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Anthropic effect on the lichen colonization in building stones from cultural heritage

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Abstract

In this study the effect of human urine on lithobiontic microorganisms colonizing dolostones from a historic quarry (Redueña, Madrid, Spain) was evaluated in situ by means of a field experiment. The application of the bioproduct was performed by directly spraying onto the rock surface of the quarry front during 8 weeks. After the treatment, the ultrastructure of *Verrucaria nigrescens* was analysed by Scanning and Transmission Electron Microscopies (SEM and TEM). The study samples showed alterations of the upper cortex fungal cells and altered algal chloroplast. On the surface of the thalli enrichment in phosphorous (P) was also detected by energy dispersive X-ray spectroscopy (EDS). The main result of the field experiment was the alteration of the upper cortex of the lichen thalli leaving unprotected algal cells exposed to environmental conditions. This process might produce in the long term a shift in the lichen community.

Key words: bioproduct; building stones; deterioration; dolostone quarry; lichen.

Introduction

Urine has never been considered as a building material decaying agent, apart from an aesthetic point of view. However, it has recently been proved that this anthropic agent is in fact a source of soluble salts. Cámara and coworkers (2014; 2015) investigated the decay process of building materials generated by human urine

in a simulation experiment. They demonstrated that the human urine was a detonation agent of salt crystallization processes under controlled laboratory conditions. When urine was in contact with granite showed a potential to precipitate a wide variety of salts including sulphates, phosphates and chlorides. Something similar was also previously observed when analyzing the interaction of pigeon droppings with

carbonate building stones (Gomez-Heras et al., 2004). These facts should be taken seriously into consideration since salt weathering is recognized as one of the most aggressive mechanisms of rock decay, especially in building stones (Rodríguez-Navarro and Doehne, 1999).

In this study, and for the first time, the effect of urine in a natural stone colonized by a well-known lichen species is investigated. The starting hypothesis for this study was based on: i) the nature and composition of urine (a bioproduct or metabolic product) with a very high proportion in water and enriched in ions and organic molecules, and ii) the increase in urinations in the historical centres of the cities as a result of changes in social behaviour, an important impact to take into consideration in the conservation of the cultural heritage. The Redueña dolostone was selected for this study for being a traditional building material in the Central area of Spain (Fort et al., 2013) and for being extensively colonized by the crustose lichen *Verrucaria nigrescens* (Cámara et al., 2011). On the other hand, *Verrucaria nigrescens* is a crustose epilithic lichen characterized by areolate thalli with extensive endolithic colonization. *Diplosphaera* sp. is known to be the photobiont component of *V. nigrescens* (Gueidan et al., 2011). This lichen species is broadly distributed in numerous European limestone buildings of the architectural heritage (Blazquez et al., 1995; Nimis and Martellos, 2008; Smith et al., 2010) and is considered a highly aggressive biodeterioration agent for causing aesthetic damage (darkening) and disintegration of stone material (Cámara et al., 2011; Speranza et al., 2012; 2013). Numerous studies have documented the physico-chemical actions of lichen thalli on a wide variety of host stones and their relevant contribution to biodeterioration processes on stone artworks (Ascaso et al., 1995, 2002; De los Ríos et al., 2004; 2009; Seaward, 2015). When evaluating damage of stone monuments it is important to

take into consideration that the alteration action of lichen on the rock substrate is not limited to its surface, since an extensive network of endolithic mycobiont hyphae could be widespread to depth areas of the rock (De los Ríos et al., 2005; Cámara et al., 2011).

Different experimental analyses were previously carried out in Redueña quarry rock to test the efficiency of physical and chemical treatments under the most real conditions to remove lithobiontic microorganisms, including *Verrucaria nigrescens* (Cámara et al., 2011; Speranza et al., 2012; Alvarez de Buergo et al., 2013; Speranza et al., 2013). This quarry constitutes a natural and open air laboratory that allows performing some testing that could not be possible in real heritage structures, but whose results can be extrapolated to real historical constructions built with this type of stone.

For the above mentioned reasons, our investigation pursues to determine the effect of urine on an existing lithobiontic microbial community on a dolostone and hypothesize the potential impact in a long term. Specifically our objective is the in situ evaluation of the alterations generated on *Verrucaria nigrescens* thalli as a consequence of the application of human urine, at the most real conditions, considering its possible influence on the lichen-rock interaction, and consequently, on the integrity of the rock substrate.

Materials and Methods

Materials

The field experiment was performed in a historical dolostone quarry (Redueña quarry, 50 km North from Madrid city, Spain; GPS coordinates of the quarry are: 40°47'39.76"N; 3°35'34.19"W. Figure 1). The Redueña stone is one of the traditional carbonate rock (from the Upper Cretaceous period) used as building material in Madrid region. This stone was used extensively from the 14th to the 18th

centuries, and quarried until the mid-20th century. Petrologically, it is a dolostone with a massive internal structure formed by dolomite [$\text{CaMg}(\text{CO}_3)_2$] and calcite (CaCO_3), with two main crystal sizes: 15-20 μm (dolomite) and 60-150 μm (calcite, from a de-dolomitization process: transformation of dolomite to calcite). Petrophysically, its open porosity or porosity accessible to water is $16.2 \pm 3.4\%$ (ranging from 9% to 48%), and its water absorption coefficient at atmospheric pressure is $5.6 \pm 1.4\%$. Regarding the capillarity absorption coefficient, it ranges from 77.8 to 88.1 $\text{g}\cdot\text{m}^{-2}\cdot\text{s}^{-0.5}$, depending on the orientation of the material according to its anisotropy (Fort et al., 2008; 2011). Pore size ranges from 100 μm to 7 mm (Fort et al., 2008; 2011).

