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Towards a calibration of the Index of Lichen Potential  
Biodeteriogenic Activity: the case of schist in the Upper  
Douro Region (NE Portugal)

Ana Cláudia Fernandes Oliveira

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# **Towards a calibration of the Index of Lichen Potential Biodeteriogenic Activity: the case of schist in the Upper Douro Region (NE Portugal)**

Ana Cláudia Fernandes Oliveira

Dissertação de Mestrado apresentada à  
Faculdade de Ciências da Universidade do Porto em  
Ecologia, Ambiente e Território  
2014





# Towards a calibration of the Index of Lichen Potential Biodeteriogenic Activity: the case of schist in the Upper Douro region (NE Portugal)

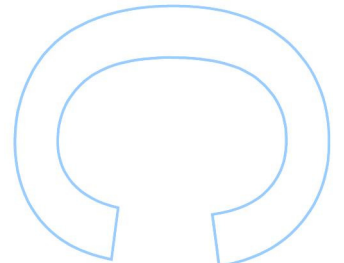
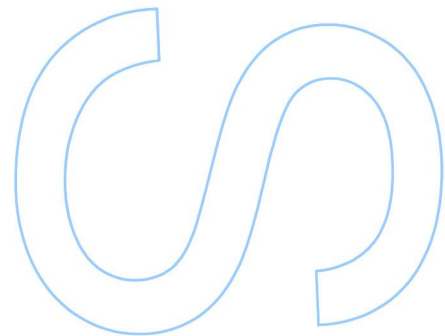
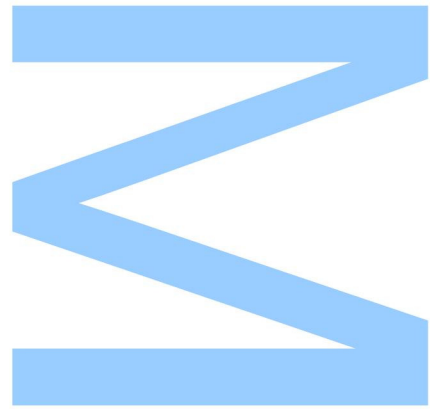
Ana Cláudia Fernandes Oliveira  
Master in Ecology, Environment and Territory  
Biology Department  
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## **Supervisor:**

Prof. Dr. Rubim Almeida, Auxilliary Professor, Faculty of  
Sciences (Biology Department), University of Porto

## **Co-supervisor**

Dr. Joana Marques, Collaborator CIBIO-InBIO,  
University of Porto

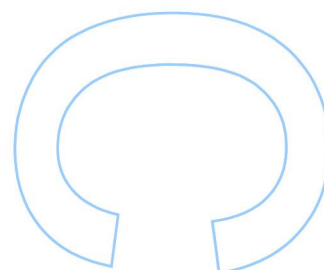
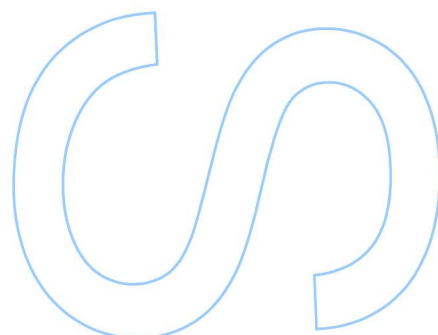
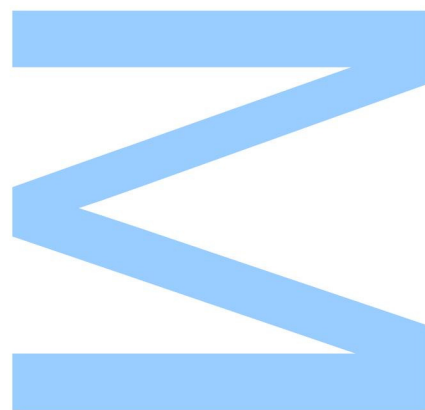




Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

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## Foreword

### List of papers:

Oliveira C, Marques J, Almeida R & Prieto B (submitted). **“Lichen-induced geochemical weathering of schist surfaces in Côa Valley Archaeological Park (NE Portugal)”**. Chemical Geology **Chapter 4**

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Oliveira C, Marques J, Paz-Bermúdez G & Almeida R. **“Relação entre padrões ambientais locais e a distribuição de espécies de líquenes dominantes no Vale do Côa (NE Portugal)” 2013**. Poster session presented at: IX Encontro Internacional de Fitossociologia – Vegetação e paisagem: uma perspectiva sócio-ecológica. May 9th-12th 2013; Parque Biológico de Gaia, Avintes, Vila Nova de Gaia.

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## Abstract | Resumo

Lichen communities are the most abundant colonizer of the vertical schist surfaces in the Upper Douro Region. Lichen organisms are closely connected to the substrate, establishing relationships with it, and they could lead to modification more or less significant. Lichen action on the rock surfaces in the Upper Douro region and especially in the Côa valley requires special attention since, in this area, the world's largest collection of open-air rock-art sites is found and the need of its conservation and preservation requires particular attention and investigation. This action could be either degradative or protective and when the substrate modification occurs, it could occur in two ways, physical or chemical, and each one is related to the colonizing species and the intrinsic substrate properties, and this phenomenon is labelled as biodeterioration. However, oversimplification and the interpretation of lichen action as exclusively degradative should be avoided once there are reports of protective action of lichen thalli and acting as a shield-like barrier between the substrate and the influence of external weathering agents (e.g.: water, wind, temperature) occurring, therefore, bioprotection. To understand how rock deterioration is related to lichen action in the study area, this work aimed to answer to various issues as: which climatic factors are related to the species occurrence and in which spatial scale would be more significant; in which way lichen colonization of surfaces is related to chemical weathering and which species contribute in a major way to that process and, lastly, in which way the index of Lichen Potential Biodeteriogenic Activity could be applied to the schist surfaces of the Upper Douro region as an evaluation tool of lichen action as biodeterioration agent.

To perform more practical and less time-consuming evaluations of the lichen action in the schist surfaces in the Upper Douro Region, four dominant species were used as representative species of the lichen assemblages present in the area. Thus, the dominant species present in the study area are the crustaceous *Aspicilia contorta* subsp. *hoffmanniana*, *Caloplaca subsoluta*, *Lecanora pseudistera* and the squamulose *Peltula euploca*. The results acquired showed that the climatic factors that influence the most in lichen occurrence are at the micro-scale level (temperature and relative humidity).

Element mobilization in colonized schist surfaces was also evaluated and the results showed that there is an aspect-related activity in element changes. In north-facing surfaces there was no evidence of lichen-induced element change. On the other side, in south-facing revealed an intense mobilization of elements being the surfaces colonized by *Caloplaca subsoluta* the ones with more statistically significant differences detected.



Allowing an evaluation of lichen action on the schist surfaces in the Upper Douro Region, the implementation of Lichen Potential Biodeteriogenic Activity was tested. The results enhanced a strong dependence of the species abundance and an improvement in the calculation of the index was proposed. Climatic variables could be incorporated to the calculation and, therefore, fill gaps related to physical and chemical action of lichens, once, often, these parameters are hard to obtain.

**Keywords:** lichens, biodeterioration, rock conservation, rock-art, climatic variables, chemical weathering, Index of Lichen Potential Biodeteriogenic Activity,

As comunidades líquénicas são os colonizadores mais abundantes das superfícies verticais de xisto da região do Douro Superior. Os líquenes são organismos que estão em contacto muito próximo com o substrato e com ele interagem, podendo provocar alterações mais ou menos significativas e extensas. A ação dos líquenes nas superfícies rochosas do Douro Superior em geral e do Vale do Côa em particular prende-se com o facto de, nesta zona, se encontrar a maior coleção mundial de arte rupestre ao ar livre e onde a sua conservação e preservação se torna no foco especial de atenção e investigação. Esta ação pode afigurar-se degradativa ou protetora. Quando há alteração do substrato por ação líquénica, esta pode verificar-se sob duas formas: alteração física e química, sendo que cada uma estará dependente quer do tipo de espécie que coloniza a rocha quer das propriedades intrínsecas do substrato, designando-se este fenómeno por biodeterioração. No entanto, não se deve generalizar e interpretar a ação líquénica exclusivamente degradativa, pois podem existir situações em que o talo funciona como um escudo protetor e forma uma barreira entre o substrato rochoso e a influência dos agentes de meteorização externos (ex.: água, vento, temperatura) ocorrendo, portanto, bioproteção.

Para inferir a melhor forma de incluir a ação dos líquenes na deterioração da rocha na área de estudo, este trabalho procurou responder a várias questões, nomeadamente: quais os fatores climáticos condicionam a ocorrência das espécies e em que escala espacial será mais relevante; de que forma a colonização das superfícies induz a meteorização química e quais são as espécies que mais contribuem para essa degradação e, por fim, em que medida se pode aplicar o índice LPBA (Lichen Potential Biodeteriogenic Activity) às superfícies xistosas do Douro Superior como forma de avaliar a ação dos líquenes como agentes de biodeterioração. Com vista a tornar mais prática e expedita a avaliação da atividade dos líquenes nas superfícies foram determinadas quais as espécies dominantes das comunidades líquénicas saxícolas do

Douro Superior. Deste modo, as espécies que se apresentam como dominantes são as do tipo crustáceo *Aspicilia contorta* subsp. *hoffmanniana*, *Caloplaca subsoluta*, *Lecanora pseudistera* e a do tipo escamoso *Peltula euploca*. Os resultados obtidos mostraram que os fatores climáticos que influenciam de forma mais evidente a distribuição das espécies são aqueles verificados à micro-escala (temperatura e humidade relativa).

Numa avaliação preliminar dos efeitos da colonização líquénica na mobilização dos elementos que constituem os minerais do xisto, os resultados evidenciaram uma ação associada à orientação das superfícies. Nas superfícies expostas a norte, não foram detetadas quaisquer alterações ao conteúdo em elementos. Por outro lado, nas superfícies expostas a sul, verificou-se uma intensa atividade nas superfícies colonizadas por *Caloplaca subsoluta* onde se destacaram como os locais onde ocorreram alterações significativas no conteúdo em vários elementos.

De forma a permitir uma avaliação da ação líquénica nas superfícies gravadas da região do Douro Superior, foi testada a implementação de um índice de potencial deterioração, LPBA (Lichen Potential Biodeteriogenic Activity). Os resultados evidenciaram uma grande dependência da abundância de espécies no cálculo e foi proposta uma melhoria do índice, incorporando variáveis climáticas de forma a colmatar falhas por parte da ação física e química das espécies, uma vez que estes parâmetros são, muitas vezes, difíceis de obter.

**Palavras-chave:** líquenes, biodeterioração, conservação da rocha, arte rupestre, variáveis climáticas, meteorização química, índice de biodeterioração.



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## List of abbreviations

**NE** – North-east

**LPBA** – Lichen Potential Biodeterioration Activity

**PAVC** – Parque Arqueológico do Vale do Côa (Côa Valley Archaeological Park)

**UNESCO** – United Nations Educational, Scientific and Cultural Organization

**XRF** – X-ray fluorescence



# Chapter 1 |

## General introduction

Lichens are defined by the International Association for Lichenology as "a permanent association between a fungus and a photosynthetic symbiont which results in a stable thallus" (Marques 2008). These organisms are very resistant to extreme environmental conditions such as dryness or freezing temperatures and they also able to colonize areas where other forms of life will fail to establish (Marques 2008; Warscheid & Braams 2000) like mountain tops (Arocena *et al.* 2003) or deserts (Viles 2005). They represent roughly 8% of dominant colonizers of the Earth surface (Paradise 1997). Its high plasticity in terms of colonization of different habitats makes possible their occurrence across a wide range of conditions and, generally, lichens can be classified as saxicolous (rocks or rock surfaces), epiphytes (on plants) or terricolous (on land) (Lisci *et al.* 2003; Marques 2008).

Lichen thallus can present itself as crustose, foliose and fruticose and it is distinguished by its form of adhesion to the substrate (Chen *et al.* 2000). In lichens of the crustacean type, the thallus adheres strongly to the surface and penetrates it, forming a sort of crust (Lisci *et al.* 2003) and, when colonized rocky substrates, can be further subdivided into epilithic (at the rock surface) and endolithic (inside the rock) (Chen *et al.*, 2000). In the case of foliose thallus, the substrate is only penetrated by rhizines that are structures similar to roots and makes easy to detach it from the rock surface (Lisci *et al.*,

2003). Finally, fruticose lichens grow in a branched form that converges into a single attaching point that connects them to the substrate (Lisci *et al.*, 2003; Marques 2008).

