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Lichen colonization and associated deterioration processes in Pasargadae, UNESCO World Heritage Site, Iran

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Abstract

Knowledge on lichen and microbial colonization as well as associated biodeterioration processes of the stone cultural heritage is needed to establish proper conservation programs, but is still poor for stonework in semi-arid regions. In this study, lichen diversity was characterized on seven monumental buildings of the Pasargadae UNESCO-world heritage site (Iran). The risk of biodeterioration processes associated to lichen occurrence on two types of limestones, and the lichen resilience to mechanical cleaning intervention were examined. Physico-chemical substrate features and climatic conditions, combined with the agricultural surrounding and tourist disturbance, supported a pervasive colonization by species-poor epi- and endolithic communities, and fast recolonization processes by nitrophytic species after mechanical removal. The endolithic growth of some lichens and the penetration of hyphal structures of epilithic ones, examined by light and electron microscopy, were associated to stone disintegration and dissolution at the lichen-rock interface. Endolithic cyanobacteria were detected under lichen thalli, likely contributing to deterioration processes. Colonization and deterioration patterns did not appear peculiar with respect to previous investigations on similar communities in different climatic regions, and were mostly related to the different examined lithologies, indicating lichens as harmful biodeteriogens of the sedimentary rock materials used in the stone cultural heritage of semi-arid regions.

Keywords: Biodeterioration, cyanobacteria, conservation, lichens, semi-arid regions

Highlights

Lichens threaten the conservation of stonework in the semi-arid Iranian region
Endolithic and nitrophytic lichens show high resilience to routinely cleaning procedures
Lichen colonization favours the formation of microhabitats for other biodeteriogenic microorganisms

1. Introduction

The archaeological sites and cultural heritage properties, besides being a representation of different civilisation period, positively involve the tourism industry and economy growth of a country (Csapó, 2012). The importance of their conservation and restoration represents an issue that has nowadays assumed a world-wide interest (Harrison and Hitchcock, 2005; Timothy and Nyaupane, 2009). Properly, the colonization by biodeteriogenic organisms is enumerated within the most acute threats for the preservation and conservation of stonework (Caneva et al., 2008; Scheerer et al., 2009). Recently, an increase of their impact has been hypothesised as potentially related to the ongoing global changes in climate and environmental/pollution conditions (Berenfeld, 2008; Viles and Cutler, 2012). Such changes may indeed determine dramatic shifts in stone bioreceptivity and colonization dynamics, and a modification of the biotic interactions with the lithic substrates in areas including vulnerable heritage structures such as the Mediterranean region, Middle East, Caribbean and Southern Africa (Gómez-Bolea et al., 2012; Viles and Cutler, 2012).

A remarkable role of lichens as biological weathering agents of rock substrata has been long recognized and widely documented on both natural outcrops and the outdoor stone cultural heritage (Adamo and Violante, 2000; Ascaso et al., 2002; de los Ríos et al., 2009; Gazzano et al., 2009; Seaward, 2015). Epilithic and endolithic lichens can indeed mechanically and chemically damage both the rock surface and interior, mostly because of the adhesion, penetration and changes of volume due to hydratation-dehydratation processes of hyphal structures and the release of metabolites having acidic and/or chelating functions (Ascaso et al., 2002; de los Ríos and Ascaso, 2005). Although lichens represent the most prominent component of lithobiontic communities, biodeterioration processes in a given site are rarely caused only by them. Associated to lichens, but also forming independent communities, different microorganisms such as cyanobacteria, green algae, free-living fungi and heterotrophic bacteria, contribute to the biodeterioration process, similarly growing both epilithically and endolithically (Bjelland et al., 2011; de los Ríos et al., 2012).

Some investigations showed that in certain conditions lichens may exert bioprotection on the stone surfaces (Carter and Viles, 2005). Bioprotective effects were documented on both natural and man-made mineral substrates under aggressive environmental conditions (Favero-Longo et al., 2009a; Mc Ilroy de la Rosa et al., 2013), for example where high atmospheric pollution occurs or in coastal areas with risk of salt-induced weathering (Mikuláš, 1994; Carballal et al., 2001). However, generalized bioprotective actions of lichens are still retained a controversial subject, needing additional studies (Mc Ilroy de la Rosa et al., 2013).