Environmental data acquisition

The microclimatic conditions (environmental temperature and relative humidity) were monitored during the experiment by means of a data logger sensor (U23Pro v2, HOBO[®]; accuracy $\pm 2.5\%$ RH/ ± 0.2 °C T^a). The data logger was set to take measurements every 30 min during the 8 weeks of duration of the experiment (13/10/2013 - 09/12/2013), and the sensor was positioned in a rock fissure shaded from the sun and in close proximity to the experimental plots. The experiment was carried out during autumn season because previous studies in this quarry concluded that the prevailing environmental conditions such as moderate temperatures and high RH values during this period could favour optimal metabolic state of thalli, and with that



Figure 1. Satellite view (Google Earth) of the Redueña quarry in the municipality of Venturada, 50 km North from Madrid city (Spain), showing different quarry fronts from which material was intermittently exploited from Roman times to the mid-twentieth century. An arrow indicates the quarry front used for the present study.

Table 1. Main chemical and physical parameters in human composite urine.

Parameters	Concentration in g/L and mg/L* except pH and bicarbonate (mEq/L)
pH	8.50
density	1.02
urobilinogen	0.20
urea	13.76
uric acid	0.35
oxalic acid	15.00
citric acid	340.00
ammonium	3.00
bicarbonate	94.00
P	0.54
Total N	7.00
F ⁻ *	40.20
Cl ⁻ *	3034.00
NO ₂ ⁻ *	79.70
PO ₄ ²⁻ *	1792.00
SO ₄ ²⁻ *	1141.00
Na ⁺ *	1998.00
K ⁺ *	1755.00
Ca ²⁺ *	94.20
Mg ²⁺ *	47.00

*Data obtained from Cámara et al. (2014).

the penetration of the bioproduct into their cells. According to our observations, lichen thalli were not completely dried in anytime of the field experiment.

Analysis of urine composition

Human composite urine was collected and mixed, under aseptic conditions, from 21 donors including 10 men and 11 women from a wide range of ages, and stored in a freezer at -30 °C until the initiation of the field experiment. The physical and chemical parameters of the human urine were analysed in a specialized laboratory (Echevarne Laboratory, Madrid, Spain).

Experimental settings

This experiment simulated the process of sporadic human urinations on stone façades of the built heritage with the aim to evaluate their effects on the existing microorganisms on the stones, specifically lichen thalli on dolostone, which colonize the quarry front rock at the most real conditions. This means that treated and control lichen thalli were evaluated in their natural state of hydration.

The experiment consisted of the application of the bioproduct (see Table 1 for ion content of urine) by directly spraying a volume of 100 ml (at a distance ~ 25 cm) onto two delimited rock square areas of 30 cm side on the vertical surface of the quarry front (Figure 2a). An additional area of similar characteristics was also delimited in the proximity as a reference or control area. The experimental areas were selected for their abundance in *Verrucaria nigrescens* (Figure 2 b,c), whose lichen thalli were previously identified according their morphological characteristics. The application was performed once a week, until saturation, during 2 months on two of the delimited areas, and the control area was not treated with any additional water supply. Note that after each application, lichen thalli were not observed completely dried. At the end of the experiment, two rock samples approximately of 2 cm³ size were collected from each treated areas along with two additional samples from the control area. The *V. nigrescens* thalli selected for this study showed similar dimensions.