Colonization of the substrate depends on several factors that can be assembled into: properties of the substrate (e.g.: rock type, surface slope, bioreceptivity) (Adamson *et al.* 2012; Carballal *et al.* 2001; Guillitte 1995; Kumbaric *et al.* 2012; Warscheid & Braams 2000) and environmental factors (e.g.: humidity, temperature, sunlight, disturbance) (Adamson *et al.*, 2012; Ellis & Coppins 2010; Prieto *et al.* 1999; Seaward *et al.* 2001; Warscheid & Braams 2000). Saxicolous lichens are considered pioneer colonizers of rocks since they have the ability to settle on rocky surfaces that have not been noticed early processes of biological change (already triggered by physical-chemical factors, bacteria, algae and fungi) (Lisci *et al.* 2003; Viles 1995).

In addition to the substrate, it is also necessary to understand how environmental conditions at different scales influence the colonization of rock surfaces. Climatic factors are closely related to surfaces aspect (Adamson *et al.* 2012). In the Northern Hemisphere, the north-facing surfaces are wetter and sometimes do not receive direct sunlight (especially in winter) and there is a tendency for environmental variations are smaller. In contrast, the south-facing slopes are more exposed to sunlight and there is a tendency for higher values of temperature (Adamson *et al.*, 2012).

Buildings and monuments are very common surfaces for lichen colonization (Chen *et al.* 2000) and stones or rocky materials are largely employed in its construction (Miller *et al.* 2012). As previously said, lichens act like pioneer colonizers of surfaces, and constructed buildings present themselves as a great spot for lichen colonization. Churches (Carballal *et al.* 2001; de los Ríos *et al.* 2009; Prieto & Silva 2003; Prieto Lamas *et al.* 1995; Villar *et al.* 2004), ancient archaeological sites (Ariño *et al.* 1995), dolmens (Prieto & Silva 2003; Romão & Rattazzi 1996) or natural monuments like “*fairly chimneys*” in Turkey (Garcia-Vallès *et al.* 2003) are some examples of structures when lichens can be found. There is a wide spectrum of rock types used in construction works of historical building and they range from plutonic rock like granite (Carballal *et al.*, 2001; de los Ríos *et al.* 2002; Prieto Lamas *et al.* 1995) to sedimentary rocks like sandstone or limestone (Ascaso *et al.* 2002; de los Ríos *et al.* 2009; Seaward 1997; Villar *et al.* 2004).

Lichens have an important task on rock deterioration and its pedogenetic role is extensively known for years (Ascaso & Galvan 1976; Ascaso *et al.* 1976; Favero-Longo *et al.* 2005; Galvan *et al.* 1981) and reports from the 19th century (Merrill 1897, cited by Burt *et al.* 2008) also enhance how lichens and lichenological colonization of rocks is important in soil formation process.

In line with the previous paragraphs, it is recognized that lichenological colonization of surfaces can induce and enhance the processes of physical or chemical

deterioration (Adamo & Violante 2000; Carballal *et al.* 2001; Chen *et al.* 2000; Lisci *et al.* 2003; Seaward *et al.* 2001; Warscheid & Braams 2000), and consequent alteration of the substrate (Carballal *et al.* 2001; Lisci *et al.* 2003; Seaward *et al.* 2001). So, from the physical point of view, the deterioration is related to the contraction and expansion of lichen thalli, which causes crevices resulting in surface disruption that increase the contact zone in the lichen-rock interface and penetration of hyphae through the rock interstices (Adamo & Violante 2000; Purvis 2008; Zambell *et al.* 2012). These important mechanisms of weathering and mechanical damage either in rocky outcrops or in buildings potentiate the effect of other agents of degradation like other fungi, vascular plants or animals (Chen *et al.* 2000). On the other hand, chemically induced biodeterioration is related to the action of lichenic acids as chelating agents of rock minerals (Adamo & Violante 2000; Adamson *et al.* 2012; Chen *et al.* 2000) allowing the neoformation of minerals like one of the most common, calcium oxalate (Chen *et al.* 2000; Edwards *et al.* 1997; Prieto *et al.* 2000; Syers *et al.* 1967) and this neoformed mineral can assume two configurations, related to its hydration state: whewellite or calcium oxalate monohydrate (Bungartz *et al.* 2004; Edwards & Perez 1999; Prieto *et al.* 2000) and weddellite or calcium oxalate dihydrate (Hernanz *et al.* 2008; Prieto *et al.* 2000; Seaward & Edwards 1997).

One of the most common lichenic acids usually identified as weathering agent in biodeterioration studies is oxalic acid (Ariño *et al.* 1995; Chen *et al.* 2000; Easton 1994; Prieto & Silva 2003; Sabbioni & Zappia 1991; Seaward 2003; Seaward 1997) once this acid is present in a large amount of lichen-forming fungi (Adamo & Violante 2000) and the reaction between the acid and calcium-bearing minerals leads to calcium oxalate formation in the lichen-rock interface (Ariño & Saiz-Jimenez 1996; Edwards *et al.* 1997; Syers *et al.* 1967). The presence of calcium oxalate mineral and other oxalates from rock-forming minerals are considered a reliable evidence of rock alteration (Adamo & Violante 2000; Chen *et al.* 2000; Warscheid & Braams 2000).

Concerning surfaces with archaeological interest, lichen colonization acts in the same way as other exposed surfaces with the same conditions for the establishment of pioneer communities. Rock colonization starts beneath the ground by organisms like bacteria (Gorbushina 2007) and when the rock is exposed, it is available for photosynthetic organisms to colonize it. Algae and cyanobacteria start to grow and they provide better conditions for others organisms to settle and the ecological succession begins with the arising of other living forms like mosses, lichens, ferns or vascular plants.

In the context of rock-art conservation and in line with what was said in the preceding paragraphs, the concepts of biodeterioration and bioprotection arise.

Biodeterioration could be defined as the lichen-induced damage caused by both chemical and physical actions (Chen *et al.* 2000). However, not all lichenological communities can be regarded as agents rock degradation (Ariño *et al.* 1995; Chen *et al.* 2000; Garcia-Vallès *et al.* 2003) and they can act as a barrier between the substrate (Seaward *et al.* 2001) and abiotic agents such as water runoff, wind abrasion, or temperature fluctuations, designating whether this phenomenon as bioprotection (Chen *et al.* 2000). This differentiation makes it clear that not all the lichen communities accelerate the weathering processes of the substrate and there comes the need to distinguish species that play a role in degradation of others that are harmless and assume a protective role (Ariño *et al.* 1995; Carter & Viles 2003, 2005; Chen *et al.* 2000; Mottershead & Lucas 2000).

River Douro and its tributaries Côa, Sabor and Tua exhibit a vast collection of open-air rock-art surfaces and they, but it is in the Côa valley where most of the engraved surfaces are located. Côa Valley Archaeological Park was classified as a World Heritage Site by UNESCO in December 2, 1998 and represents the largest collection of open-air rock-art site from the Paleolithic in the Western Europe (Baptista 2001) with more than 1000 exposed engraved surfaces identified (Fernandes 2012; Marques 2013). Due to these discoveries and their undeniable scientific interest (Baptista 2001), it is necessary to adopt conservation techniques to preserve this heritage for future generations (Fernandes 2004, 2012).

Recently, Marques *et al.* (2014) assessed the lichen and bryophyte abundance in rock-art surfaces in the Upper Douro region, focused on schist panels in the Côa Valley Archaeological Park and it was concluded that these groups were the dominant colonizers of the rock surfaces. The study was conducted to evaluate the abundance of species in opposite aspects (north-facing *versus* south-facing surfaces) and lichens dominate over bryophytes and taking this information into account, seems clear the need to include lichen action in the rock-art preservation and conservation plans.

To assess how rock weathering could be assigned to lichen colonization it is necessary to estimate how much of the designed rock weathering is attributed to lichens rather than environmental factors as, for instance, rain, temperature or wind. To quantify lichen action on rock surfaces, Gazzano *et al.* (2009) purpose the implementation of an index – Index of Lichen Potential Biodeterioration Activity – applied to the evaluation of the lichen action in stonework, where species and substrata features were taken into account, namely: cover (abundance in the substrate), reproductive potency of lichen species, the physical action of the species on the substrate, depth of hyphal penetration and spread in the colonized interface, physical and chemical action, and bioprotection effects reported in literature. These parameters need to be adapted to the particular

conditions observed in the Upper Douro region therefore providing a useful tool to be employed by researchers involved in the preservation of the cultural heritage as the open-air rock-art sites.

Efforts are being made there to understand the weathering dynamics acting on the schist outcrops that support the rock-art, integrating biological, geophysico-chemical and environmental data in order to prevent major damages to the engraved surfaces (Fernandes 2004). There is general consensus about the combination of physical (mechanical) and chemical changes brought about by lichens to rock surfaces but the extent and relative contribution of their weathering action is a central question – still unanswered – in the Upper Douro Region as in the field of rock-art conservation in general.

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## Chapter 2 |

### Aims and thesis organization

The main goal of this work is to provide insights about the lichen colonization contribution to potential rock weathering in the Upper Douro region and incorporate this information in conservation and protection of open-air rock-art in the region.

During the course of the work, the following specific goals were achieved:

- ◆ to assess how patterns of climatic variables affect the abundance and distribution of lichen species in the Upper Douro Region;
- ◆ to identify useful species as indicators of environmental factors relevant to rock conservation;
- ◆ to evaluate of the role played by lichen in the chemical weathering of schist surfaces and implication in rock-art deterioration in the Upper Douro region;
- ◆ to contribute to the validation and calibration of the Index of Lichen Potential Biodeteriogenic Activity (LPBA) on schist.

### Conceptual structure and thesis organization

To accomplish the upper-cited goals, the work was planned in a three-level outline, regarding singular subjects implied in the study.

**Level I.** *The role of multi-scale climatic variables on the abundance and distribution of saxicolous lichens in the Upper Douro Region (NE Portugal)*

Assessing which environmental variables are related to patterns of occurrence in vertical schist surfaces in the Upper Douro Region. This chapter complements prior works concerning only Côa Valley Archaeological Park and the results previously obtained were verified in a larger area.

**Level II.** *Lichen-induced geochemical weathering of schist surfaces in Côa Valley Archaeological Park (NE Portugal)*

Study of the chemical changes in element mobility of colonized schist surfaces and how those changes could be assigned to lichen colonization. This approach is linked to previous studies where the analysis was restricted to mineralogical changes on schist.

**Level III.** *Modelling the vulnerability of schist surfaces to lichen-induced deterioration in the Upper Douro Region (Portugal)*

Employing information of prior studies and new data obtained with this work in the calibration and validation of the Index of Lichen Potential Biodeteriogenic Activity in the schist surfaces of the study area.

## Chapter 3 |

# The role of multi-scale climatic variables on the abundance and distribution of saxicolous lichens in the Upper Douro Region (NE Portugal)

### Abstract

The study was focused on a multi-scale approach (macro-, meso- and micro-scale) related to climatic factors, specifically, temperature, relative humidity, and precipitation. A multivariate analysis (DCA and CCA) was performed to establish the relationship between species distribution and climatic variables and a partition of variance (pCCA) was used to assess the relative contribution of each variable to general patterns of distribution. The results obtained revealed that, for the total species data, all variables were significant in the species distribution in the study area. As lichen assemblages are usually highly species rich, the study of lichen distribution is invariably focused on a few target species, usually the dominant or more characteristic ones. Concerning the dominant species, the crustose *Aspicilia hoffmanniana*, *Caloplaca subsoluta* and *Lecanora pseudistera* and the squamulose *Peltula euploca*,



only the micro-scale factors, temperature and relative humidity, were significant to explain their distribution. Since the dominant species are widely spread in the study area and their distribution is intimately related to climatic conditions observed in the substrate it seems more important to monitor climatic conditions at the schist surface lichen distribution on exposed schist surfaces and to give useful information for archaeologists and conservators in the Upper Douro region about the features of surface colonization and the need of including it in the risk assessment and conservation programmes of open-air rock-art on schist once lichens are the pioneer communities in this sort of substrate.