Due to their efficiency in accumulating nutrients and their resistance to desiccation and temperature extremes and fluctuations, lichens colonize rock substrates in a wide range of habitats, including those normally hostile to other life forms (e.g. de los Ríos et al., 2014). Accordingly, investigations on lichen interactions with the lithic substrate have been performed through a wide geographical range, from polar to tropical areas, revealing different bioweathering and bioprotection patterns depending on different climate conditions, lithologies and lichen communities (Seaward, 2015). In parallel, lichen colonization of stonework has been also sparsely explored up to remote areas (St. Clair and Seaward, 2004), but many combinations of “lithology × lichen community × climate conditions” have hitherto been understudied, even where lichen occurrence has been generally recognized as a potential threat in conservation/restoration plans.

Lichen colonization of the stone cultural heritage in arid/semi-arid environments has been less deeply characterized respective to that in the temperate and Mediterranean bioclimatic areas (Mohammadi and Krumbein, 2008). The poorness of information appears particularly serious as the few available reports revealed extremely contrasting effects of the lichen colonization, varying from severe deterioration (Ascaso et al., 2004; Knight et al., 2004; Paradise, 2013) to significant bioprotection (Souza-Egipsy et al., 2004; Özvan et al., 2015). Counteracting processes below the same lichen species were even reported in the case of the endolithic *Verrucaria rubrocincta* on limestone, actively deteriorating its substrate, but concomitantly forming a micrite layer which mitigates the destructive effects (Garvie et al., 2008).

In Iran, five world-heritage sites [Armenian Monastic Ensembles of Iran (West and East Azerbaijan provinces), Bisotun (Kermanshah province), Pasargadae (Fars province), Persepolis (Fars province), Takht-e Soleyman (West Azerbaijan province)] built by stone materials are heavily colonized by lichens (Sohrabi, personal observation). However, although the diversity of lichens and lichenicolous fungi has been recently surveyed in the natural environments of different areas of Iran (e.g. Seaward et al., 2004; Westberg and Sohrabi, 2012), the characterization of the lichen colonization of monuments is largely deficient. Mohammadi and Krumbein (2008) remarkably explored biodeterioration of Persepolis monuments, where they generally described hyphal penetration patterns and pitting effects of the epi- and endolithic lichen components. However, they did not associate the observed phenomena with the different lithologies characterizing the archaeological site and they did not analyze the lichen diversity to properly correlate different species with the observed penetration/deterioration patterns. On the other hand, in the recent years, the lichen diversity has been surveyed on the brick surfaces of the Gonbad-e Qabus, UNESCO world Heritage site (Sohrabi et al., unpublished technical report) and in other minor sites (e.g. on the Tangivar stone inscriptions: Sohrabi and Abbas-Rouhollahi, 2012), but related deterioration patterns were not investigated. Lichen colonization has been also recently listed as a risk factor for the conservation of the World Heritage Site of Pasargadae, which is located in a rural plain of South-Western Iran and notably includes the Mausoleum of Cyrus the Great (Rafiee-Fanood and Mehdizadeh-Saradj, 2013). On this latter monument, conservation and restoration interventions were performed in 2006, including the cleaning of the rock surfaces by mechanical methods (water and plastic or wire brushes). However, lichen diversity and the associated deterioration patterns had been not preventively examined to evaluate their effective impact on the rock surfaces.

In this paper, we first survey (a) the lichen diversity on the stone monuments of the World Heritage Site of Pasargadae and we examine (b) the lichen-lithic substrate interface of epilithic and endolithic lichen species to infer their potential effect on limestones with different quartz contents. The colonization patterns and associated mineral-microorganism interactions observed in Pasargadae are then discussed in comparison to those previously reported from arid/semi-arid regions to the most studied Mediterranean/temperate regions, where lichens have been variously associated to biodeterioration or bioprotection effects.

2. Material and methods

2.1. Study site

The archaeological site of Pasargadae is located in the Madar-e-Soleyman rural district, Pasargadae county, in the valley of the river Polvar, on the Dasht-e Morghab (the plain of Morghab), approximately 25 kilometres long and 12 kilometres wide (N30° 11’ 37.788” E53° 10’

2.244) (Fig. 1). The site, one of the oldest residences of the Achaemenid kings, founded by Cyrus the Great (r. 559-530 BC), was inscribed in 2004 in the UNESCO World Heritage List (Mozaffari 2014; http://whc.unesco.org/en/list/1106). Its 2×3 km^2 area includes seven monumental buildings, (Fig. 1C, 1-7 locations). A beige limestone (<5% quartz content, BL) from the Sivand Quarry (*c.* 30 km far from the site), also used in Persepolis, was mostly used in the Cyrus Mausoleum and in the other buildings, but a more dark-grey arenaceous variety of limestone (15-20% quartz content, GL), and other lithologies were also abundantly used (Bonazza et al., 2007; Emami, 2010; Mozaffari, 2014). In BL, calcite clasts are embedded in a very fine calcite matrix (diam. c. 1 um), while in GL, calcite and quartz grains (diam. c. 50-100 um) are embedded in a slightly coarser calcite matrix (diam. c. 1-5 um), detemining a higher porosity (Supplementary material 1).