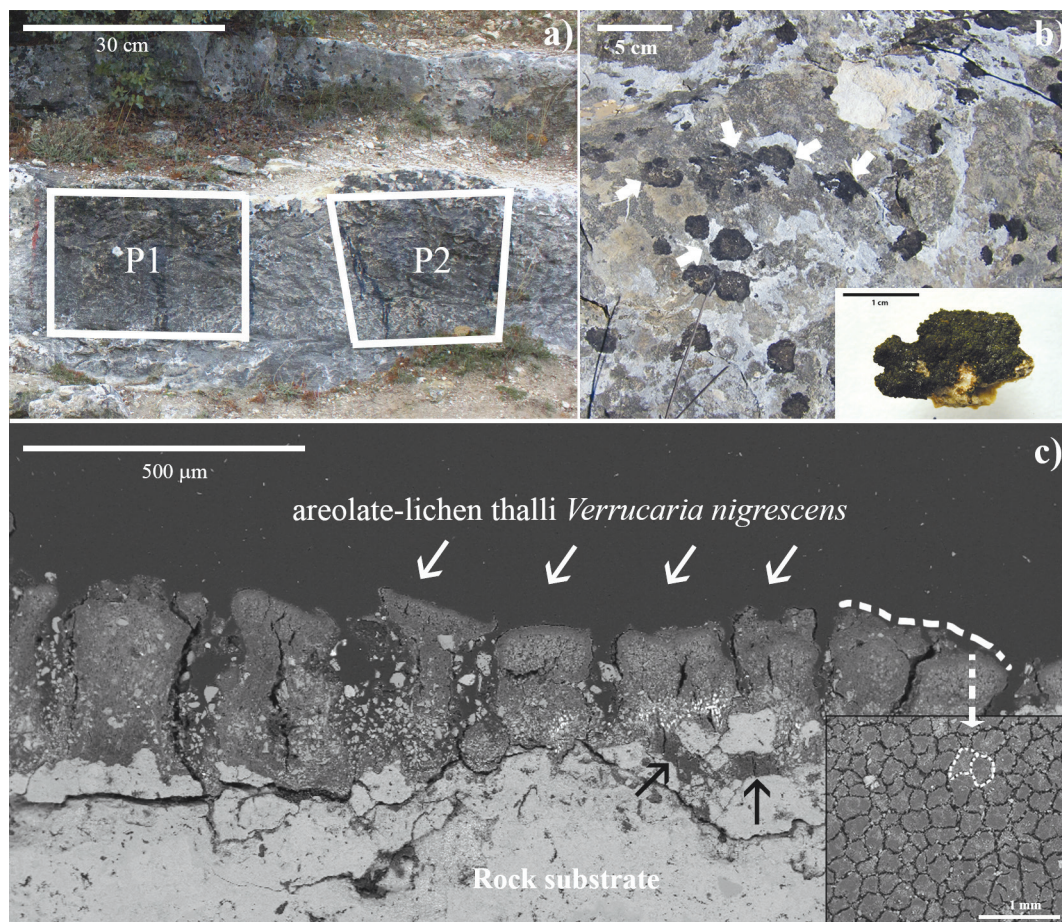


Figure 2. a) General view of the dolostone quarry front (~ 30 cm of height) showing the experimental plots P1 and P2 during one of the bioproduct application sessions; b) Detailed view of the control area extensively colonized by *Verrucaria nigrescens* (arrows and figure inset), the lichen species selected to evaluate the interaction urine-lichen; c) SEM-BSE image of a transverse section of the lichen thalli-rock interface revealing its areolate structure (white arrows) and extensive mycobiont's hyphal penetration (black arrows). The inset is an ESEM-BSE image showing the upper surface view of the areolate *V. nigrescens* lichen thalli.

A Dremel power tool (Robert Bosch Tool Corporation, Racine, WI) was used to extract the samples from the front. Transversal sections were prepared from the six pieces of material to be processed for SEM-BSE studies and smaller parts corresponding to individual areoles from young and old areas of the thalli were simultaneously prepared for TEM analyses.

Scanning Electron Microscopy in back-scattered electron mode (SEM-BSE)

Scanning electron microscopy in back-scattered electron mode was used to study the *Verrucaria nigrescens*-rock interface of treated and control dolostone samples. For that, six sections of rock samples (~ 2 cm²) were processed according to the method developed

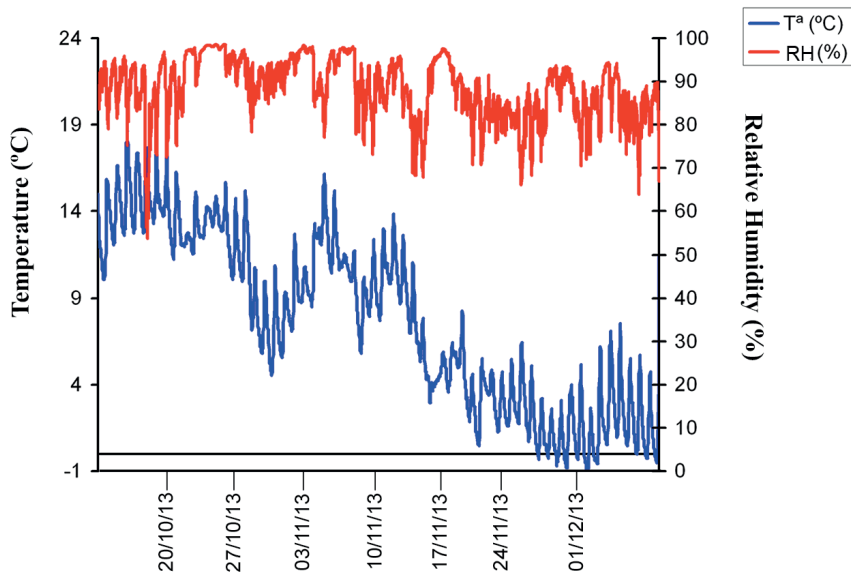


Figure 3. Microclimate diagram (blue- temperature, T^a ; red- relative humidity, RH) during field experiment (from October to December 2013) with RH values between 80 and 100% and T^a values ranging from 19 °C to -4 °C.

by Wierchos and Ascaso (1994) and observed with a Zeiss DMS 960 SEM equipped with a four-diode semiconductor BSE detector and an ISIS Link EDS microanalytical system.

Transmission Electron Microscopy (TEM)

Several areoles of *V. nigrescens* thalli from treated and untreated experimental plots were processed according to the procedure described elsewhere (Ascaso and Galvan, 1976; De los Ríos and Ascaso, 2002). The biological samples, within one day of collection, were fixed, dehydrated and embedded in Spurr's resin. Finally, ultrathin sections were stained with lead citrate and observed in a Zeiss Leo EM910 transmission electron microscope at 80kV.

Environmental Scanning Electron Microscopy (ESEM)

An Inspect FEI environmental scanning electron microscope, in back-scattered and

secondary electron modes, equipped with an Oxford Instrument Analytical 7509 energy dispersive X-ray spectroscopy (EDS), was used to observe the upper surface of treated and untreated *V. nigrescens* lichen thalli, in their natural state of hydration without a previous processing step, in order to determine the presence of any crystalline phase precipitated after the bioproduct application.

Results

Environmental data

The relative humidity (RH) values registered from 13th October to 9th December 2013 were constantly high during the experiment ranging between 80 and 100% (red line in Figure 3) with a mean RH value of 88.36 % (± 7.09). The temperature values ranged from 14 °C (at the beginning of the experiment) to 4 °C (at the end) (blue line in Figure 3) with a mean