**Keywords:** saxicolous lichens, schist surfaces, multivariate analysis, rock-art preservation, partition of variance

## Introduction

Lichen colonization of exposed rock surfaces has been a field of growing interest in the last few decades (Adamson *et al.* 2012; Armstrong 2002; Carballal *et al.* 2001; Dale 1995; de los Ríos *et al.* 2002; Prieto Lamas *et al.* 1995; Syers *et al.* 1967), especially in the context of stone conservation, owing to the realization that many buildings and other monuments of historical meaning are hotspots of lichen diversity and the general consensus about the weathering ability of these organisms (Viles 1995). Mellor (1921) provided the first studies on lichen-induced weathering in ancient stained-glassed windows, after the pioneer works of Muntz (1890) and Sollas (1880) on biological weathering. Since then several authors have provided increasingly detailed observations and explanations of the actions of various lichen species on a range of rock types (Gorbushina 2007; Prieto *et al.* 2000; Sabbioni & Zappia 1991; Seaward & Edwards 1997)

The presence of rock-dwelling lichens on monuments and archaeological remains is usually regarded as negative both for aesthetic and conservative reasons, due to the physical and chemical changes they are known to cause on rock surfaces (Seaward 2003). On the other hand, some authors (Ariño *et al.* 1995; McIlroy de la Rosa *et al.* 2013b; Mottershead & Lucas 2000) defend the protective role of such organisms, once they provide a barrier between substrate (e.g.: limestone, sandstone, granite) and agents of deterioration such as rain, wind or temperature (Warscheid & Braams 2000).

Colonization of rock surfaces by lichens has special requirements in terms of pH of the substrate, humidity and luminosity (Lisci *et al.* 2003), just to name a few, and the presence or absence of certain species can be a good indicator of the conditions observed in a certain region (Prieto *et al.* 1999). Climatic conditions are very important for species occurrence, abundance and distribution and even a slight change in their range could lead to changes in species assemblages in a certain area (Hauck 2009). To evaluate those changes it is central to understand how each variable contributes to explain those changes (Legendre & Legendre 1998). For instance, variations of temperature and water availability related with surface aspect led to significant differences in biological colonization by *Rhizocarpon geographicum* in opposite orientations in North Wales (Armstrong 2002).

Multi-scale studies take into account that ecological events happen at various scales (Hespanhol *et al.* 2010; Lalley *et al.* 2006) and each scale contributes in a major or minor way to answer to specific questions (Cushman & McGarigal 2002). In line with multi-scale approach, partition of variance presents itself as a good method (Ellis & Coppins 2010; Hespanhol *et al.* 2011; Jüriado *et al.* 2009; Legendre & Legendre 1998) to evaluate the contribution of each variable to the explained variability and allows to understand how of each phenomena affects the organisms studied. Ecological studies about the influence of large and small-scale environmental factors on the structure of lichen species assemblages on rock surfaces have been undertaken by several researchers in different parts of the world (Kershaw & Macfarlane 1980; Lalley *et al.*, 2006; Orwin 1972; Pentecost 1979). The effect of lichen colonization on stone monuments must be analysed with caution as the role lichen species play on rock surface weathering might be different for each particular situation depending on the climatic factors acting at different scales (Pope *et al.* 1995; Viles 2001).

Viles (1995) developed a conceptual model showing how the effectiveness of biological weathering changes, depending upon the stress of the environment. Change can be in terms of large and small-scale environmental drivers, the first related with latitudinal and altitudinal gradients in climate while the latter depicted by microclimate determined by local variation of factors such as aspect and slope. The scale-related issues can become critical for rock conservation since lichen species distribution (McIlroy de la Rosa *et al.* 2013a) and activity (Giordani *et al.* 2003) is restricted to areas with suitable microclimates even if regional climatic conditions are less suitable.

Lichens form the pioneer communities inhabiting the engraved surfaces in the Upper Douro region (Marques *et al.* 2014) and provide the conditions necessary to support other organisms in the next stages of ecological succession. The Upper Douro

region holds one of the world's largest collections of open-air rock-art dating from the Upper Paleolithic spread throughout the slopes and riverbanks of river Douro and its main tributaries in the region: Côa, Sabor and Tua. The majority of engraved surfaces is located in the Côa Valley Archaeological Park (Vila Nova de Foz Côa, north-east Portugal) which is a UNESCO classified world heritage site since 1998 (Baptista 2001).

To assess the contribution of biological colonization in the preservation or degradation of open-air rock-art surfaces in the Upper Douro region, it is necessary, firstly, to understand which drivers are responsible for the establishment of these lichen-dominant communities in a multi-scale approach.

This study aims to provide information about the environmental requirements of lichen assemblages in the engraved surfaces in the Upper Douro region. Specifically, we ask: 1) Which are the dominant species colonizing exposed vertical schist surfaces in the Upper Douro region? 2) How are environmental variables at three different spatial scales related to lichen colonization of schist surfaces? 3) How do environmental variables at three different spatial scales affect the abundance patterns of dominant lichen species in schist surfaces?

## Material and methods

### *Study area*

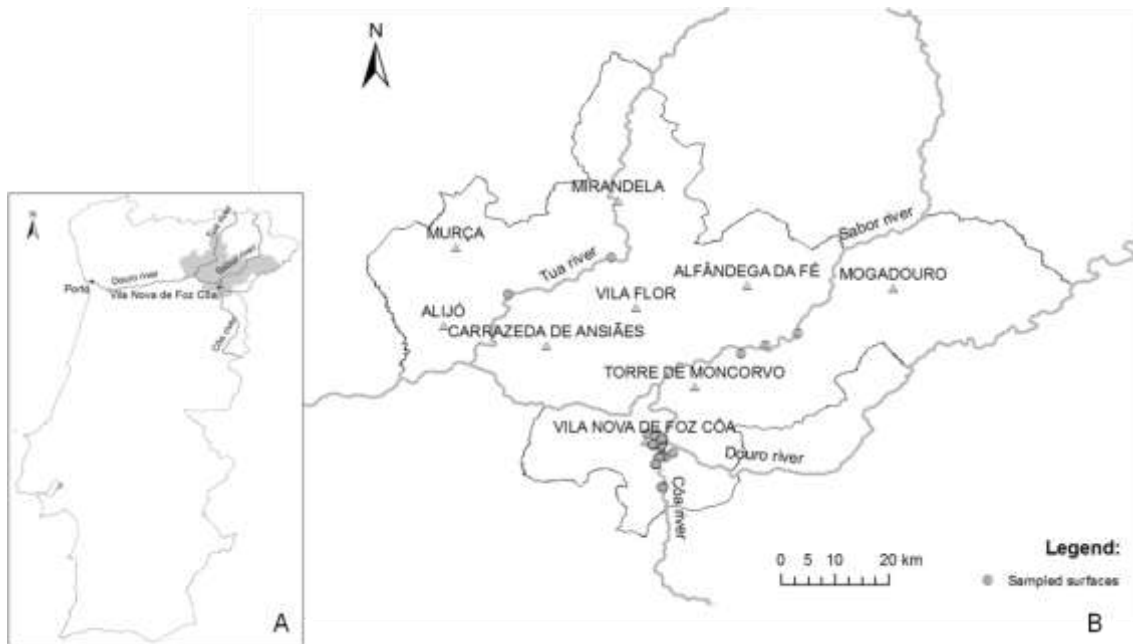
Sampling strategy was initially focused in the schistose areas of the Côa Valley, where most rock-art sites are located, however, soon it became clear that the neighbouring areas shared characteristics like climate (AEMET & IM 2011), geology (Oliveira *et al.* 1992), biogeography (Franco 1994), landscape history (Andresen 2006), lichenological interest (Marques 2013) and occurrence of rock-art of the same period (Bicho *et al.* 2007). The limits of the study area are centred on the valley of River Douro and the valleys of its tributaries Côa, Sabor and Tua and respective municipalities (Figure 1).

The climate is predominantly Mediterranean with very hot and dry summers and cold winters. The study area is located between the mountainous areas of Alvão-Marão, Montemuro and Montesinho and, in fact, this causes a barrier from moist winds from the Atlantic Ocean and extreme values of temperature are easily reached (AEMET & IM 2011; Marques 2013). Vila Nova de Foz Côa municipality, which is located in the mouth of the river Côa is the hottest of Portugal, with mean temperatures of the warmest month reaching 34.5°C (Marques 2013).

The area is dominated by schist from the schist-greywacke complex from Trás-os-Montes and there are large amounts of outcrops dispersed through the region and these outcrops are characterised by natural occurring vertical schist surfaces.

### *Sampling design*

The study was performed by recording the percentage of lichen cover in 66 exposed vertical schist surfaces spread throughout Tua river valley (3), Sabor river valley (4) and Côa river valley (59) through visual inspection. The analysis was performed using the total species assemblage and, afterwards, the analysis was also conducted with a subset of the original data containing only the dominant species.



**Figure 1:** Study area. A: Location of the study area in north-east Portugal. B: Study area in detail with reference to administrative areas (municipalities) where sampling took place, rivers (Douro and its main-tributaries in the region: Côa, Sabor and Tua) and location of the sampled surfaces (grey dots).

### *Environmental variables*

Vertical schist surfaces in the Upper Douro region were selected to perform the analysis once they are representative of engraved surfaces. Surface aspect was obtained from a digital elevation model (DEM) in ArcMap 10.1 (ESRI 2012) and only four classes were considered (North, South, East and West). Three scales were defined for the available environmental variables: micro-, meso- and macro-scale. To acquire micro-scale environmental data, eleven data loggers (Maxim Integrated, Sunnyvale (CA) USA) were spread throughout 65 strategic vertical schist surfaces in the Côa valley with almost a four-year series (September 2010 to June 2014) of data collected concerning temperature ( $^{\circ}\text{C}$ ) and relative humidity (%) on an hourly or half-hourly recording frequency. Data loggers were placed to obtain measurements of all orientations (number of data loggers per orientation: north = 3; south = 6; east = 1; west = 1). For each data logger, the mean value of temperature and relative humidity

was calculated and the results were paired according to aspect. Data obtained from these data loggers reflects the micro-scale variation in temperature and relative humidity at the level of the vertical schist surfaces. For meso-scale environmental data, three weather stations located in the Côa river valley with a 4-year data series (March 2010 to June 2014) that collected, among other parameters, temperature and relative humidity. To obtain a surface with these variables, an interpolation method was performed (Inverse Distance Weighting) in ArcMap 10.1 (ESRI 2012) and the values were extracted to sampling surfaces for acquiring information at the valley level. Finally, macro-scale environmental variables, annual mean temperature (°C) and annual mean precipitation (mm) were obtained from the Iberian Climate Atlas (AEMET & IM 2011) and the values were extracted to sampling points and this broader perspective gives information at the macro-scale level.

#### *Data analysis*

113 species of lichens were identified of which 26 were single occurrences, i.e., species only found once in a sampling surface. The mean species sampled in the schist surfaces was 13 with a minimum of 2 and maximum of 27 lichen species. Detrended Correspondence Analysis (DCA) with detrending by segments (Hill's scaling) was performed to provide an estimation of the length of gradient in the data set and afterwards to clarify the relationships among sampling sites based on the lichen percentage cover. The value of the length of gradient considering the whole dataset was 4,393 and for the analysis with the dominant species was 2,771. Taking these results into account, the most appropriate method to carry out the multivariate analysis was the unimodal method (Lepš & Šmilauer 2003). Afterwards, the data was analysed by means of canonical correspondence analysis (CCA) to enhance the relationships between lichen percentage coverage of schist surfaces and the variables considered. The analysis was carried out with Hill's scaling focused on inter-sample distances and downweighting of rare species after exclusion of single occurrences. A partial CCA (Ellis & Coppins 2010; Hespanhol *et al.* 2011; Lepš & Šmilauer 2003) was used to assign the explained variability in colonized surfaces by lichens to each scale of climatic variables. To perform the partial CCA, climatic variables were tested under different scenarios and they were employed or as variable (when the effect was intended to be interpreted) or covariable (when the effect was needed to take into account). The statistical significance of the environmental variables was tested using Monte-Carlo permutation test (499 permutations). Species codes in the ordination diagrams are according Table I and nomenclature follows Index Fungorum (2013). All

multivariate analyses were performed using CANOCO ver. 4.5 (Ter Braak & Šmilauer 2002).