Mean annual rainfall for Pasargadae is over 350 mm, the prevailing direction of rain-bearing winds being west to southwest (Talebian, 2014).

2.2. Lichen diversity survey

Lichen diversity was surveyed in the period 2005-2015 on the following monumental buildings in Pasargadae (Fig. 2): (A) The Mausoleum of Cyrus the Great, (B) The Madrasseh or Mozafari Caravanserai, (C) Gate R (Gate House), (D) Palace P (Residential Palace), (E) Palace S (Audience Hall), (F) The Zendan (Zendan-e Soleyman / Solomon’s Prison), (G) The Tall-e Takht (Takht-e Soleyman). The whole surface of each monument was surveyed. The Mausoleum of Cyrus was examined both before and after the cleaning intervention in 2006 (Fig. 3). Non-destructive techniques were applied to sample small thallus fragments. All lichen samples were determined in the laboratory of Iranian research organization for science and technology (IROST) following monographic keys and descriptions. Besides morphological observations, chemical analyses of lichen secondary metabolites were performed by thin layer chromatography according to Orange et al. (2011). Nomenclature of species follows Arup et al. (2013) for Teloschistaceae, Otálora et al., (2014) for Collemataceae, Zhao et al., (2015) for *Lecanora dispersa* group and Kirk et al. (2008) for other lichens. The relative importance of components of γ-diversity, i.e. similarity (S), relativized richness difference (D), and relativized species replacement (R), was evaluated for the set of monumental buildings with SDR Simplex software using the Simplex method (Podani and Schmera, 2011). With few exceptions, photographs, acquired using a Camera DSC-H20 (2009, 3" LCD, 10.1 megapixels, 10x optical zoom (Japan), are kept as voucher specimens instead of original specimens due to restrictions of sampling in the world heritage site. Digital files are deposited in the

MYCOLICH website (www.mycolich.ir) and all printed photographs are kept at the Iranian Cryptogamic Herbarium (ICH) located in the Iranian Research Organization for Science and Technology (IROST).

2.3. Light and scanning electron microscopy

Millimetric limestone fragments, already detached from the monumental rock blocks and colonized by epilithic and/or endolithic lichens, were collected for performing microscopical observations at the lichen-rock interface. The collected BL fragments hosted the epilithic species *Calogaya biatorina* (A. Massal.) Arup, Frödén & Søchting and the endolithic *Pyrenodesmia erodens* (Tretiach, Pinna & Grube) Søchting, Arup & Frödén; the GL fragments were colonized by the epilithic *Acarospora* sp. and the endolithic *Rinodina immersa* (Körb.) Zahlbr. The sampling of only previously detached fragments led to the examination of a limited number of samples for each species (two or three samples). Ex situ sampling on natural outcrops colonized by lichens in the Sivand Quarry area was excluded, because it was preferred to focus the analyses on the rock facies under the Pasargadae environmental conditions (e.g. specific climate and microclimate conditions, influence of anthropic activities such as tourism and agriculture).

Polished cross sections were obtained after including the rock fragments in a polyester resin, stained using the periodic acid-Schiff method (PAS; Whitlach and Johnson, 1974) to highlight hyphae penetrating the lithic substrate and observed under reflected light microscopy (LM) using an Olympus SZH10 stereomicroscope (Favero-Longo et al., 2005). Along the colonized transect, measuring points were established at every millimetre from the cross-section vertex; the maximum depth of hyphal penetration was then measured beneath each measuring point perpendicularly to the colonized transect. Occurrence and depth of the photobiont partners of the endolithic species were evaluated before the PAS-staining. Petrographic thin-cross sections were also prepared and observed under transmitted light using a Nikon Eclipse 50i polarizing microscope.