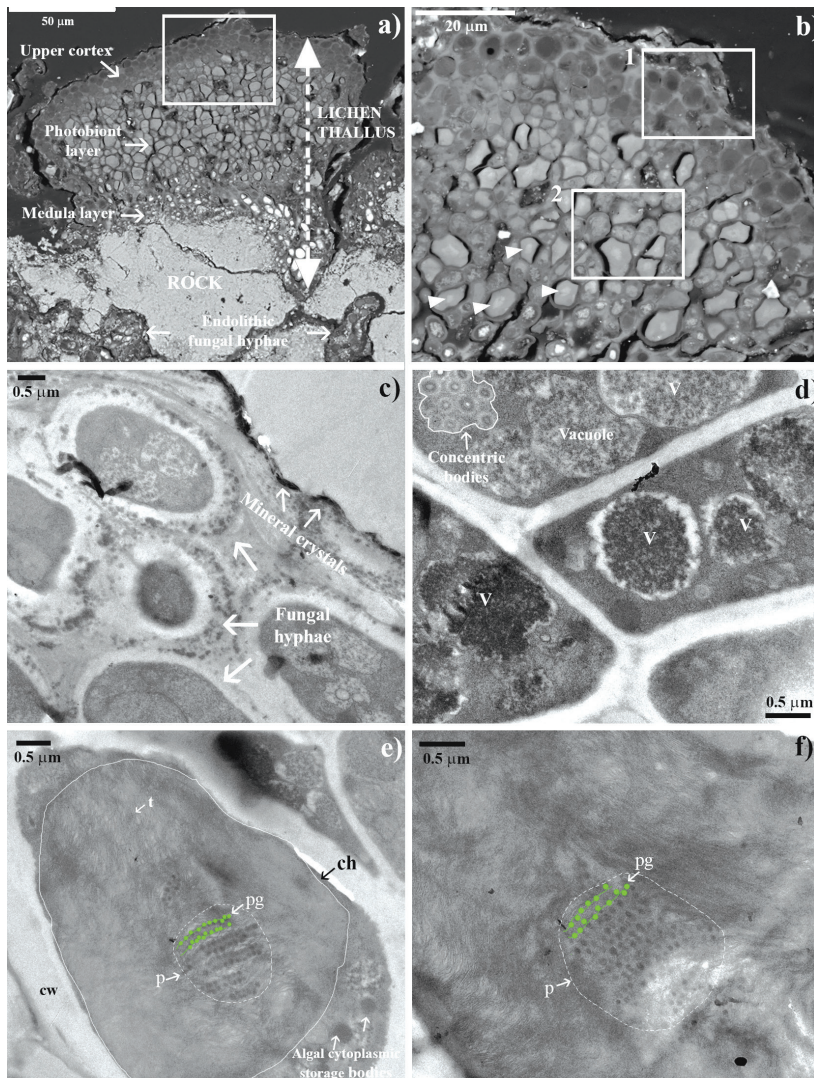


Figure 4. SEM-BSE (a,b) and TEM (c-f) images of *Verrucaria nigrescens* lichen thalli collected from a control area; a) Transverse section of a lichen thallus-rock interface with its typical aerolate and heteromeric structure. Note mycobiont's fungal hyphae occurring in pore spaces below the rock surface; b) Detailed image (area marked in 4a) of the continuous upper cortex (1) and photobiont layer (2) of *V. nigrescens*. Note that the pyrenoid area of algal cells is easily distinguishable (light grey area indicated by arrowheads); c) Ultrastructure of the upper cortex of *V. nigrescens* lichen thalli showing the typical plectenchyma tissue structure; d-f) Transversal section of the photobiont layer showing (d) fungal cells with concentric bodies and vacuoles, and (e,f) *Diplosphaera* sp. algal cells of normal appearance. Note the interior of the chloroplast (ch) in algal cells where the gelatinosa-type pyrenoid (p) shows perfectly thylakoid lamella (t) and numerous round-shaped pyrenoglobulli (pg).

temperature of 7.73 °C (\pm 5.04) and contrasting daily temperature variations especially since November. Temperature values below zero degrees were registered some days of the last week of the experiment, indicating the onset of winter season.

Physical and chemical parameters of the urine

The main physical and chemical parameters determined in the human composite urine used for the field experiment (Table 1) showed that the nature of the urine is basic (pH = 8.50) with a high content of inorganic salts and N-enriched organic compounds (urea, urobilinogen, uric acid, ammonium). The ion content of the urine was determined by ion chromatography and revealed that the most abundant anions and cations were chlorides, phosphates and sulfates, and sodium and potassium, respectively.

*Alterations of *Verrucaria nigrescens* thalli due to urine interaction*

The observation of control (untreated) and treated samples revealed alteration of the structure of *Verrucaria nigrescens* thalli and cellular alteration of biological components after bioproduct application.

The SEM-BSE images of control samples (Figure 4) showed a good structural appearance of the lichen thalli. Discrete areoles of healthy crustose lichen thalli with high extension of hyphal penetration in the rock substrate can be observed in Figures 2c and 4a. In general, the control lichen thalli showed an intact thallus with most of the algal and fungal cells of viable appearance. The upper cortex constitutes a continuous layer (Figure 4 a,b). The cells both in the upper cortex and photobiont layer, revealed intact cellular ultrastructure such as preserved cell walls, concentric bodies and vacuoles in mycobiont's fungal hyphae (Figure 4 c,d). The chloroplast of *Diplosphaera* sp. algal cells, the photobiont component of *V. nigrescens*, occupied almost the entire cell and showed a

well-organized structure. The gelatinosa-type pyrenoid can be clearly distinguished in the central area of the chloroplast showing a parallel arrangement of thylakoid lamellas with a lining up of round-shaped pyrenoglobuli (Figure 4 e,f).

In contrast, in treated *Verrucaria nigrescens* thalli (Figure 5), the effects of the bioproduct application were observed mainly in the upper cortex and in the photobiont layer, although preserving the areolate structure of the thalli (Figure 5a). In some treated lichen thalli, the upper cortex showed breakages with dead fungal cells in its structure (arrow in Figure 5b). In others, additional effects were detected in the photobiont layer showing a higher proportion of plasmolized algal (asterisks) and fungal cells (arrowheads) in comparison to the control (Figure 5c). TEM observations revealed the ultrastructure of dead fungal hyphae in the upper cortex showing only their cell walls (Figure 5d). In the photobiont layer, most of the fungal cells showed cellular disorganization (Figure 5e). Additionally, the algal cells also revealed disorganization in the area of the gelatinose-type pyrenoid, being especially evident for the disorganization of thylakoidal lamellae, and the loss of the typical size and shape of their pyrenoglobuli (Figure 5f). In both lichen thalli components, the cells walls were preserved.