**Table I:** List of lichen taxa found in the study area with the respective codes used in the diagrams. Nomenclature follows Index Fungorum.

Code	Species
ACAHIL	<i>Acarospora hiliaris</i> (Dufour) Arnold
ACAUMB	<i>Acarospora umbilicata</i> Bagl.
ASPCRE	<i>Aspicilia crespiana</i> V. J. Rico
ASPHOF	<i>Aspicilia contorta</i> (Hoffm.) Kremp. subsp. <i>hoffmanniana</i> S. Ekman & Fröberg
ASPVIR	<i>Aspicilia viridescens</i> (A. Massal) Hue
BUEDIS	<i>Buellia dispersa</i> A. Massal.
BUESP	<i>Buellia</i> sp.
CALCON	<i>Caloplaca conversa</i> (Kremp.) Jatta
CALCRE	<i>Caloplaca crenularia</i> (With.) J. R. Laundon
CALDEM	<i>Caloplaca demissa</i> (Flot.) Arup & Grobe
CALFLA	<i>Caloplaca flavescens</i> (Huds.) J. R. Laundon
CALPEL	<i>Caloplaca pellodella</i> (Nyl.) Hasse
CALPER	<i>Caloplaca percrocata</i> (Arnold) J. Steiner
CALSUB	<i>Caloplaca subsoluta</i> (Nyl.) Zahlbr.
CANVIT	<i>Candelariella vitellina</i> (Ehrh.) Müll. Arg.
CLAPYX	<i>Cladonia pyxidata</i> (L.) Hoffm.
CLASRA	<i>Cladonia rangiformis</i> Hoffm.
COLRYS	<i>Collema rysssoleum</i> (Tuck.) A. Schneider
DERMIN	<i>Dermatocarpon miniatum</i> (L.) W. Mann
DIPACT	<i>Diploschistes actinostomus</i> (Ach.) Zahlbr.
DIPCAN	<i>Diploicia canescens</i> (Dicks.) A. Massal.
DIPINT	<i>Diploschistes interpidens</i> (Schreb.) Norman
DIPSCR	<i>Diploschistes scruposus</i> (Schreb.) Norman
ENDLOS	<i>Endocarpon loscosii</i> Müll. Arg.
ENDPUS	<i>Endocarpon pusillum</i> Hedw.
FUSMED	<i>Fuscopannaria mediterranea</i> (Tav.) P. M. Jørg.
GLYLIG	<i>Glyphopeltis ligustica</i> (B. de Lesd.) Timdal
LASPUS	<i>Lasallia pustulata</i> (L.) Mérat
LECMUR	<i>Lecanora muralis</i> (Schreb.) Rabenh.
LECORO	<i>Lecanora orosthea</i> (Ach.) Ach.
LECPSE	<i>Lecanora pseudistera</i> Nyl.
LEPCYA	<i>Leptogium cyanescens</i> (Rabenh.) Körb.
LEPGEL	<i>Leptogium gelatinosum</i> (With.) J. R. Laundon
LEPMIC	<i>Leprocaulon microscopicum</i> (Vill.) Gams ex D. Hawksw.
LEPPLI	<i>Leptogium plicatile</i> (Ach.) Leight.
LEPSCH	<i>Leptogium schraderi</i> (Bernh.) Nyl.
LEPSP	<i>Leptogium</i> sp.
LICCRI	<i>Lichinella cribellifera</i> (Nyl.) P. P. Moreno & Egea

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LICNIG	<i>Lichinella nigritella</i> (Lettau) P. P. Moreno & Egea
LICSTI	<i>Lichinella stipatula</i> Nyl.
LOBRAD	<i>Lobothalia radiosa</i> (= <i>Aspicilia radiosa</i> (Hoffm.) Poelt & Leuckert) <i>Melanelixia fuliginosa</i> (Fr. Ex Duby) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch
MELFUL	
MOENEB	<i>Moelleropsis nebulosa</i> (Hoffm.) Gyeln.
NEOLOX	<i>Neofuscelia loxodes</i> (Nyl.) Essl.
NEOPUL	<i>Neofuscelia pulla</i> (Ach.) Essl.
OCHPAR	<i>Ochrolechia parella</i> (L.) A. Massal.
PARTIL	<i>Parmelina tiliacea</i> (Hoffm.) Ach.
PELBOL	<i>Peltula bolanderi</i> (Tuck.) Wetmore
PELEUP	<i>Peltula euploca</i> (Ach.) Poelt ex Ozenda & Clauzade
PELFAR	<i>Peltula farinosa</i> Büdel
PELOB	<i>Peltula lobata</i> J. Marques, M. Schultz & Paz-Berm.
PELOBS	<i>Peltula obscurans</i> (Nyl.) Gyeln.
PELPAT	<i>Peltula patellata</i> (Bagl.) Swinscow & Krog
PELPLA	<i>Peltula placodizans</i> (Zahlbr.) Wetmore
PELSP	<i>Peltula</i> sp.
PELZAL	<i>Peltula zahlbruckneri</i> (Hasse) Wetmore
PERLEU	<i>Pertusaria leucosora</i> Nyl.
PHAORB	<i>Phaeophyscia orbicularis</i> (Neck.) Moberg
PHYADS	<i>Physcia adscendens</i> (Fr.) H. Olivier
PHYENT	<i>Physconia enteroxantha</i> (Nyl.) Poelt
PHYTRI	<i>Physcia tribacia</i> (Ach.) Nyl.
PLARUF	<i>Placidium rufescens</i> (Ach.) A. Massal.
PLATRE	<i>Placynthium tremniacum</i> (A. Massal.) Jatta
PSODEC	<i>Psora decipiens</i> (Hedw.) Hoffm.
PTEAFF	<i>Pterygiopsis affinis</i> (A. Massal.) Hessen
PYRTRI	<i>Pyrenopsis triptococca</i> Nyl.
RHIGEO	<i>Rhizocarpon geographicum</i> (L.) DC.
RHIRIC	<i>Rhizocarpon richardii</i> (Nyl.) Zahlbr.
RINBEC	<i>Rinodina beccariana</i> Bagl.
RININT	<i>Rinodina intermedia</i> Bagl.
RINTEI	<i>Rinodina teichophila</i> (Nyl.) Arnold
RINTRA	<i>Rinodina trachytica</i> (A. Massal.) Bagl. & Carestia
RINVEZ	<i>Rinodina vezdae</i> H. Mayrhofer
SOLHOL	<i>Solenopsora holophaea</i> (Mont.) Samp.
SOLVUL	<i>Solenopsora vulturiensis</i> A. Massal.
SQUCON	<i>Squamarina conrescens</i> (Müll. Arg.) Poelt
TEPATR	<i>Tephromela atra</i> (Huds.) Hafellner
TONARO	<i>Toninia aromatica</i> (Turner) A. Massal
TONCIN	<i>Toninia cinereovirens</i> (Schaer.) A. Massal.
TONSED	<i>Toninia sedifolia</i> (Scop.) Timdal
TONSQU	<i>Toninia squalida</i> (Ach.) A. Massal.

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TONTOE	<i>Toninia toepfferi</i> (Stein) Navàs
TRAFLE	<i>Trapeliopsis flexuosa</i> (Fr.) Coppins & P. James
TRAGYM	<i>Trapeliopsis gymniata</i> Aptroot & Schumm
VERNIG	<i>Verrucaria nigrescens</i> Pers.
XANSOM	<i>Xanthoparmelia somloensis</i> (Gyeln.) Hale
XANTIN	<i>Xanthoparmelia tinctina</i> (Maheu & A. Gillet) Hale

## Results and discussion

### *Selecting the dominant lichen species*

For the total surfaces sampled, the mean value of the species registered in the schist surfaces was 13 with a minimum of 2 and maximum of 27 lichen species. Regarding species abundance, the minimum value achieved was 1% by 84 species and the maximum 70% by only one species, *Peltula euploca*, with a mean value of 5%. A total of 87 species was used for the analysis, however, only 8 of these were present in more than 20% of the surfaces sampled: *Aspicilia contorta* (Hoffm.) Kremp. subsp. *hoffmanniana* S. Ekman & Fröberg (*Aspicilia hoffmanianna* hereafter), *Caloplaca subsoluta* (Nyl.) Zahlbr., *Lecanora pseudistera* Nyl., *Peltula euploca* (Ach.) Poelt ex Ozenda & Clauzade, *Pertusaria leucosora* Nyl., *Pterygiopsis affinis* (A. Massal.) Hessen, *Pyrenopsis triptococca* Nyl. and *Xanthoparmelia tinctina* (Maheu & A. Gillet) Hale.

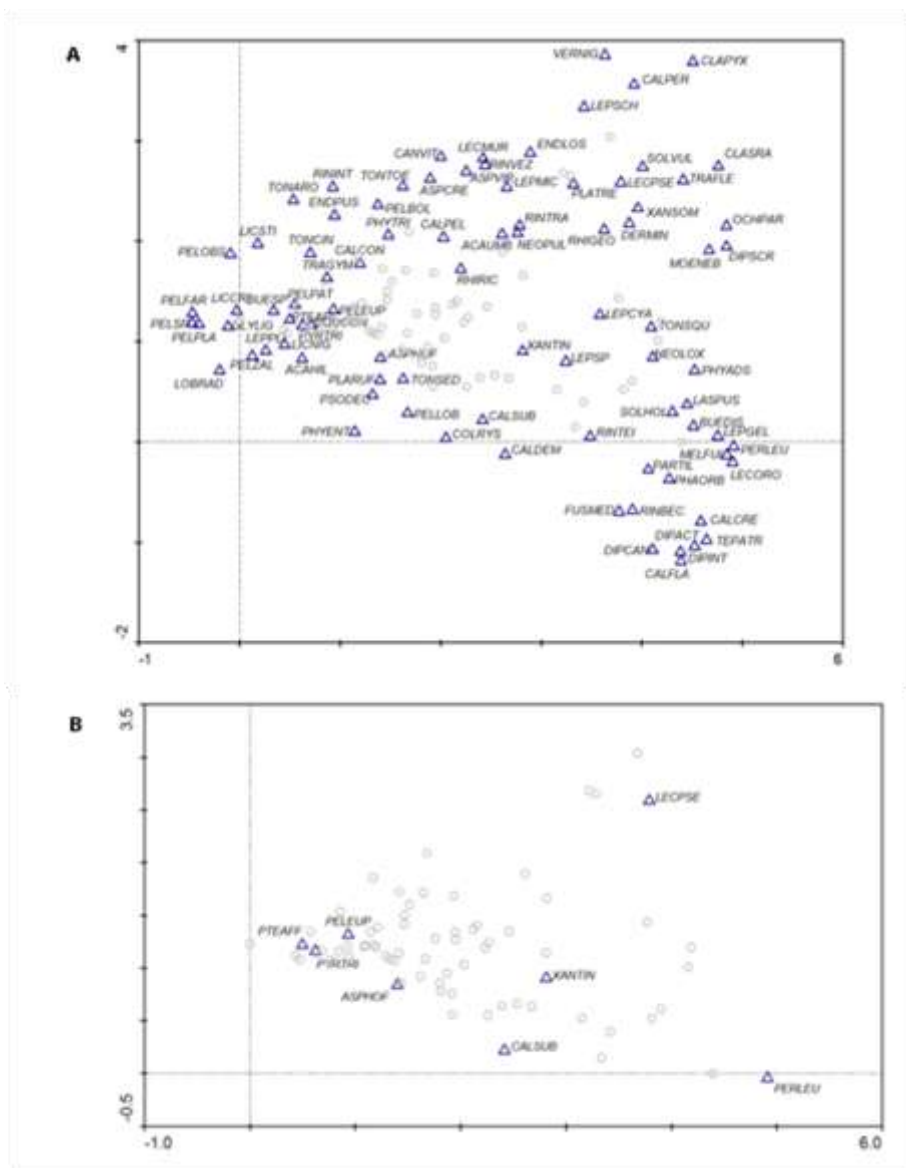
Sampled surfaces (Figure 2) were split into a group well defined on the left side of the diagram and a sparse group on the right side. This distribution seems to be related with a “south versus north” variation and reflects a humidity gradient. This gradient is, therefore, related to drier conditions in the south-facing surfaces with, roughly, high temperatures and low relative humidity values and the opposite is verified for the north-facing surfaces. Concerning only the dominant species, *Peltula euploca*, *Aspicilia hoffmanianna*, *Pterygiopsis affinis* and *Pyrenopsis triptococca* seems to be closely related to conditions observed in the south-facing surfaces, *Lecanora pseudistera* and *Pertusaria leucosora* occur on north-facing surfaces where wetter and colder conditions are usually present and *Caloplaca subsoluta* and *Xanthoparmelia tinctina* occurrence seems to be indifferent in terms of surface orientation.