The BL fragments colonized by *C. biatorina* were also prepared according to a procedure developed for observing the rock-microorganism interface by scanning electron microscopy with backscattered electron imaging (SEM-BSE) (Wierzchos and Ascaso, 1994). The pieces of rock were fixed in glutaraldehyde and osmium tetroxide solutions, dehydrated in a graded ethanol series and embedded in LR-White resin. Blocks of resin-embedded rock samples were finely polished, carbon coated and observed using a FEI INSPECT SEM microscope. Microprobe analyses were performed using an Oxford Instruments INCA X-Ray Energy Dispersive Spectrometer (EDS) microanalytical system during SEM observation.

3. Results

3.1. Lichen flora on the Pasargadae monumental buildings

A total of 28 lichen species was observed on the monuments of the Pasargadae World Heritage site, including 5 species with endolithic thallus, 22 species with epilithic thallus (20 crustose and 2 foliose) and one lichenicolous lichen (*Verrucaria biatorinaria* growing on *Calogaya biatorina*) (Table 1). Species diversity on the different monuments ranged between 22 (Tall-e Takht) and 4 (the Madrasseh) species, the resulting communities displaying high similarity (S=46) and richness difference (D=44), and low species replacement (R=10). Species with Holarctic or with European-Asian distribution were dominant, with a minor presence of species common in eastern North-America and East Asia (e.g. *Anaptychia elbursiana*, *Myriolecis percrenata*). The nitrophytic *Calogaya biatorina*, mainly growing on the upper parts of limestone boulders that are mostly exposed to direct sunlight and wind, was the most common species through the whole archaeological site (photographs of dominant and remarkable detected species in Supplementary Material 2). Other two nitrophytic species (*Acarospora cervina, Calogaya decipiens)* and the lichenicolous *Verrucaria biatorinaria* were also observed on all the investigated buildings.

Before the last restoration intervention in 2006-2008, lichen colonization on the Cyrus Mausoleum was more developed on the North-West wall. Procedures of mechanical cleaning carried out in 2006 mostly removed the epilithic lichens (Fig. 3A-D), while endolithic ones were quite undisturbed because of their growth form (Fig. 3E and F). Lichen epilithic recolonization was already evident in 2010, *C. biatorina* being again the dominant species. The most prominent recolonization process affected the South-West façade, often completely wetted by rain associated with dominant westerly winds and more characterized by stone alveolization (Fig. 4A-C), and the North-West one, the most colonized before the restoration work. The grooved surface of large blocks used for restoration in 1970s (Rafiee-Fanood and Mehdizadeh-Saradj 2013) remarkably displayed high recolonization (Fig. 4D). Only two [*Acarospora laqueata* and *Anaptychia elbursiana*] out of the 19 species observed previous to the cleaning intervention were not observed on the limestone surfaces in 2010-2015 (Table 1).

3.2. Lichen colonization patterns on/within the limestone substrates

Different patterns of endolithic growth were observed for *Pyrenodesmia erodens* and *Rinodina immersa* colonizing beige limestone (<5% quartz content, BL) and dark-grey limestone (15-20% quartz content, GL), respectively.

Surface depressions up to 3 mm deep characterized the BL surface where *P. erodens* endolithically grew (Fig. 5A). Observations under reflected light showed a diffuse growth of the photobiont partner of *P. erodens* within the upper 200 µm layer of the colonized BL (Fig. 5B). PAS staining showed photobiont cells localized within small canals, from perpendicular to parallel to the surface, occupied also by the mycobiont hyphae (Fig. 5C and D). The shape and size of these canals point to the involvement of active dissolution processes in their formation. Only few sections of the investigated surfaces displayed a more continuous hyphal network, penetrating up to 500 µm into the lithic substrate through intergranular porosity (Fig. 5E). Observations under transmitted light showed that the upper layer of the BL colonized by *P. erodens* was characterized by partially dissolved calcite crystals (not shown).

The photobiont layer of *R. immersa* similarly occupied the upper 200 µm layer of the colonized GL (Fig. 5F). In this case, however, the PAS staining showed an abundant occurrence of hyphal bundles penetrating between grains, down to 1 mm (Fig. 5G). From the surface down to 3 mm in depth, a diffuse PAS halo was also observed, likely related to extracellular polymeric substances and/or organic deposits accumulated in the upper rock layer. No surface depression was observed where *R. immersa* colonized the GL blocks, while traces of pitting were observed where fruiting bodies had decayed.