The ESEM images showing the upper surface view (inset in Figures 2c and 6) of lichen thalli were obtained from samples in their natural state of hydration collected from treated and control areas (Figures 2c and 4a). Here, biological material is represented by the dark grey areas, meanwhile minerals show a brighter BSE signal because of their high atomic number. On the upper surfaces of areolate thalli and in the spaces occurring between them, in control (Figure 6 a,b) and treated samples (Figure 6 c,d), abundant mineral microcrystals (asterisks) were observed by ESEM-BSE, completely intermixed with biological material. The EDS microanalysis of these areas in control samples showed the

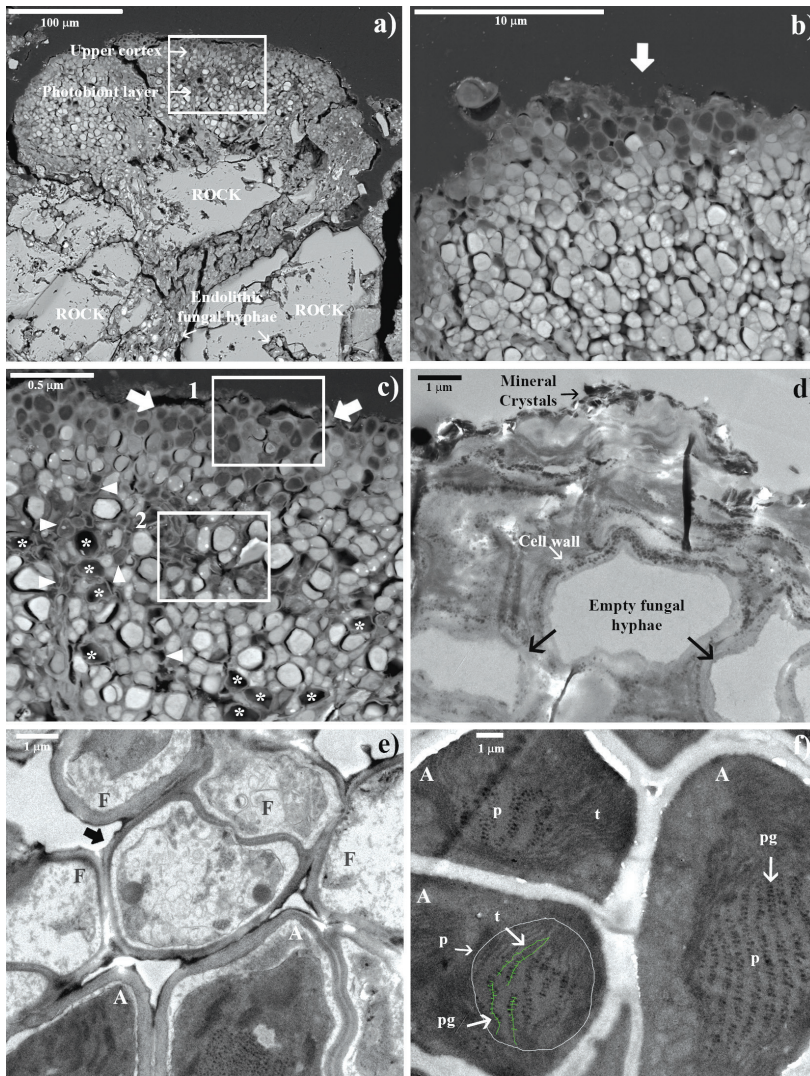


Figure 5. SEM-BSE (a-c) and TEM (d-f) images of treated *V. nigrescens* lichen thalli after 8 weeks of bioproduct application; a) Transverse section of a lichen thallus-rock interface showing cellular damage in the upper cortex and photobiont layer (arrows in marked area). Note the presence of intact fungal hyphae of the mycobiont in rocks fissures at ~ 200 µm depth; b) Detailed view of the area marked in 5a showing a *V. nigrescens* lichen thallus with some dead fungal cells (arrow) in the outer part of the upper cortex; c) Alteration effects in the upper cortex (1: fungal hyphae) and in the photobiont layer in others *V. nigrescens* lichen thalli (2: dead fungal hyphae- arrowheads; dead algal cells- asterisks); d) TEM image of the area 1 marked in 5c showing remains of dead fungal cells in the upper cortex; e,f) TEM images corresponding to the photobiont layer of treated lichen thalli (area 2 marked in 5c), e) showing cellular disorganization in fungal cells (F), and f) disorganization of thylakoid lamella (t) and loss of the typical round shape of pyrenoglobuli (pg) in the pyrenoid area (p) of *Diplosphaera* sp. algal cells (A).