The DCA ordination of the species and sampled surfaces reveals a strong relationship between the first axis and surface aspect with an eigenvalue of 0,612, which explained the variability observed in the diagram.

These results corroborate earlier knowledge about the study area by Marques *et al.* (2014), carried out with less sampling surfaces and in a more restricted area. The



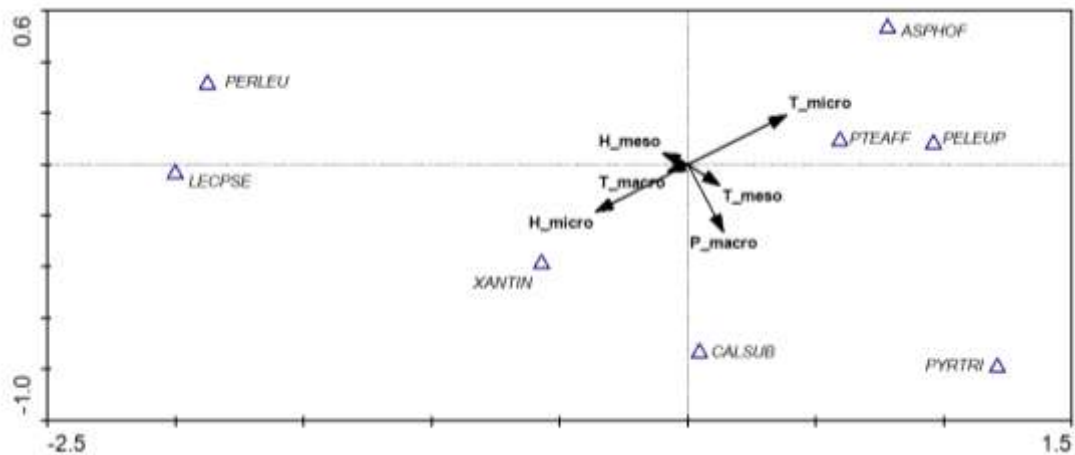
suggestion in that study of including, at least, three dominant crustose lichens in the assessment of lichen colonization of the schist surfaces in the Côa Park Archaeological Park is maintained for the whole region. The dominant species used to perform the analysis were the crustose species *Aspicilia hoffmanianna* (mean cover = 10,44%) as the representative of lichen assemblages of north-west facing surfaces, *Lecanora pseudistera* (mean cover = 4,90%) as the representative of lichen assemblages of south-east facing surfaces, *Caloplaca subsoluta* (mean cover = 7,03%) as crustose species dominant in both exposures and *Peltula euploca* (mean cover = 13,8%) as the representative of the most abundant lichen community in the study area (Llimona & Egea 1985).



**Figure 2:** Ordination diagram of DCA with detrending by segments. The grey circles represent the sampled surfaces. **A** all species used in the analysis plotted. **B** Only dominant species (occurring in >20% of sampled surfaces) are displayed. Species codes are according to Table 1.

*How are environmental variables at three different spatial scales related to lichen colonization of schist surfaces?*

To evaluate the contribution of each variable to the total species sampled in the schist surfaces, a canonical correspondence analysis (CCA, Figure 3) was carried out. All variables showed statistically significant relationships with lichen cover ( $p < 0,05$ ).



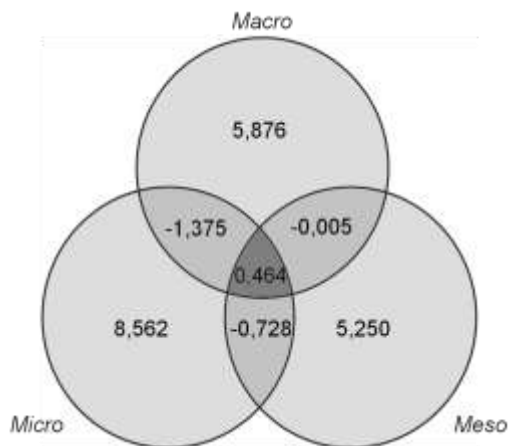
**Figure 3:** Canonical Correspondence Analysis biplot representing lichen *taxa* and the environmental variables. *T* stands for temperature in °C, *H* for relative humidity in % and *P* for precipitation in mm. *Macro*, *meso* and *micro* refers to the three scales used in the study. (Note: the CCA was performed with all species, but for an easier analysis only species with >20% of occurrences were plotted).

The CCA with all significant variables explained 17,01% of the total variance (Total Variance Explained, TVE) in lichen cover in the sampled surfaces, which is reasonable considering the variability inherent to ecological data. Macro- and meso-scale variables explain 4,96% of total variability in the dataset each, while that explained by micro-scale variables was 6,97%.

The CCA biplot of species and environmental variables expresses a change in species distribution along the first axis of ordination which can also be explained by a humidity gradient ("south *versus* north" variation, Figure 3). Species found in the dryer areas showed the highest positive correlation with axis 1 (e.g.: *Aspicilia hoffmanianna* and *Peltula euploca*) and species related to a high relative humidity values shows the highest negative correlation with axis 1 (e.g.: *Lecanora pseudistera* and *Pertusaria leucosora*).

The partial CCA results (Figure 4) indicate that the largest amount of variance in the abundance of lichens in the colonized schist surfaces was explained by the micro-scale variables (temperature and relative humidity at the schist surface level) with 8,562% of TVE when macro- and meso-scale variables were used as co-variables, followed by macro-scale variables (5,876% of TVE) and with meso- and micro-scale

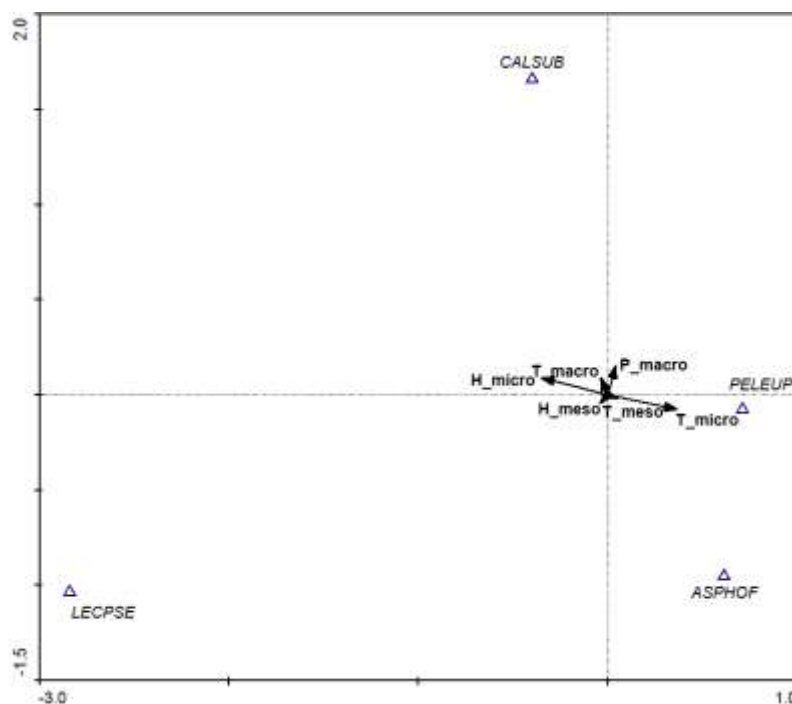
variables as co-variables and, at last, meso-scale variables (5,250% of TVE) with macro- and micro-scale variables as co-variables.



**Figure 4:** Venn diagram showing partitioned variation in lichen abundance (%) explained by multi-scale climatic variables (at macro-, meso- and micro-scale).

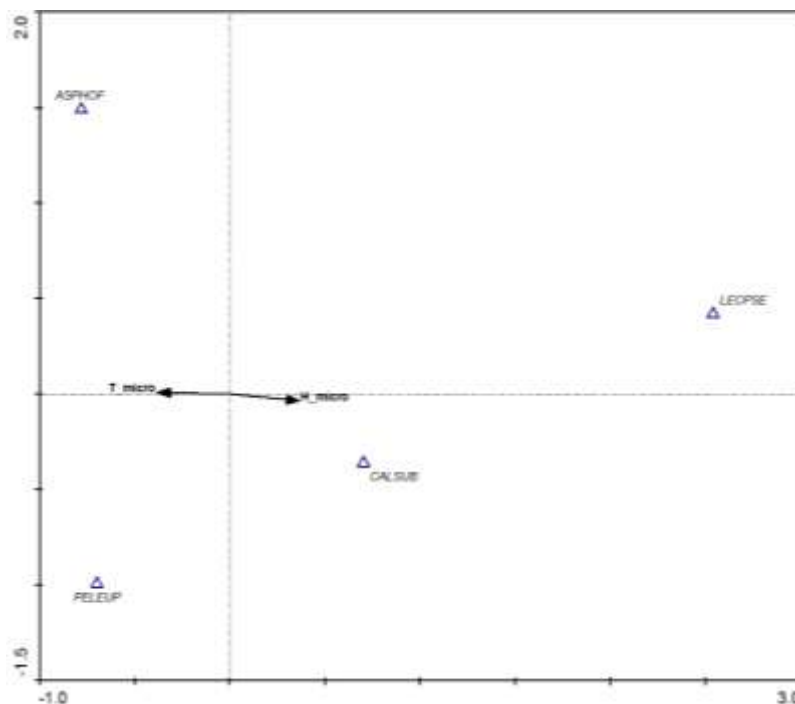
*How do environmental variables at three different spatial scales affect the abundance patterns of dominant lichen species in schist surfaces?*

Axis 1 of the CCA biplot (Figure 5) is still reflecting the same humidity gradient as the one based on the total dataset, since the highest positive score is shown by the north-west dominant *Lecanora pseudistera*.



**Figure 5:** CCA biplot with dominant species and all climatic variables. *T* stands for temperature in °C, *H* for relative humidity in % and *P* for precipitation in mm. *Macro*, *meso* and *micro* refers to the three scales used in the study.

In the CCA procedure (Figure 6), the only statistically significant effect in explaining the variability in the abundance of dominant lichen species on the studied schist surfaces were micro-scale variables ( $p < 0,05$ ;  $p\text{-value} = 0,002$ ), i.e. temperature and relative humidity at the level of the schist surface.



**Figure 6:** CCA biplot with the dominant species and micro-scale variables.

This scale is closely related to the strong relationship between the colonizing species and the substrate (de los Ríos *et al.*, 2002). Slightly variations in surface temperature and relative humidity can produce significant changes in species assemblages in terms of species frequency and abundance. The variance explained by the micro-scale variables in the occurrence of dominant species reached almost one fifth percent of total variation (19,57% of TVE).

These results support previous work by Marques *et al.* (2014) where it was proved that the colonization of schist surfaces by lichens at the Côa Valley Archaeological Park was highly related with slope aspect which is strongly dependent on micro-scale variables but is somewhat surprising in the lack of effects of meso- and macro-scale variables.

## Conclusions

This study provides an insight into the relationship between saxicolous lichens colonizing of vertical schist surfaces and particular environmental conditions (relative

humidity, temperature and precipitation) observed in the Upper Douro Region, northeast Portugal. Although there are statistically significant multi-scale effects of climatic variables in the patterns of total lichen species abundance on vertical schist surfaces, when it comes to the abundance of dominant species, the most important drivers of lichen occurrence and distribution are micro-scale factors related with surface temperature and relative humidity. To provide a simple, prompt and cost-efficient method by considering the hypothesis of monitor only the dominant species in the area, especially in the Côa Valley Archaeological Park there should be no need to consider meso- to macro-scale effects of environmental variation on major patterns of lichen-induced deterioration at the regional level.

Having in mind the need of incorporate the lichenological component in the risk assessment of the engraved surfaces, since lichen communities are the major colonizers of schist surfaces. Their action is quite uncertain in this kind of rock and seems to be necessary to account the potential damage or benefit of their role in the schist surfaces with cultural interest.