Different penetration patterns also characterized the epilithic lichen species examined on the two lithologies. The epilithic thalli of *C. biatorina* did not show a conspicuous hyphal penetration component within the colonized BL: hyphal bundles were only sporadically observed to penetrate down to 150 µm beneath the thalli (Fig. 6A and B). On the other hand, the observation of the cross-sectioned thalli displayed the occurrence of a prominent mineral component/deposit cementing the epilithic lobes. By contrast, *Acarospora* sp., having less prominent thalli than C. *biatorina*, displayed remarkable deeper hyphal penetration components, penetrating down to 800-1600 µm in depth between the quartz granules of the GL (Fig. 6C and D).

The SEM-BSE study of the *C. biatorina*-lithic substrate interface has revealed a close attachment of the lichen areoles to the lithic substrate and associated disaggregation of the BL surface (Fig. 7A). It is possible to observe mineral fragments separated from the lithic substrate, which have been incorporated into the lichen medulla (white head-arrows in Figs. 7A and B). Hyphal bundles observed penetrating in the rock substrate (black arrows in Figs. 7A-C) induce the formation of holes in the BL surface increasing the disaggregation (Fig. 7A and B). Further individual fungal hyphae were also observed penetrating the lithic substrate through intergranular porosity (black head-arrow in Fig. 7C).

Under the lichen thalli, some ellipsoid-shaped cavities were observed (white arrows in Fig. 7B, D and E), which were occupied by different cyanobacteria morphotypes. These cyanobacterial colonized cavities were found to a depth of approx. 300 µm of the rock surface (Fig. 7A). The formation of the cavities in BL could be favoured by the moldic porosity of the colonized limestone. The localization of these cavities colonized by cyanobacteria under the lichen thalli (Fig. 7A and B) points to the influence of lichen thalli in their development. Endolithic algal-fungal cells associations (asterisk in Fig. 7F) and heterotrophic bacteria (white thin arrow in Fig. 7F) were also detected on weathered stone surface and in the ellipsoid-shaped cavities.

4. Discussion

Calcareous sedimentary rocks of monumental remains in Pasargadae have been found particularly vulnerable to lichen colonization and associated biodeterioration processes. Physico-chemical substrate features and climatic and microclimatic conditions, combined with the influence of agricultural surroundings and disturbances associated to tourism, support a widespread colonization by species-poor epi- and endolithic communities, and fast recolonization processes after cleaning intervention. Signs of deterioration phenomena, such as pitting and granular disaggregation, are frequently observed in different parts of the archaeological site colonized by lichen communities. The endolithic growth of some lichens and the penetration of hyphal structures of epilithic ones seem to lose the surface of the stone, inducing its disaggregation, and favouring the colonization by other epilithic and endolithic microorganisms. The nature of the lichen growth form and the close attachment to the lithic substrate observed, together with the mycobiont-mineral interactions established, suggest that the lichens can be considered harmful biodeteriogens in the study area and potentially in other semi-arid regions.

4.1. Lichen colonization patterns

The composition of lichen communities on the stone Cultural Heritage is related to the specificity to certain substrates of the different species and to their physiological and morphological adaptation to macro- and microclimatic conditions and other environmental pressures to which the rock surfaces are exposed (St. Clair and Seaward, 2004; Seaward 2015). Investigations on lichens of the Pasargadae site, characterized by high community similarity and nestedness, indicate diversity poorness as remarkable feature of monumental surfaces in rural areas of the semi-arid regions, probably responding to specific environmental conditions and anthropogenic disturbances of the archaeological site too.

Only 75% of collected species turned out to be common outside the historical site, occurring in the surrounding mountains and hills of Pasargadae (Sohrabi, unpublished data). Moreover, although the rich Iranian lichen flora includes several species known only for SW Asia, including tens of taxa based on Iranian type specimens (Seaward et al., 2004), the contribution of these latter to the Pasargadae flora is negligible. The observed lichen flora differences could be attributed to the different climate and microclimate conditions between the archaeological site located in a flat plain and the reliefs in the surroundings and other Iranian regions. In addition, lichen communities of anthropic ecosystems, including rural areas, generally show low diversity since conditions derived from human activities act as strong filters (Clair and Seaward, 2004; Cámara et al., 2015). Thus, a wide set of nitrophytic species with wide distributional ranges is dominant on the Pasargadae monuments, as in rural-monumental sites of the temperate region (Nimis et al., 1992; Caneva et al., 2008) with a prominent occurrence of species of *Caloplaca* s.l., well adapted to alkaline substrata and sunny environment (Ariño et al., 1995). As widely described in other areas with regard to the composition of epiphytic lichen communities (Frati et al., 2008 with refs. therein), the agricultural activity favours the selection of few dominant species tolerating a high eutrophication level. In Pasargadae, a similar pattern is compatible with the agricultural activity in the surrounding plain and with frequent events of wind-storms (personal communications with local people), favouring transport and deposition of nitrogen compounds and dust (Bonazza et al. 2007). Tourist flows also contribute a more local dust mobilization. Droppings by birds, frequently observed on the monumental surfaces, may also positively affect the nitrophytic colonization (Caneva et al., 2008).