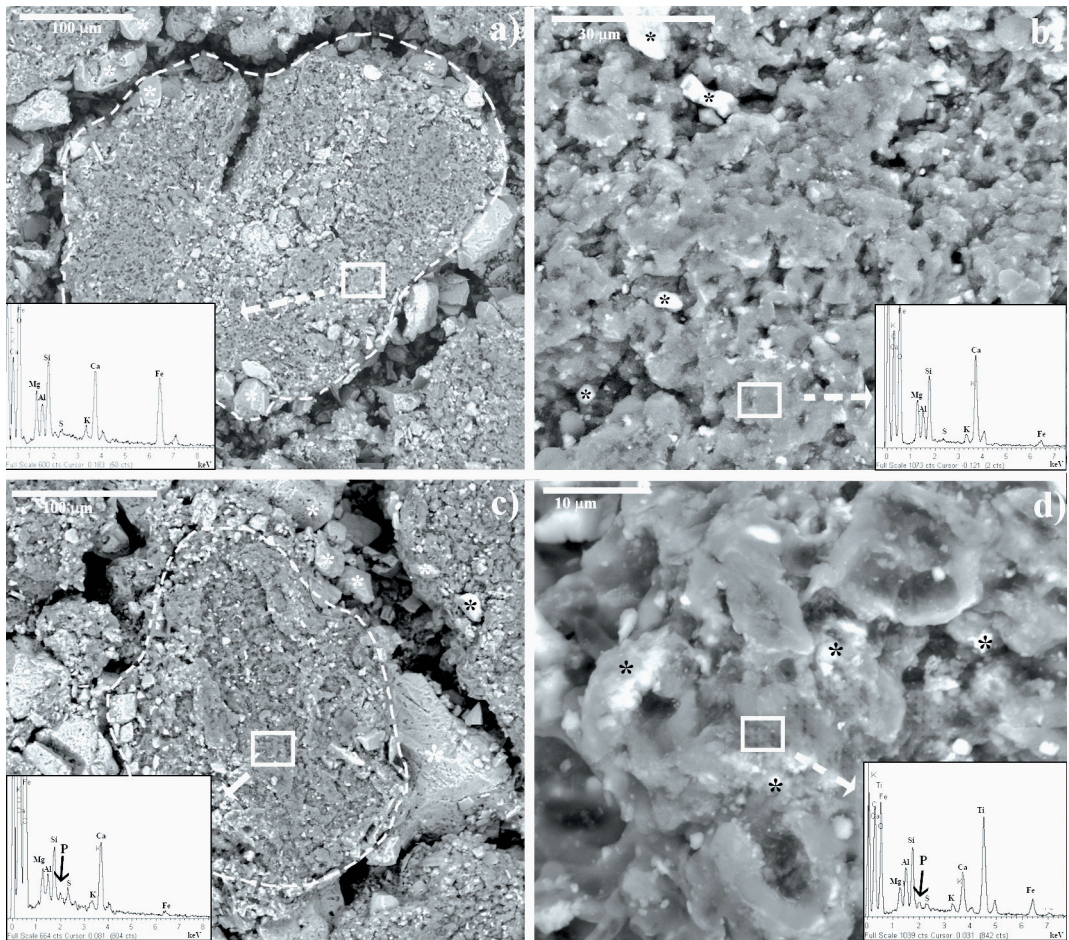


Figure 6. ESEM (BSE mode) images of the upper surface in control (a,b) and treated areolate *Verrucaria nigrescens* lichen thalli (c,d) with their EDS microanalysis spectra (asterisks- mineral crystals; dark grey areas- biological material); b and d are the corresponding detailed images of the areas marked with squares in a and c. Note the detection of P element in treated lichen thalli (insets of EDS spectra in c,d) in addition to the elements present in the host Cretaceous dolostone.

presence of Ca, Mg, Si, Al, K and Fe elements from the host dolostone (insets in Figure 6 a,b). Additionally, the EDS microanalysis on the upper surface of treated lichen thalli allowed detecting an accumulation of phosphorus (P), apart from above mentioned elements of the dolostone (insets in Figure 6 c,d). The ESEM methodology does not allow observing if there

are changes in lichen structure and cellular ultrastructure.

Discussion

The application of human urine during 8 weeks on the lithobiotic lichen *Verrucaria nigrescens* resulted in the structural alteration

of thalli and cellular damage of their symbiotic partners (mycobiont and photobiont components). The integrity of their cell membranes and ultrastructural changes were successfully determined by ultrastructure studies and electron imaging. Similar effects were observed in previous studies with biocide and laser treatments on *V. nigrescens* (Cámara et al., 2011; Speranza et al., 2012; Alvarez de Buergo et al., 2013) such as disorganization of cytoplasmic content on mycobiont's fungal hyphae of the medulla area and on their endolithic counterparts. In the *Diplosphaera* sp. algal cells, disorganization of the thylakoidal lamellae and pyrenoglobuli of the pyrenoid area were some of the most common effects observed in treated thalli. Damaged and dead cells of the mycobiont and photobiont components were recognized as dark grey areas and empty spaces in the upper cortex and photobiont layer in SEM-BSE images (Figure 5 a,c insert). The dark grey colour in these areas was due to the presence of dead or damaged cells whose biological compounds were not effectively contrasted by osmium tetroxide during the processing of samples for SEM-BSE observations. Moreover, the lack of any organic material from cells caused the presence of some empty spaces. Other techniques such as pulse amplitude modulation (PAM) which detects chlorophyll-a fluorescence could provide complementary information in relation to the functionality of the photosynthetic apparatus of the algal symbiont (Tretiach et al., 2010; Speranza et al., 2012). In fact, Speranza et al. (2012) pointed out that the combination of physiological and imaging techniques could constitute an ideal strategy for the in situ evaluation of biocide treatments on building heritage, focusing on the optimization of protocols and on the selection of the most effective biocide dose. However this technique will not provide information about the influence on the fungi component and will only give partial information of the effect. It was not used in this

experiment as we supposed a physical external effect of the urine on the lichen communities.

Verrucaria nigrescens a crustose lichen with wide ecological requirements. It has been described as a subcosmopolitan lichen inhabiting urban and natural habitats with neutral to basic pH and a broad spectrum of eutrophication levels (Nimis and Martellos, 2008), and dominating lichen communities in later successional stages (Nascimbene et al., 2009). This makes us to think that *Verrucaria nigrescens* is a lichen species characterized by a high adaptive capacity to almost any environmental condition. The human urine used in this experiment is a bioproduct with an alkaline pH (8.5), high water content (~ 90%) and enriched in inorganic salts and N-organic molecules (urea, ammonium, uric acid and urobilinogen). To our knowledge there are no studies that report the sensitive or tolerance of *Verrucaria nigrescens* to nitrogen enrichment conditions. However, the alterations observed in this study after urine treatment could indicate its low nitrogen tolerance. For instance, if the ultrastructure alterations observed in the algal symbiont cells persisted over time, this could compromise the integrity of the lichen for being the photobiont the organic carbon supplier for the mycobiont component (Friedl and Büdel, 2008). The way the cells are damaged is more probably due to a change in the pH of their environment, than to the N-compounds themselves, as determined by Frati et al. (2007) in a study on monitoring the effects of nitrogen deposition in lichens along a transect from a pig stockfarm. This is also supported by Armstrong (1984; 1994) who pointed out that the growth and/or survival of lithobiontic lichens could be altered due to changes of their microenvironmental pH when interacting with bird droppings. In a scenario with high levels of N-compounds, nitrophytic lichens (those tolerant to high levels of ammonia, displaying an increase in growth and cover, Pinho et al., 2011), which showed a

