlorolichens result more thoroughly
474 adapted to the semi-arid climate conditions of the site, while bryophytes and biofilms of
475 cyanobacteria and black fungi only characterize wind-sheltered architectural elements. Both
476 endolithic and epilithic lichens are agents of biodeterioration, by physically and chemically
477 affecting the rock surface, causing aesthetic damage or supporting lithobiontic succession. Their
478 removal is thus recommendable, particularly for the antique surfaces already affected by long-term
479 exposure to biotic and abiotic weathering factors. However, the preliminary devitalization practices,
480 necessary for an effective restoration intervention, may encounter remarkable tolerance and
481 resilience. Indeed, even the application by poultice of the widely used biocide BZC, usually
482 effective against lithobionts in temperate conditions, may be ineffective to devitalize lichens
483 adapted to the stress conditions of semi-arid climates. If the adopted application method does not
484 guarantee a pervasive biocide diffusion through a metabolically active thallus, the efficacy of the
485 treatment is incomplete and mechanical cleaning operations risk to scatter, rather than control, the
486 lithobiontic colonization.

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493 *Compliance with ethical standards*

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1495 *Research involving human participants.* N/A

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496 *Informed consent.* N/A

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706 **Tables**

707 Table 1 Lichen diversity and abundance on the architectural elements (arch. el.) in the site of the
 708 Tomb of Lazarus: antique churches (i), Monastery (ii), modern church (iii). Growth forms of lichen
 709 species (crustose endolithic, Cr. en.; crustose epilithic, Cr. ep.; foliose, Fol.; squamulose, sq) and
 710 eutrophication index (1, not resistant to eutrophication; 2, resistant to a very weak eutrophication; 3,
 711 resistant to a weak eutrophication; 4, occurring in rather eutrophicated situations; 5, occurring in
 712 highly eutrophicated situations) according to Nimis (2016).

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Lichen species	Growth form	Eut	Av. frequency per arch. el. (% of plots)			Av. frequency per plot (% of quadrats)			Maximum cover (%)		
			(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(iii)
<i>Bagliettoa baldensis</i> (A. Massal.) Vězda	Cr. en.	1	50.0	16.7	-	13.8	0.8	-	20	+	-
<i>Calogaya</i> cfr. <i>decipiens</i> (Arnold) Arup, Frödén & Søchting [§]	Cr. ep.	4-5	10.0	16.7	-	0.4	7.4	-	+	2	-
<i>Calogaya pusilla</i> (A. Massal.) Arup, Frödén & Søchting [§]	Cr. ep.	2-3	-	-	50.0	-	-	28.0	-	-	1
<i>Candelariella aurella</i> (Hoffm.) Zahlbr.	Cr. ep.	2-4	80.0	33.3	-	24.6	8.2	-	18	1	-
<i>Circinaria calcarea</i> (L.) A. Nordin, Savić & Tibell	Cr. ep.	2-3	20.0	33.3	25.0	5.0	15.6	2.0	6	16	+
<i>Circinaria contorta</i> (Hoffm.) A. Nordin, Savić & Tibell	Cr. ep.	3-5	20.0	16.7	-	9.2	0.8	-	15	+	-
<i>Flavoplaca polycarpa</i> (A. Massal.) Arup, Frödén & Søchting [§]	Cr. ep.	1-3	20.0	-	-	2.9	-	-	+	-	-
<i>Lepraria</i> sp.	Cr. ep.	-	60.0	16.7	-	27.5	1.6	-	20	1	-
<i>Myriolecis albescens</i> (Hoffm.) Sliwa, Zhao Xin & Lumbsch	Cr. ep.	3-4	40.0	16.7	25.0	7.1	6.6	5.0	2	6	+
<i>Myriolecis</i> gr. <i>dispersa</i> (Pers.) Sliwa, Zhao Xin & Lumbsch	Cr. ep.	2-4	10.0	66.7	25.0	1.3	24.6	2.0	+	3	+
<i>Myriolecis pruinosa</i> (Chaub.) Sliwa, Zhao Xin & Lumbsch	Cr. ep.	2-4	30.0	50.0	-	12.1	10.7	-	2	4	-
<i>Physconia muscigena</i> (Ach.) Poelt	Fol.	2-4	30.0	16.7	-	9.2	0.8	-	+	+	-
cfr. <i>Pyrenodesmia erodens</i> (Tretiach, Pinna & Grube) Søchting, Arup & Frödén [§]	Cr. en.	1-2	-	16.7	25.0	-	9.8	4.0	-	2	+
<i>Squamarina</i> cfr. <i>gypsacea</i> (Sm.) Poelt	Sq.	1-3	-	16.7	-	-	1.6	-	-	+	-
<i>Variospora aurantia</i> (Pers.) Arup, Frödén & Søchting [§]	Cr. ep.	3-4	30.0	100.0	25.0	12.1	63.9	4.0	12	23	+
<i>Verrucaria ochrostoma</i> (Leight.) Trevis.	Cr. en.	4-5	70.0	83.3	-	43.3	41.0	-	20	12	-
<i>Xanthocarpia lactea</i> (A. Massal.) A. Massal. [§]	Cr. en.	2-3	70.0	66.7	-	12.1	32.8	-	1	4	-