Remarkably, nitrophytic species are also known as related to fast re-colonization processes, even following deep cleaning treatments coupling mechanical and chemical approaches (Nascimbene et al., 2009). Lichen recolonization of dolostones after 16 months of mechanical and biocide treatment has been reported in experiments carried out in quarry rocks (Cámara et al., 2011). Accordingly, it is not surprising that a rapid recolonization process followed the mechanical cleaning of the Cyrus Mausoleum, which left quite undisturbed the endolithic species and only partially affected the epilithic forms. On the North-West wall, in particular, weathering micromorphologies, as surface alveoles, acted as refugees for epilithic lichen thalli during the cleaning intervention. Thereafter, the more direct exposure to rainfall events, related to dominant western winds (Talebian, 2014), likely made these thallus remains significant sources of reproductive propagule short-distance dispersal, as described in the temperate region for Teloschistaceae species, supporting the recolonization (Favero-Longo et al., 2014). Nevertheless, it is worth noting that the conservation history of Pasargadae is rather unknown, especially with regard to restoration works possibly performed during 1970-2006, approximately 10-45 years ago, thus preventing a full comprehension of colonization dynamics.

4.2. Biodeterioration patterns

The deterioration effects of lichen colonization are generally a combination of both geophysical and geochemical actions induced mainly by mycobiont hyphae (Ascaso et al., 2002; De los Ríos et al., 2009; Seaward, 2015). In the material analyzed here, several damaging mechanical and chemical processes, which induce granular disaggregation of the dark-grey limestone (higher quartz content, GL) and disaggregation and dissolution of the beige limestone (BL), attributed to epilithic crustose and endolithic lichen species colonization, could be distinguished.

LM images revealed the mechanical and chemical impact on the colonized stones generated by the penetration of mycobiont hyphae through the stone porosity. Deeper hyphal penetration was observed within GL with respect to BL, generally characterized by a lower total porosity. More porous rocks, indeed, generally support a higher hyphal penetration and are generally considered more bioreceptive (Cámara et al., 2008; Miller et al., 2006; Favero-Longo et al., 2009b).

The SEM-BSE study showed that even the crustose lichen species *Calogaya biatorina*, with a much loose association to the colonized lithic substrate shown by LM, induced clear physical damages in the colonized BL. Observations clarified that even a hyphal penetration limited to few tenths/hundreds of microns within the substrate may be associated to a significant disaggregation of the stone surface and the consequent entrapment of mineral grains within the medulla layer. Mineral disaggregation related to hyphal penetration, widely documented for several sedimentary lithotypes through a wide range of climatic areas, from the polar (Ascaso et al., 1990; Chen and Blume, 2002) to the tropical (Bartoli et al., 2014) regions, is thus here shown for stonework in the semi-arid Iranian region. These results support previous reports by Paradise (1997, 2013) on a lichen-driven acceleration of surface recession in other semi-arid/arid regions (Red Mountains, Arizona, USA; Petra, Jordan). Oppositely, evidences for a bioprotective role of lichen, previously reported on sandstone in semi-arid environments around the Mediterranean basin (Viles and Pentecost, 1994; Ariño et al., 1995; Souza-Egipsy et al., 2004), were not recognized on the Pasargadae monuments. Although lichen thalli and the prominent mineral deposit cementing the epilithic lobes of *C. biatorina* (Fig. 6A) may possibly exert an umbrella effect with respect to abiotic deteriogenic factors, as wind, they are simultaneously weakening the stone structure.