low cation exchange capacity, seemed to be more protected against the possible effects of nitrogen due to the limited possibility of this element to bind with their cell walls (Gaio-Oliveria et al., 2001). In addition to this, it has been shown in several air quality monitoring studies (Fрати et al., 2007; Pinho et al., 2011) that an increase exposure to NH_3 pollution led to a decrease of oligotrophic lichen species and an increase of nitrophytic lichen species. Considering the above mentioned, the behaviour of *V. nigrescens* observed in this study seems to be more related to non-nitrophytic lichen species than to nitrophytic lichen species, although experiments with long term exposures are needed.

Regarding the rock substrate, it is possible that lichen cover might act as a protective layer for dolostone absorbing most of urine. However, we cannot rule out potential consequences on the substrate itself in a long term exposure such as mineral disaggregation due to salt crystallization (Winkler, 1973; Price, 1996). In fact, an accumulation of phosphorous element on the surface of treated lichen samples was observed by ESEM and EDS. This could constitute nucleation sites for potential crystallization processes after only two months experiment, although no signs of any crystal salts precipitated were detected on the lichen surface, in contrast to what it could be expected from results derived in laboratory experiments (Cámara et al., 2014).

The effects of urine treatment on lichen and rock substrate should be assessed in a long term field experiment. From the biological point of view, we cannot discard that this new scenario could generate a shift in the lichen community composition producing the disappearance of some lichens and inducing the presence of nitrophilous ones. On the other hand, taking into account results obtained from Cámara et al. (2014; 2015) a prolonged urine exposure could also have serious consequences for the host rock substrate derived from precipitation of salt crystals into their pores. Additionally, the

biodeterioration processes generated by the loss of initial lichen cover could also accelerate this process.

For all these reasons, urine should be considered as additional source of nitrogen deposition and soluble salts, constituting a biodeterioration agent for the cultural built heritage whose alteration effects should never be underestimated.

Conclusive remarks

Some of the most common effects detected after the urine application on the lichen thalli colonizing dolostones analysed here were breakages of the upper cortex and increased presence of decaying fungal cells and altered algal cells chloroplast. This process will lead to expose unprotected algal cells to environmental conditions.

The P element content detected by EDS microanalysis on the upper surface of treated lichen thalli, could derive for future crystallization processes in a long-term bioproduct application.

Urine should be considered, from now, as agent of biodeterioration of cultural heritage, especially in urban areas which are exposed to human urinations.

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References

- Alvarez de Buergo M., Gomez-Heras M., Fort R., Ascaso C., De los Ríos A., Pérez Ortega S., Speranza M., Wierzchos J., Sanz M., Oujja M. and Castillejo M. (2013) - Assessment of laser treatment on dolostones colonized by microorganisms and lichens. In: Science and Technology for the Conservation of Cultural Heritage, edited by Rogerio-Candelera M.A., Lazzari M. and Cano E., Taylor & Francis Group, London, 173-176.
- Armstrong R.A. (1984) - The influence of bird droppings and uric acid on the radial growth of five species of saxicolous lichens. *Environmental and Experimental Botany*, 24, 95-99.
- Armstrong R.A. (1994) - The influence of bird droppings on the growth of lichen fragments transplanted to slate and cement substrates. *Symbiosis*, 17, 75-86.
- Ascaso C. and Galvan J. (1976) - The ultrastructure of the symbionts of *Rhizocarpon geographicum*, *Parmelia conspersa* and *Umbilicaria pustulata* growing under dryness conditions. *Protoplasma*, 89, 409-418.
- Ascaso C. and Wierzchos J. (1995) - Study of the biodeterioration zone between the lichen thallus and the substrate. *Cryptogamic Botany*, 5, 270-281.
- Ascaso C., Wierzchos J., Souza-Egipsy V., De los Ríos A. and Rodrigues J.D. (2002) - In situ evaluation of the biodeteriorating action of microorganisms and the effects of biocides on carbonate rock of the Jeronimos Monastery (Lisbon). *International Biodeterioration and Biodegradation*, 49, 1-12.
- Blazquez F., Calvet F. and Vendrell M. (1995) - Lichen alteration and mineralization in calcareous monuments of northeastern Spain. *Geomicrobiology Journal*, 13, 223-47.
- Cámara B., Álvarez de Buergo M., Fort R., Ascaso C., De los Ríos A. and Gomez-Heras M. (2014) - Another source of soluble salts in urban environments due to recent social behaviour pattern in historical centres. In: Science and Technology for the Conservation of Cultural Heritage, edited by Rogerio-Candelera M.A., Lazzari M. and Cano E., Taylor & Francis Group, London, 89-94.
- Cámara B., Álvarez de Buergo M. and Fort R. (2015) - Crystallization processes derived from the interaction of urine and dolostone. In: Geophysical Research Abstracts, EGU General Assembly, 17, EGU2015-6878-1.
- Cámara B., De los Ríos A., Urizal M., Álvarez de Buergo M., Varas M.J., Fort R. and Ascaso C. (2011) - Characterizing the microbial colonization of a dolostone quarry: implications for stone biodeterioration and response to biocide treatments. *Microbial Ecology*, 62, 299-313.
- De los Ríos A. and Ascaso C. (2002) - Preparative techniques for transmission electron microscopy and confocal laser scanning microscopy of lichens. In: Protocols in lichenology, edited by Kranner I., Beckett R.P. and Varma A.K., Springer, Berlin, 87-151.
- De Los Ríos A., Galván V. and Ascaso C. (2004) - In situ microscopy diagnosis of biodeterioration processes occurring in the convent of Santa Cruz la Real (Segovia, Spain). *International Biodeterioration and Biodegradation*, 54, 113-120.
- De los Ríos A. and Ascaso C. (2005) - Contributions of *in situ* microscopy to the current understanding of stone biodeterioration. *International Microbiology*, 8, 181-188.
- De los Ríos A., Cámara B., Garcia del Cura M.A., Jimenez Rico V., Galvan V. and Ascaso C. (2009) - Deteriorating effects of lichen and microbial colonization of carbonate building rocks in the Romanesque churches of Segovia (Spain). *Science of the Total Environment*, 407, 1123-1134.
- Fort R., Fernandez-Revuelta B., Varas M.J., Alvarez de Buergo M. and Taborda M. (2008) - Effect of anisotropy on Madrid-region Cretaceous dolostone durability in salt crystallization processes. *Materiales de Construcción* 58, 161-77.
- Fort R., Varas M.J., Alvarez de Buergo M. and Martin-Freire D. (2011) - Determination of anisotropy to enhance the durability of natural stone. *Journal of Geophysics and Engineering*, 8, S132-S144.
- Fort R., Alvarez de Buergo M., Perez-Monserrat E.M., Gomez-Heras M., Varas-Muriel M.J. and Freire D.M. (2013) - Evolution in the use of natural building stone in Madrid, Spain. *Quarterly Journal of Engineering Geology and Hydrogeology* 46, 421-428.
- Fрати L., Santoni S., Nicolardi V., Gaggi C., Brunialti G., Guttova A., Gaudino S., Pati A., Pirintzos S.A. and Loppi S. (2007) - Lichen biomonitoring of ammonia emission and nitrogen deposition around