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## **Chapter 4 |**

# **Lichen-induced geochemical weathering of schist surfaces in Côa Valley Archaeological Park (NE Portugal)**

### **Abstract**

The study was carried out in the Côa Valley Archaeological Park (Vila Nova de Foz Côa, northeast Portugal), where is located one of the most important sites of open-air rock-art on schist surfaces and classified by UNESCO as World Heritage. It is necessary to assess the risk of deterioration of engraved surfaces to implement conservation techniques and prevent future damages. Lichens are the most abundant colonizers of schist surfaces in the park and the assessment of their impact on a geochemical point of view is lacking. Four dominant species were selected and colonized rock samples (and non-colonized samples used as controls) were collected from non-engraved surfaces to assess the potential effect on major element mobility.

Preliminary results of aspect related chemical weathering reveal that this phenomenon occurs in the south-facing surfaces and it is very reduced or potentially

absent in the north-facing surfaces, affected by physical weathering in a higher extent. Results evidence significant differences between colonized and non-colonized rocks in the south-facing surface. *Caloplaca subsoluta* is the species that affects the highest number of the assessed elements.

**Keywords:** chemical weathering, lichens, schist surfaces, rock-art preservation, element mobilization

## Introduction

The study of lichen-induced weathering has been carried out in various types of rocks such as granite (Carballal *et al.* 2001, Prieto *et al.* 1999), limestone (McIlroy de la Rosa *et al.* 2013) or sandstone (Ariño *et al.* 1995). It is a matter of real concern since stone-built cultural heritage is often highly colonized by lichen dominant communities whose action in the substrate has been broadly accessed (e.g.: Adamo & Violante 2000, Chen *et al.* 2000, Garcia-Rowe & Sáiz-Jiménez 1991, Seaward 2003, Warscheid & Braams 2000) but still a matter of discussion. Some authors defend that the role played by lichens could be either protective acting as shield-like barriers against abiotic deterioration agents like rain, wind or temperature fluctuations (Ariño *et al.* 1995, Garcia-Vallès *et al.* 2003, Warscheid & Braams 2000) or deteriorative (Chen *et al.* 2000, Seaward *et al.* 2001), by increasing the rate of disintegration of rock-forming minerals. Alterations produced on the rock surface by lichens may actually arise from a combination of physical and chemical effects. The penetration of lichen hyphae and subsequent expansion and contraction through hydration and dehydration can result in the dislocation of particles of the substratum and be the main cause of lichen-induced physical weathering. Furthermore, the differential growth of the thallus can generate an upward pressure that produces a blistering effect on the rock surface (Ascaso *et al.* 1990, Chen *et al.* 2000, Warscheid & Braams 2000). Chemical weathering has been mainly attributed to the metabolic production and excretion of lichen acids, including polyphenolic compounds.

The lichen-rock interface is a place of particularly intense chemical activity (Ascaso *et al.* 1990) and it is the ideal spot to evaluate lichen action on rock surfaces (Adamo & Violante 2000). The chemical action of lichen substances can lead to the alteration of the original minerals and mineral neoformation. The most commonly observed products of mineral neoformation are oxalates, associated with the secretion of oxalic acid (Chen *et al.* 2000).

Some examples include calcium oxalates (whewellite and weddellite) (e.g.: Edwards *et al.* 1997, Hernanz *et al.* 2008) in sandstone colonized by *Caloplaca aurantia* (Edwards *et al.* 1997) and granite colonized by *Ochrolechia parella* (Prieto Lamas *et al.* 1995), copper oxalate (mooloonite) in brochantite colonized by *Lecidea inops* (Chisholm *et al.* 1987) or magnesium oxalate (glushinskite) in serpentinite colonized by *Lecanora atra* (Wilson *et al.* 1980). The magnitude of the impact of these processes is related to the colonizing species (de los Ríos *et al.* 2002, Prieto *et al.* 1999) and the weathering environment (Ascaso *et al.* 1990, Bungartz *et al.* 2004, Chen & Blume 1999, Chen *et al.* 2000).

Concern about all aspects of schist weathering has arisen from the discovery of the Côa Valley's rock-art surfaces with more than one thousand engraved flat vertical surfaces (Fernandes 2004, Marques *et al.* 2014) that are mainly located in the Côa Valley Archaeological Park. Open-air rock-art surfaces and other exposed rock surfaces in the Côa Valley are colonized by various organisms including free-living cyanobacteria, lichens, bryophytes and vascular plants. Lichens are the most abundant colonizers of the vertical schist surfaces in the Upper Douro region (Marques 2013; Marques *et al.* 2014) but characterization of schistose rocks in terms of their geochemical weathering is still incomplete (but see Marques *et al.* submitted). Ongoing studies (Marques 2013) have analysed the general patterns of lichen-induced physical and chemical weathering of schist surfaces exposed to contrasting weathering environments in the Côa Valley Archaeological Park but some aspects of lichen-induced geochemical alterations are still poorly known, namely how individual elements are mobilized.

The goal of this study was to evaluate the extent to which lichens are implicated in elemental mobilization in schist surfaces in the Côa Valley Archaeological Park. This information should be useful when deciding whether the presence of lichens will be beneficial to schist surfaces conservation or their removal should be considered.

## Material and methods

The studied rock has been traditionally called schist (Noronha *et al.* 2012) or slate (Aires *et al.* 2012) but is in fact a relatively low-grade metamorphic (greenschist facies) phyllite consisting of thin alternating layers of whitish psammitic and dark pelitic components (Aires *et al.* 2011). The psammitic component is sometimes more abundant and the rock is then classified as a metaquartzwacke instead of a phyllite. For a matter of simplicity, this phyllite-metaquartzwacke sequence will be addressed

under the broad term schist. This schist is mainly composed of quartz, sericite and/or muscovite, chlorite and biotite minerals, as well as plagioclase feldspars (mostly albite) in variable amounts depending of the psammitic contribution. Calcite is usually present in the matrix of these rocks in sufficient amounts to produce effervescence when treated with dilute hydrochloric acid. Magnetite and, more sporadically, pyrite crystals are present in both the psamitic and pelitic levels as accessory constituents (Sousa 1982). Both calcite and pyrite are potentially highly alterable (Prieto *et al.* 2011). Additional accessory minerals are illite, kaolinite, montmorillonite, graphite, turmaline, zircon, apatite, epidote, hematite, leucosene and some alkali feldspars, such as microcline and orthoclase (Aires *et al.* 2011, Gomes & Almeida 2003, Sousa 1982).

The evaluation of lichen-induced element mobilization was focused on a few target lichen species. Target species selection was based on their frequency and abundance on the vertical schist surfaces of the Côa Valley Archaeological Park, following Marques *et al.* (2014). The dominant species used in this analysis were the four: *Aspicilia contorta* (Hoffm.) Kremp. subsp. *hoffmanniana* S. Ekman & Fröberg (*Aspicilia hoffmanniana* hereafter), *Caloplaca subsoluta* (Nyl.) Zahlbr., *Lecanora pseudistera* Nyl. and *Peltula euploca* (Ach.) Poelt ex Ozenda & Clauzade. Dominant species distribution is closely related to surface aspect. The north-west facing surfaces are dominated by *Lecanora pseudistera*, while *Peltula euploca* and *Aspicilia hoffmanniana* are predominant on south-east facing surfaces. *Caloplaca subsoluta* is highly abundant on both orientations. Two hundred and three samples colonized by the target species were taken from non-engraved schist surfaces, located at representative rock-art sites in the Côa Valley Archaeological Park (

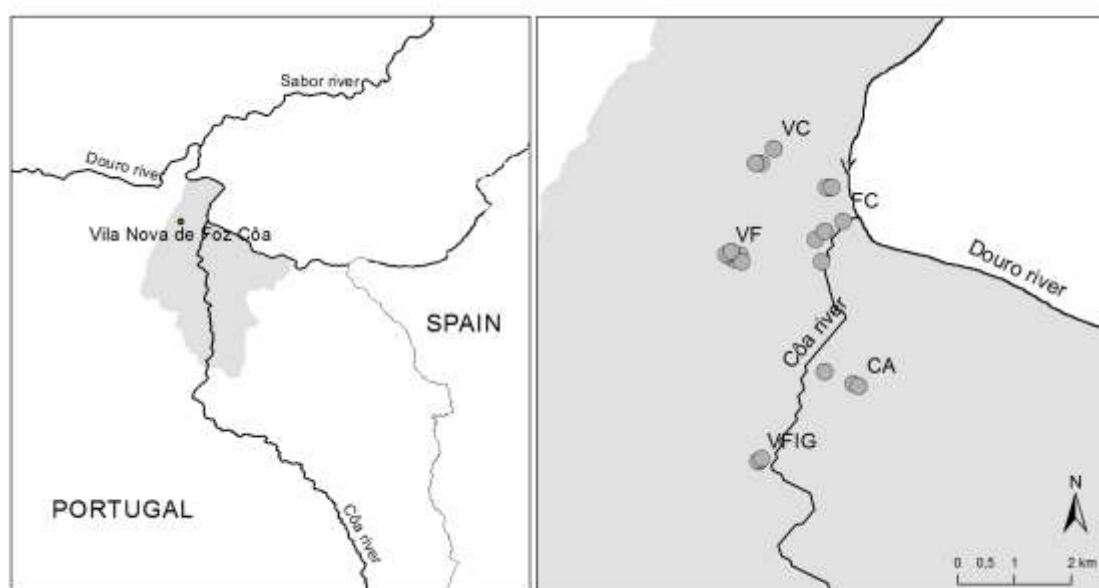
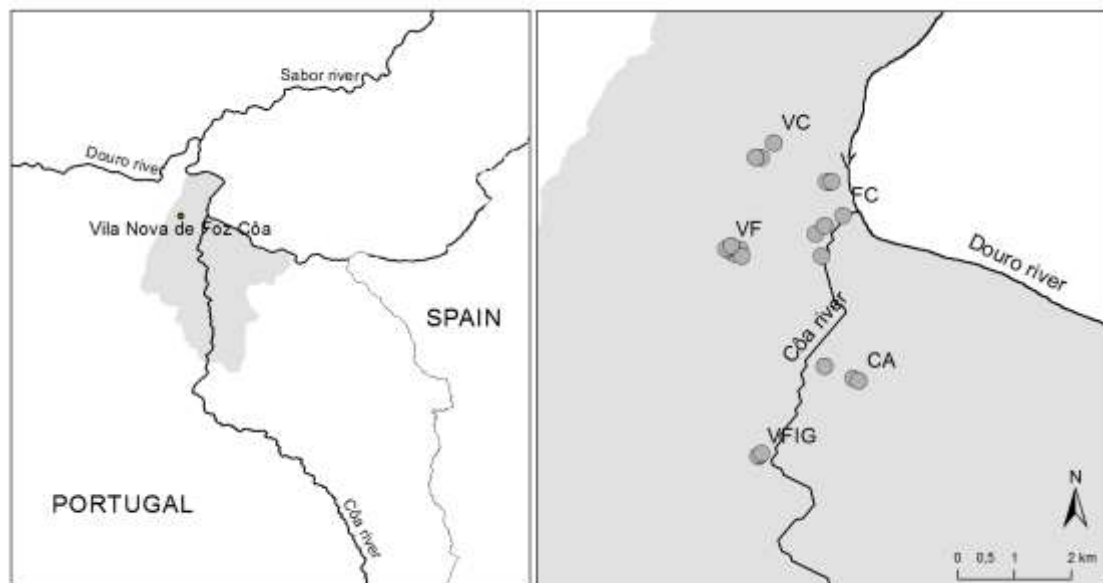


Figure 7). Sampling was focused on south-east facing surfaces, where rock-art is most

frequent. Non-colonized rock samples were also taken from the source outcrops to be used as controls.