Biophysical stone degradation by lichens results primarily from the penetration of the fungal attachment structures into pores, preexisting cracks, and fissures in the stone. The colonized cavities and fissures may subsequently widen due to an increase in the mass of the thallus during growth (de los Ríos and Ascaso, 2005). Mechanical disaggregation may be combined with chemical processes induced by lichen chemicals, especially in carbonate rocks (Ascaso et al., 2002, 2004). This mixture of physical and chemical effects is clear in the case of endolithic species, as *Pyrenodesmia erodens*, described as euendolith (i.e. actively dissolving calcite; sensu Golubic et al., 1981) in dry sites of the Mediterranean region on exposed, subvertical faces of limestone and dolomite rocks, including old monuments (Tretiach et al., 2003; Vondrák et al., 2012). Endolithic thallus development in surface depressions, with photobiont cells within small canals also occupied by the mycobiont, revealing an euendolithic behaviour, is observed here in the semi-arid conditions of Pasargadae, in agreement with previous descriptions of thalli from the Mediterranean area (Pinna and Salvadori, 2000). The deep hyphal penetration within GL of *Rinodina immersa*, also known as euendolithic lichen on calcareous rocks of the Mediterranean area (Pinna et al., 1998), seems here mostly associated to physical penetration through the high intrinsic porosity of this lithotype than to chemical processes.

The presence of cyanobacterial colonies also contributes to the deterioration of the colonized stone and can be associated to micropitting phenomena. Cyanobacteria observed under lichen thalli occupied cavities following colony outline, typical behaviour of the euendolithic niche (Golubic et al., 1981). Chemical actions of these cyanobacteria on the colonized stone are probably involved in the formation of the observed cavities (Ascaso et al., 2002) and consequently in the disaggregation of the stone surface. However, a previous action of fungal hyphae penetrating through moldic porosity cannot be discarded. Mycobiont penetration through spaces left behind by preexisting shell has been reported previously for bioclastic limestones (de los Rios et al., 2009).

The colonization of certain microorganisms in the analysed calcareous stones could be conditioned by the presence and activity of lichens due to they can actively contribute to the formation of microhabitats and chemical microenvironments in lithic substrates (de los Ríos et al., 2002). In fact, here not only cyanobacteria, but also heterotrophic bacteria and algal-fungal associations have been detected in cavities associated to the mycobiont hyphae penetration. The lichen thalli not only alter the surface of the stone but also modify the microclimate of the rock surface on which they settle (Carter and Viles, 2004). *Calogaya biatorina* could favour the microbial colonization observed by facilitating moisture holding and the protection given against strong radiation to the microorganisms situated under the thalli. Further, it has been shown that some lichen-forming fungi primarily associated with green algae also show different degrees of interactions with cyanobacteria (cyanotrophy) (Poelt and Mayhofer, 1988). Thus the association of endolithic cyanobacterial colonization and crustose lichens observed in Pasargadae could be related to a specific adaptation to the arid conditions of the area. These results point to that the contribution of the lichens to the formation of microhabitat for other lithobiontic biodeteriogenic microorganisms have to be take in account in order to understand the role of lichens in biodeterioration processes and design treatments to protect the stone Cultural Heritage.

5. Conclusions

Lichen colonization and deterioration patterns on the Pasargadae monuments, as a model of the stone cultural heritage in the semi-arid region of Iran, do not appear peculiar with respect to what previously described for temperate and (semi-)arid areas around the Mediterranean basin and mostly related to the different examined lithotypes. Observed damaging action suggests that lichen communities should be considered a potential threat for the conservation of stonework in the semi-arid region, as in the temperate and Mediterranean ones. The high resilience of endolithic and nitrophytic community to routinely performed mechanical cleaning indicates the need of proper control and removal programs. These conservation measurements should consider (i) the autoecology of deteriogenic/dominant species, to understand factors determining their local distribution, (ii) the specific interactions with the rock-substrate and the associated microbial consortia, to evaluate the overall impact on the stone durability, (iii) the optimization of biocidal and other chemical and/or physical removal approaches, to chose the most appropriate treatments. Such knowledge is still widely missing for the stone cultural heritage in semi-arid regions, as Iran, and further detailed investigations need to be stimulated.

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Figure captions

Fig. 1. Location and map of the Pasargadae UNESCO World Heritage Site, Iran. A-B. Location of Pasargadae in the Fars Province, SW Iran. C. Monumental buildings of the Pasargadae site: (1) The Mausoleum of Cyrus the Great; (2) The Madrasseh or Mozafari Caravanserai; (3) Gate R (Gate House); (4) Palace P (Residential Palace); (5) Palace S (Audience Hall); (6) The Zendan (Zendan-e Soleyman / Solomon's Prison); (7) The Tall-e Takht (Takht-e Soleyman / Solomon's Throne.