- a pig stockfarm. *Environmental Pollution*, 146, 311-316.
- Friedl T. and Büdel B. (2008) - Photobionts. In: Lichen Biology, edited by Nash TH., Cambridge University Press, Cambridge, UK, 9-26.
- Gaio-Oliveira G., Branquinho C., Máguas C. and Martins-Loução M.A. (2001) - The concentration of nitrogen in nitrophilous and non-nitrophilous lichen species. *Symbiosis*, 31, 187-199.
- Gomez-Heras M., Benavente D., Alvarez de Buergo M. and Fort R. (2004) - Soluble salt minerals from pigeon droppings as potential contributors to the decay of stone based cultural heritage. *European Journal of Mineral* 16, 505-509.
- Gueidan C., Thüs H. and Pérez-Ortega S. (2011) - Phylogenetic position of the brown algae-associated lichenized fungus *Verrucaria tavaresiae* (Verrucariaceae). *Bryologist*, 114, 563-569.
- Nascimbene J., Salvadori O. and Nimis P.L. (2009) - Monitoring lichen recolonization on a restored calcareous statue. *Science of the Total Environment*, 407, 2420-2426.
- Nimis P.L. and Martellos S. (2008) - The information system on Italian lichens. In: Version 4.0., edited by University of Trieste DoB, IN4.0/1. <http://dbiodbs.univ.trieste.it/>.
- Pinho P., Dias T., Cruz C., Sim Tang Y., Sutton M.A. Martins-Loução M.A., Máguas C. and Branquinho C. (2011) - Using lichen functional diversity to assess the effects of atmospheric ammonia in Mediterranean woodlands. *Journal of Applied Ecology*, 48, 1107-1116.
- Price C.A. (1996) - Stone Conservation: An Overview of Current Research. Getty Institute, Sta. Monica, 88 p.
- Rodríguez-Navarro C. and Doehne E. (1999) - Salt weathering: influence of evaporation rate, supersaturation and crystallization pattern. *Earth Surface Processes and Landforms*, 24, 191-209.
- Seaward M.R.D. (2015) - Lichens as agents of Biodeterioration. In: Recent advances in Lichenology. Modern methods and approaches in biomonitoring and bioprospection, edited by Upreti D.K., Divakar P.K., Shukla V. and Bajpai R., Springer India, New Delhi, I, 189-211.
- Smith C.W., Aptroot A., Coppins B.J., Fletcher A., Gilbert O.L., James P.W., Wolseley, P.A. and Orange A. (2009) - The lichens of Great Britain and Ireland. British Lichen Society, London, 1046 p.
- Speranza M., Sanz M., Oujja M., De los Ríos A., Wierzchos J., Pérez-Ortega S., Castillejo M. and Ascaso C. (2013) - Nd-YAG laser irradiation damages to *Verrucaria nigrescens*. *International Biodeterioration & Biodegradation*, 84, 281-290.
- Speranza M., Wierzchos J., De los Ríos A., Perez-Ortega S., Souza-Egipsy V. and Ascaso C. (2012) - Towards a more realistic picture of *in situ* biocide actions: Combining physiological and microscopy techniques. *Science of the Total Environment*, 439, 114-122.
- Tretlach M., Bertuzzi S. and Salvadori O. (2010) - Chlorophyll a fluorescence as a practical tool for checking the effects of biocide treatments on endolithic lichens. *International Biodeterioration & Biodegradation*, 64, 452-460.
- Wierzchos J. and Ascaso C. (1994) - Application of back-scattered electron imaging to the study of the lichen-rock interface. *Journal of Microscopy*, 175, 54-59.
- Winkler E.M. (1973) - Stone: Properties, Durability in Man's Environment. Springer-Verlag, Berlin, 230 p.

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