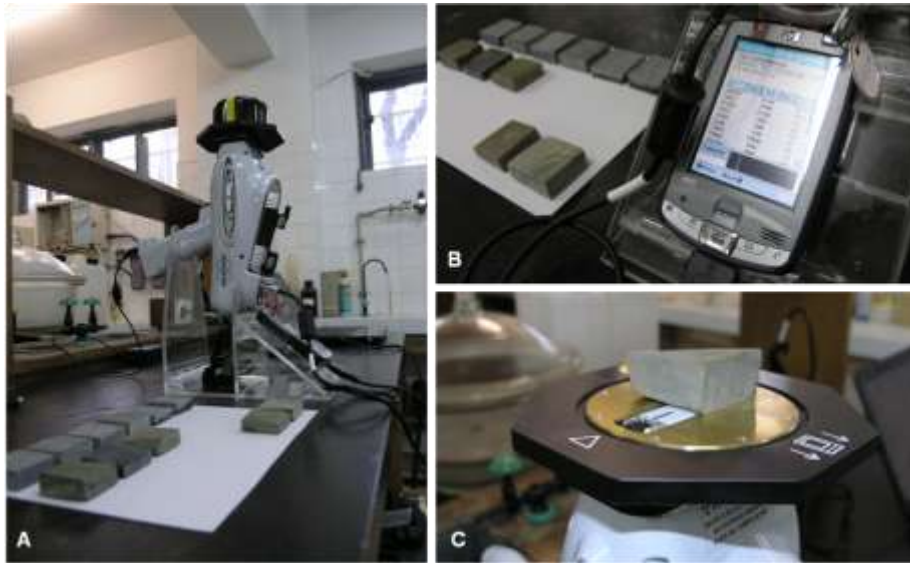
For a preliminary assessment of the effect of surface exposure, and related micro-environmental constraints on lichen-induced element mobilization, samples colonized by *Caloplaca subsoluta* and respective controls were also collected from north-west facing non-engraved surfaces. This species was chosen given its abundance is quite similar on both aspects, allowing for comparisons (Marques *et al.*, 2014).

Analysis of elemental changes is based on X-ray fluorescence (XRF) performed with a Tracer IV handheld XRF analyser (Bruker, Billerica (MA), USA) adapted to the benchtop (Figure 8).



**Figure 7:** **A** Location of the Côa Valley Archaeological Park, Vila Nova de Foz Côa municipality and the main nearby rivers. **B** Location of the sampled surfaces in the study area: CA: Canada do Amendoal, FC: Foz do Côa, V: Vermelhosa, VC: Vale de Cabrões, VF: Vale do Forno and VFIG: Vale Figueira (yellow dots).

Colonized samples were therefore studied *ex situ* with the lichen thallus kept intact. XRF measurements were taken on three fractions of collected samples: 1) the lichen-rock interface of colonized samples, 2) the surface of non-colonized control samples and 3) the interior of non-colonized control samples. Data analysis is based on a minimum of ten measurements per fraction after some data cleaning and removal of measurement errors. Differences between colonized and non-colonized schist samples in terms of major element content were tested by means of linear mixed-effect models with “lme4” (Bates *et al.* 2014) package in R version 2.14.



**Figure 8:** X-ray fluorescence analyser. A: general view. B: Screen displaying measurement results. C: Schist sample ready to perform the analysis (during the analysis, the samples are protected to avoid X-ray exposure by the operator).

## Results and discussion

The results of the major element content of all target species and rock interior are depicted in Table II. Major element content was the same at the surface and interior of non-colonized schist samples indicating that the surfaces of the studied lithotype were not severely weathered.

**Table II:** Major element content (mean, in %) determined by X-ray fluorescence in colonized and non-colonized rock samples. Asterisks represent statistically significant differences in element content between colonized and non colonized samples.

	MgO	Al <sub>2</sub> O <sub>3</sub>	SiO <sub>2</sub>	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	CaO	TiO <sub>2</sub>	MnO	Fe <sub>2</sub> O <sub>3</sub>
South-facing surfaces									
<b><i>A. hoffmanniana</i></b>	1,915	21,940	75,390	0,031*	4,473	1,620	0,335	0,010	9,575
<b><i>L. pseudistera</i></b>	2,201	21,475	74,200	0,026	4,256	1,463	0,084	0,010	9,416
<b><i>P. euploca</i></b>	2,122*	21,228	75,566*	0,022	4,127	1,559	0,537	0,009	9,175
<b><i>C. subsoluta</i></b>	2,211*	20,065*	76,882*	0,035*	3,858*	2,399*	0,432	0,010	7,958
<b>Non-colonized rock (interior)</b>	2,107	22,743	73,350	0,023	4,633	1,597	0,620	0,008	9,421
<b>Non-colonized rock (surface)</b>	1,811	21,902	73,230	0,022	4,441	1,494	0,452	0,009	9,413
North-facing surfaces									
<b><i>C. subsoluta</i></b>	-	19,260	49,260	0,033	3,320	1,380	0,279	0,077	7,640
<b>Non-colonized rock (interior)</b>	-	18,960	62,080	0,016	3,040	1,140	0,069	0,070	6,680
<b>Non-colonized rock (surface)</b>	-	17,225	49,375	0,036	2,900	1,250	0,065	0,087	7,350

There were statistically significant changes in the content of all elements induced by at least one species with the exception of Fe, Ti and Mn.

The results obtained in the analysis showed that surfaces colonized by *Aspicilia hoffmanniana* (p-value <0,001) and *Caloplaca subsoluta* (p-value <0,001) lead to statistically significant differences in phosphorous content that increases in the colonized surface regarding the control. Increase phosphorus content at the lichen-rock interface is consistent with P geochemistry since any mobilized phosphorous leached from the rock would also precipitate or adsorb on secondary minerals (Gardner 1990).

Magnesium and silicium contents were significantly higher from the statistical point of view in the surfaces colonized by *Peltula euploca* (p-value (Mg) = 0,047; p-value (Si) = 0,030) and *Caloplaca subsoluta* (p-value (Mg) = 0,033; p-value (Si) <0,001) relative to the control. A possible explanation for the increase of Mg content at the lichen-rock interface is the protection provided by lichen thalli against the loss of Mg salts through leaching as stated by Silva *et al.*, 1999 in lichen-induced geochemical weathering in granite. Regarding Si, the main element of quartz, which is the main component of schist, and reported as being extremely resistant to weathering (Pope 1995), the identified differences could be assigned to major detection at the lichen-rock interface after the other components were leached away.

Calcium oxalates (weddelite) had been previously detected at the lichen-rock interface of samples colonized by *Caloplaca subsoluta*, *Lecanora pseudistera* and *Peltula euploca* and assumed to be the result of mineral neoformation (Marques 2013). Ca content was therefore expected to be higher in colonized samples when compared to control samples due to a retention of Ca ions in the form of calcium oxalates, as it seems to happen with Mg. In fact, surfaces colonized by *Caloplaca subsoluta* showed a statistically significant increase in Ca content suggesting that Ca is retained in the form weddelite. The lack of statistically significant differences in calcium content in samples colonized by *Lecanora pseudistera* and *Peltula euploca* relative to the control suggests a balance between lichen-induced Ca retention and leaching mechanisms.

Al and K contents were significantly lower (p-value (Al) <0,001; p-value (K) <0,001) in samples colonized by *Caloplaca subsoluta* which is attributable to the leaching of these elements. This suggests that these species are affecting the weathering of Al and K-rich minerals, most probably biotite, exhibited a distinct loss in K content compared to unaffected areas by lichen action (Wierzbos & Ascaso 1998). Evidence therefore suggests that dominant lichens occurring on the exposed schist surfaces of the Côa Valley Archaeological Park are protecting against the leaching of Si, Mg, Ca and P but causing the loss of Al and K.



### *Preliminary assessment of surface exposure in elemental mobility*

South-east dominant lichen species protected against the loss of P (*Aspicilia hoffmanniana*), Mg and Si (*Peltula euploca*) or Mg, Ca, Si, P (*Caloplaca subsoluta*). However, the analysis of samples colonized by *Caloplaca subsoluta* taken from both exposures revealed an effect of surface exposure. Although responsible for some element loss (Al and K) and gain (Mg, Ca, P and Si) at south-east facing surfaces no statistically significant differences were detected between the element content relative to the control at north-west facing surfaces. Furthermore, the analysis of the content of major elements of surfaces colonized by *Lecanora pseudistera* a north-west dominant species, revealed no statistically significant differences between colonized and non-colonized surfaces, in any of the detected elements. Previous work by Marques (2013) inferred that lichen-induced physical weathering in the Côa Valley is species-specific and stronger on north-east facing surfaces, whereas lichen-induced chemical action is microclimatically controlled and more severe on south-east facing surfaces. There is probably some variation in the relative abundance of alteration minerals and calcium oxalates at different portions of the samples but according to present evidence, the lichens currently dominant on the vertical schist surfaces in the Côa Valley are unlikely to be responsible for the differential weathering, and distribution pattern of engraved schist surfaces.

## Conclusions

Intense mobilization of elements was detected in the rock-lichen interface of colonized surfaces in the Côa Valley Archaeological Park. These changes reflected an increase in the content of some elements such as phosphorus, magnesium, silicon and calcium and, on the other hand, aluminium and potassium contents decreased relative to the control samples. Those changes were related to the element leaching, adsorption or neoformation of minerals in the rock-lichen interface. Lichen-induced weathering is case-dependent and seems necessary to consider the effect of each species individually once their consequence on element content was quite heterogeneous. Samples colonized by *Lecanora pseudistera* had no detected influence on element content, contrasting with the strong activity in surfaces colonized by *Caloplaca subsoluta*. The preliminary assessment on surface exposure supports the previous hypothesis that the chemical weathering is more evident in the south-east

facing surfaces rather north-facing surfaces, where the physical weathering seems to be stronger.

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## **Chapter 5 |**

# **Modelling the vulnerability of schist surfaces to lichen-induced deterioration in the Upper Douro Region (Portugal)**

### **Abstract**

Conservators of cultural heritage currently lack practical tools to diagnose and quantify the impact of different (micro-)organisms on stonework, a key element in the planning and management of conservation programmes. The severity of deteriorative activity of many lichen species is well known, and thus public authorities generally opt for the physical and/or chemical removal of “disfiguring” lichens from stonework. However, in some cases these organisms may protect the substratum beneath and also have a positive aesthetic significance and biodiversity value, thus being worthy of conservation. A research network of lichenologists is establishing a standardized methodology to quantify the relative damaging impacts of lichens on monuments by means of the Index of Lichen Potential Biodeteriogenic Activity (LPBA). This study is focused on a LPBA assessment of schist surfaces supporting prehistoric open-air rock-

art in the Upper Douro region, considering four locally dominant lichens, and on the development of a model that is able to predict lichen-induced deterioration in the region. The results showed a strong dependence of LPBA on species cover and a weaker contribution of the chemical and physical action related parameters that suggests the need for a calibration of some of its parameter scales. The use of climatic variables combined with information about species cover was nevertheless quite accurate at predicting LPBA, indicating that its interpretation can be effectively and appropriately disseminated to public authorities, managers and professionals engaged in conservation work.

**Keywords:** LPBA, schist surfaces, rock conservation, climatic variables, chemical weathering, physical weathering, biodeterioration

## Introduction

The Upper Douro region in north-east Portugal is probably one of the most outstanding regions in the country in terms of natural and cultural heritage, bringing together two world heritage sites: the Alto Douro wine region, and the prehistoric rock-art of the Côa Valley. Schist is the common denominator of both sites. It is used to build the steeped walls that support the vineyards and provided the perfect canvas for rock-art, currently preserved on approximately 1000 vertical surfaces dating back to the Upper Palaeolithic (Zilhão 1995). The world heritage rock-art sites are concentrated within the boundaries of the Côa Valley Archaeological Park, named after that tributary of the Douro, along the last 22 km of the Côa River Valley and extending into a portion of the Douro River Valley. Some remnants of prehistoric rock-art can also be found outside the classified area, towards the north-west, along the Tua and Sabor River Valleys, two main tributaries on the right margin of the Douro.

When a site is inscribed on the World Heritage List awareness grows concerning the importance of protecting and preserving its features. Ambitious goals included in the Strategic Action Plan for the Implementation of the World Heritage Convention 2012-2022 (UNESCO 2011) have further emphasized the need for heritage protection and conservation considering present and future environmental, societal and economic needs and for researchers to move from conceptual models that provided a common theoretical background for researchers and practitioners (Viles 1995) to field-based applications.

Rock-art research has been mainly focused on field surveys, documentation and dating. Interpretations of the multitude of factors that affect rock-art preservation

have been few (Chaloupka 1978, Fernandes 2012) despite the recognition of the risks posed by natural and human-related hazards. Evidence suggests that rates of open-air rock-art deterioration may be increasing, due to the continuous action of several erosion processes associated with biological activity as well as physical and chemical phenomena, although it is unclear whether this is due to local factors or wider environmental influences accelerated by climate change (Giesen *et al.* 2014). A very prominent and important research task in rock-art conservation is to find inexpensive and non-expert based tools to assess biological colonization in a multi-scale manner, from the engraved surface to the rock-art site or region (Oliveira *et al.* in prep.).