Fig. 2. Main monumental buildings of the Pasargadae UNESCO World Heritage Site, Iran. (A) The Mausoleum of Cyrus the Great; (B) The Madrasseh or Mozafari Caravanserai; (C) Gate R (Gate House;); (D) Palace P (Residential Palace;); (E) Palace S (Audience Hall;); (F) The Zendan (Zendan-e Soleyman / Solomon's Prison;); (G) The Tall-e Takht (Takht-e Soleyman / Solomon's Throne).

Fig. 3. Lichen colonization on the Mausoleum of Cyrus before and after the cleaning intervention in 2006-2008. The North-West side of the Mausoleum walls (A, B), the Arabic inscription at the stone-block of the Northern wall (C, D) and an area colonized by *Pyrenodesmia erodens* (E, F) in 2005 (A, C, D) and 2012 (B, D, F), respectively. Arrows in Figs. A-D indicate areas where endolithic lichens persisted after the cleaning intervention, while in Figs. A-B highlight the removal of an epilithic *Circinaria* sp., surrounded by a persistent thallus of *P. erodens*.

Fig. 4. Persistence and recolonization of epilithic lichens on the Cyrus Mausoleum. (A) Surface alveolization (arrows) on the South-West wall. (B) Thallus of *Calogaya biatorina* (arrow) after the cleaning intervention, repaired by the alveolar surface structure. (C) Young thalli of *C. biatorina* (arrows) on an exfoliated block of the SW wall in 2013. (D) Epilithic recolonization on the grooved surfaces of blocks of the SW wall, used in 1970s restorations.

Fig. 5. Endolithic lichen colonization of different lihtologies used in Pasargadae (polished cross-sections observed under reflected light microscopy before - A-B, F - and after, - C-E, G - the staining with PAS). (A-E) Endolithic growth of *Pyrenodesmia erodens* within beige limestone (<5% quartz content), developing millimetric deep hollows and characterized by hyphae which surround photobiont cells (§) within small canals or, subordinately, sparsely penetrate within the substrate (*). (F-G) Endolithic growth of *Rinodina immersa* within dark-grey limestone (15-20% quartz content), showing a remarkable hyphal penetration component (*), including hyphal bundles (#), below the photobiont layer (§) and sparse, epilithic apothecia. Scale bars: 1.5 mm (A), 1 mm (B-C, F), 250 µm (D-E, G).

Fig. 6. Epilithic lichen colonization of different lithologies used in Pasargadae (polished cross-sections observed under reflected light microscopy before, A-C, and after, B-D, the staining with PAS). (A-B) Thallus of *Calogaya biatorina* on beige limestone (<5% quartz content), displaying a remarkable mineral deposit/accumulation (#) between its areolae and an inconspicuous development of its hyphal penetration component (*). (C-D) Thallus of *Acarospora* sp. on dark-grey limestone (15-20% quartz content), displaying a conspicuous hyphal penetration component (*), and associated endolithic phototrophic microorganisms (§). Scale bars: 1 mm (A, C), 500 µm (B-D).

Fig. 7. SEM-BSE images of *Calogaya biatorina*-lithic substrate interface at beige limestone (<5% quartz content, BL). A) Disaggregation of the surface of the BL associated to the lichen thallus attachment, especially to hyphal bundle penetration (black arrow). White head-arrow notes mineral fragments separated from the lithic substrate and incorporated in lichen medulla B) ellipsoid-shape cavities (white arrows) present under lichen areolae. White head-arrow note mineral fragments incorporated in lichen medulla and black arrow the penetration of a hyphal bundle and white arrows ellipsoid-shape cavities. C) A detail view of the formation of a hole by penetration of a hyphal bundle from the surface (black arrow) and endolithic colonization of individual fungal hyphae through intergranular porosity (black head-arrows). D) A detail view of the colonization of ellipsoid-shape cavities by cyanobacteria (white arrow). E) Area of the weathered BL showing multiple ellipsoid-shaped cavities of different size colonized by different morphotypes of cyanobacteria (white arrows). F) Weathered surface of the BL showing colonization by heterotrophic bacteria (white arrow) and endolithic algal-fungal association (asterisk).