715 Fig. 1 The site of the Tomb of Lazarus. **a** Walls of the Crusaders' Monastery in the foreground, and
 716 view of Mosque of al-Uzair (left) and the modern Church of Saint Lazarus (right) in the
 717 background. **b** Lower "Plaza", with the pillars of the Byzantine and Crusaders' antique churches in
 718 the foreground, and the façade of the modern church in the left background. **c** Walls of the
 719 Crusaders' Monastery, with carved capitals in the foreground. **d** Lithobiontic community including
 720 chlorolichens, bryophytes and a black cyanobacterial biofilm on blocks of the pillars of the antique
 721 church. **e** Upper surface of a carved capital of the Crusaders' Monastery, covered by lichens (mostly
 722 orange thalli of *Variospora aurantia*) partially hidden by dust deposits, but made visible by wetting
 1723 their thalli (left side). **f** Black fungi, in part overgrowing chlorolichens, on the façade of the modern
 1724 church (the 50×50 cm square grid used for relevés is visible on the vertical surface).

14 725 Fig. 2 Frequency (av. % per plot, **a**) and cover (% **b**) of lithobionts (lichens, white columns; black
 15 726 biofilm, dark grey columns; bryophytes, light grey columns) on the investigated architectural
 1727 elements in the site of the Tomb of Lazarus. Maximum (upper whisker), 75th percentile (top box),
 1728 mean (square), median (transversal line), 25th percentile (bottom box), minimum (lower whisker).

20 729 Fig. 3 Biofilm forming microorganisms from the Jerusalem stone surfaces of the Tomb of Lazarus.
 21 730 **a** Coccoid cyanobacteria surrounded by a polysaccharide sheath. **b** Cyanobacterial mat, locally
 22 731 including green algae (§) and black yeasts (#). **c** Black yeasts (#) and meristematic (*) fungi within
 23 732 the medulla and **d** on the cortex of chlorolichens. Scale bars: 50 µm (a), 100 µm (b-d).

26 733 Fig. 4 Interface between lichens and the Jerusalem stone (cross sections observed by RLM). **a** Free
 27 734 colonies of dematiaceous fungi (#) beneath a thallus of *Variospora aurantia*. **b-e** Hyphal
 28 735 penetration component of epilithic and endolithic lichens (cross sections stained by PAS,
 29 736 visualizing biological structures in violet): **b**, *Variospora aurantia* (*, hyphal penetration limited to
 30 737 a crack), **c**, *Pyrenodesmia erodens* (left) and *V. aurantia* (right), **d**, *Bagliettoa baldensis* (§,
 31 738 perithecia), **e**, *Verrucaria ochrostoma* (§, perithecia). Scale bars: 0.5 mm (**a**, **c**, **e**), 1 mm (**b**, **d**). **f**
 32 739 Quantitative characterization of the specific structural organization (av. thallus thickness, massive
 33 740 and maximum depth of hyphal penetration component). For each parameter, columns which do not
 34 741 share letters are significantly different (ANOVA, Tukey's test, p<0.05).

38 742 Fig. 5 Lichen effect on the total porosity of the Jerusalem stone (sections observed by SEM-BSE). **a**
 39 743 Petrographic thin section of a fresh fragment, showing porosity internal to bioclats (§). **b**
 40 744 Quantitative characterization, based on image analysis, of the total porosity of: a 300 µm layer
 41 745 beneath the surface showing the epilithic *Variospora aurantia*, the endolithic *Pyrenodesmia*
 42 746 *erodens*, and absence of lichens (polished cross section 1); a 300 µm layer beneath the surface
 43 747 showing the endolithic *Bagliettoa baldensis* and absence of lichens (section 2); 300 µm layers at
 44 748 more than 2 mm from the surface (sections 1 and 2); 300 µm layers of fresh fragments as control.
 45 749 For each section, columns which do not share letters are significantly different (ANOVA, Tukey's
 46 750 test, p<0.05). **c-e** BED-C images of section 1: **c**, rock surface (arrow) not colonized by lichen thalli,
 47 751 **d**, rock surface (arrow) covered by a *V. aurantia* thallus (#), **e**, rock surface (arrow) dissolved by *P.*
 48 752 *erodens* (°). **f** BED-S image of a polished cross sectioned fresh fragment, with bioclats (§)
 49 753 embedded in a fine-grained calcite matrix. **g-h** BED-S images of section 2: **g**, rock surface (arrow)
 50 754 penetrated by *B. baldensis* (*), with hyphae invading the internal porosity of bioclats
 51 755 (magnification in **h**). Scale bars: 100 µm (**a**, **c-g**), 25 µm (**h**).

56 756 Fig. 6 Epifluorescence observations run on hand cut transverse sections of thalli of *Variospora*
 57 757 *aurantia* upon biocide treatments. **a-b** Samples collected from block-1 at the end of the application
 58 758 of 1.5% BZC by cellulose-sepiolite poultice (**a**) and 0.25% BZC by brush (**b**); **c-d** Samples

759 collected at the end of the application of BZC 1.5% by nebulizer, without rinsing (c), and after 40
760 days (d). Scale bars: 40 μm.

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Figure 1

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