Lichens are the dominant colonizers of the exposed schist surfaces in the Côa Valley. They may become a defence against other weathering agents and protect the surface beneath (Ariño *et al.* 1995, Carter & Viles 2005, Garcia-Vallès *et al.* 2003, Warscheid & Braams 2000) or represent a serious threat to surface stability due to physico-chemical changes directly promoted by lichen colonization (Adamo & Violante 2000, Carballal *et al.* 2001, Chen *et al.* 2000, Seaward *et al.* 2001). Rhizine penetration and thallus expansion and contraction are the most important mechanisms involved in biogeophysical weathering, whereas a large group of substances frequently referred to as “lichen acids” are the most significant chemical compounds of potential importance in biogeochemical weathering (Chen *et al.* 2000). Many species create microclimates at the lichen-rock interface, particularly in terms of water retention, which undoubtedly leads to mechanical damage of the rock surface in ten years or less (Viles 2005).

There are few tools available to evaluate the effect of the entire and usually highly species-rich community on rock surfaces, but several steps have been taken towards a holistic assessment of lichen-induced rock deterioration. The most promising approach is the recently proposed Index of Lichen Potential Biodeteriogenic Activity (LPBA), a numeric descriptor to measure the impact of different lichen species on rock surfaces (Gazzano *et al.* 2009) that is versatile enough to account for the entire lichen assemblage without complete data on the physical and chemical action of every species. The index is currently under validation (Favero-Longo *et al.* 2012) and has never been adequately evaluated on schist surfaces. Moreover, none of the previous applications have attempted to provide spatial or temporal predictions of rock-art vulnerability to lichen-induced deterioration. To do so, a set of statistical tools is needed, capable of describing lichen-induced deterioration as a function of environmental drivers of lichen distribution patterns. Key variables for the distribution of saxicolous lichens include type of rock (Ariño *et al.* 1997; Prieto Lamas *et al.* 1995), rock weathering state (Guillitte 1995), other abiotic variables such as altitude and slope



(Adamson *et al.* 2012, John & Dale 1990, Marques *et al.* 2014), and climatic variables (McIlroy de la Rosa *et al.* 2013, Viles 2005). Rock type, rock weathering state and other abiotic variables are quite homogeneous in the study area (Marques 2013) and only the climatic variables vary, in a multi-scale manner.

The suitability of interpolated large-scale climatic variables to model lichen-induced deterioration has not yet been tested, and so it is not known whether they are superior to meso- (Fernandes 2012; Oliveira *et al.* in prep.) and small-scale variables (Oliveira *et al.* in prep.). Climatic variables are much easier to quantify than some of the LPBA's parameters, and they may be used as a complementary tool when quantifying lichen-induced deterioration. In addition, we hypothesize that the predictions will be much better if we combine the abundance of dominant lichen species and environmental variables to model lichen-induced deterioration than using only environmental variables. The rationale is that owing to the high dependence of LPBA estimation from lichen species abundance, abundance of dominant lichens will reflect micro-scale variations of lichen effect which are not accounted for by the usually rather coarse-scale environmental variables.

We sought to apply LPBA assessment at several open-air rock-art sites in the Upper Douro region (NE Portugal) and test thoroughly the possibility of predicting the degree of rock-art deterioration by the evaluating different sets of lichen and environmental predictors. Specifically, we have addressed the following questions:

1. Is the abundance of dominant lichen species related to lichen-induced deterioration?
2. Which of the three sets of environmental variables (macro, meso and micro-scale variables) better predicts lichen-induced deterioration?
3. Does a combination of the two data sets perform better?

## Materials and methods

LPBA estimation is based on the weighting of seven parameters: cover, reproductive potency, depth of hyphal penetration, physical action, chemical action, hyphal spread and putative bioprotection effects (Figure 9).

$$LPBA = \log \sum_{i,j=1}^n \left\{ a_{ij} b_i \left[ c_{ij} (d_{ij} + e_i) f_{ij} \right]^{g_{ij}} \right\}$$

**Figure 9:** Index of Lichen Potential Biodeteriogenic Activity. The parameters a-g are summarized in the Table III and the *i* stands for the species in the *j* substrate.

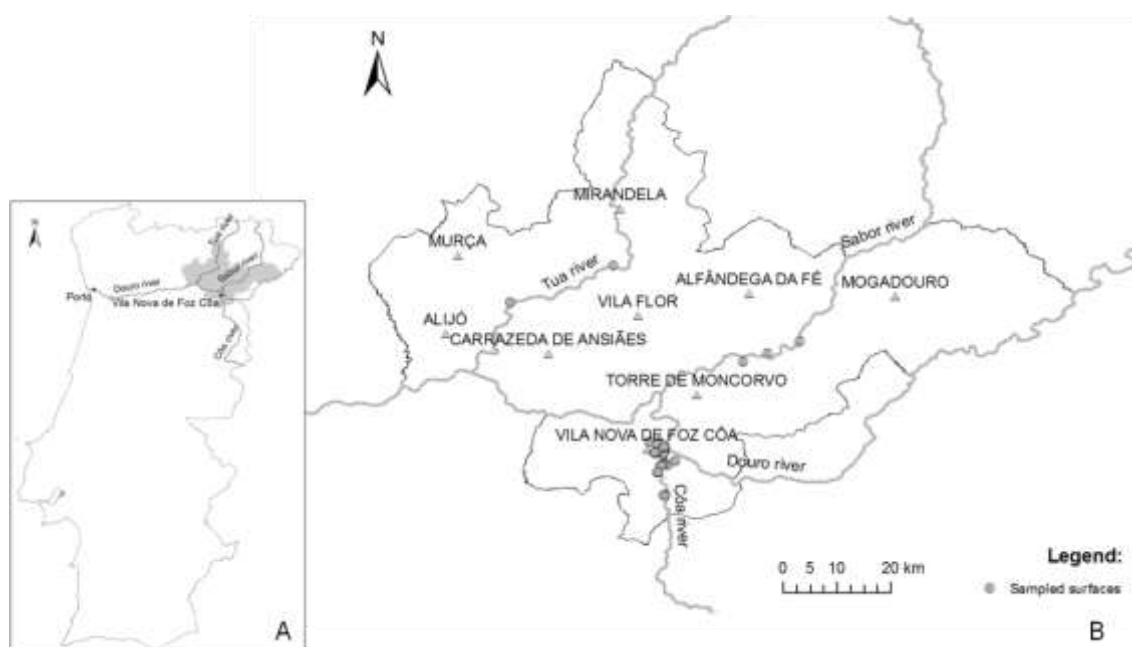
For a detailed explanation of the LPBA estimation procedure the reader is reported to the original work by Gazzano *et al.* (2009).

A brief description of the LPBA estimation procedure is given here focusing on the adaptations to the specificities of the study area and applied analytical techniques. LPBA estimation in the study area was based on four lichen species considered because of their dominant occurrence in the vertical schist surfaces of the study area (Oliveira *et al.* in prep.) namely *Aspicilia hoffmaniana*, *Caloplaca subsoluta*, *Lecanora pseudistera* and *Peltula euploca*. The first two parameters (cover and reproductive potency) were collected prospectively in 65 vertical schist surfaces at 22 open-air rock-art sites in the Upper Douro region (Figure 10).

**Table III:** Summary of the parameters applied in the calculation of the LPBA index (for detailed explanation, see Gazzano *et al.* (2009))

Parameter	Meaning	Contribution to the index
<b>a.</b> <i>Cover</i>	Surface covered by lichen colonization	0 to 100%
<b>b.</b> <i>Reproductive potency</i>	Production of vegetative propagules tends to present more reproductive potency than those displaying sexual reproductive structures	<b>10:</b> species displaying asexual reproduction <b>5:</b> species displaying sexual reproduction and having fertile ascocarps <b>1:</b> species displaying sexual reproduction but young ascocarps without spores or no ascocarps
<b>c.</b> <i>Depth of hyphal penetration</i>	Growth of the fungal hyphae within the substratum through intragranular and intergranular voids	<b>10:</b> > 2000 µm <b>5:</b> between 2000 and 500 µm <b>1:</b> < 50 µm
<b>d.</b> <i>Physical action</i>	Mechanical action related to the more or less significant disaggregation or fragmentation of the rock immediately below the thallus	<b>5:</b> thallus detachment of rock fragments is appreciable at the macroscopic scale <b>3:</b> medullar or hyphal inclusion of lithic fragments deriving from the disaggregation of the substratum is observed by light microscopy <b>1:</b> no disaggregation is appreciable at both the macroscopic and microscopic scale
<b>e.</b> <i>Chemical action</i>	Secretion of primary or secondary metabolites affecting the mineral stability (especially oxalic acid)	<b>5:</b> species secreting oxalic acid <b>3:</b> species secreting secondary metabolites having mineral-leaching effect and species having pitting effect caused by unknown chemical processes <b>0:</b> species secreting secondary metabolites not having mineral-leaching effects or species not secreting any secondary metabolite
<b>f.</b> <i>Hyphal spread</i>	Volume of rock occupied by hyphae	<b>1:</b> > 50% <b>0,7:</b> 50-20% <b>0,3:</b> 20-5% <b>0,1:</b> < 5%
<b>g.</b> <i>Bioprotection</i>	Lichen thalli acting like a shield-barrier between the substrata and external weathering agents	<b>0:</b> reports or evidence of biocovering effects <b>1:</b> no reports or evidence of biocovering effects

Fifty nine of the studied rock-art sites are located in the last 22 km of the valley of river Côa, inside the borders of the Côa Valley Archaeological Park. The remaining rock-art sites are located along the slopes of other tributary rivers of the Douro - 3 in the Sabor river valley and 3 in the Tua river valley. Cover is the percentage of the schist surface that is colonized by each species, estimated through visual inspection. Evaluation of the reproductive potency of each species was based on field observations and expert knowledge. When both fertile and sterile individuals occurred in the same site, the higher value was adopted to avoid an underestimation of the phenomenon as recommended by Gazzano *et al.* (2009). The predominant orientation of all selected schist surfaces was also registered in the field with a compass, in order to later verify its conformity with corresponding slope aspect.



**Figure 10:** Study area. A: Location of the study area in north-east Portugal. B: Study area in detail with reference to administrative areas (municipalities) where sampling took place, rivers (Douro and its main tributaries in the region: Côa, Sabor and Tua) and location of the sampled surfaces (white dots).

Evaluation of the parameters reflecting lichen-induced physical and chemical deterioration (depth of hyphal penetration, hyphal spread, physical and chemical action) was based on the results of a previous assessment by Marques *et al.* (submitted).

To estimate the hyphal penetration component used to calculate the parameter  $c$ , transverse sections of the lichen-schist interface were prepared for each species, with a minimum size of 3 cm wide, were impregnated in resine and cut with a rock saw using an oil–water emulsion lubricant. These surfaces were then dry-polished using carborundum papers of progressively finer grade. Polished sections were stained using

the periodic acid-Schiff method (PAS) (Whitlatch & Johnson 1974) to highlight the hyphal penetration component (HPC) according to Favero-Longo *et al.* (2005). Three polished cross-sections were prepared for each case-study (i.e. different sites, different species) and respective control (no colonization). Microphotographs (150 dpi) of the stained sections were acquired at  $\times 10$  magnification under reflected light microscopy using a stereomicroscope (Nikon SMZ1000) equipped with a digital camera (Nikon DS Fi1). The acquired images were analysed using free and open-source software packages namely: *ImageJ* (Abràmoff *et al.* 2004) for pre and post-processing procedures and generation of calibration points, *MaZda* (Szczypiński *et al.* 2009) for texture analysis and *R* for colour feature extraction, data analysis and classification; in order to obtain data on the spread of hyphal penetration, length of penetration and area weathering rind. As lichen penetration through schist follows the path along plans of weakness (Marques 2013) it is difficult, if not impossible, to distinguish an area of massive HPC like the one observed in calcareous rocks (Gazzano *et al.* 2009).

Considering the physical action (parameter d) related to the four species studied, maximum attributed value was 3 since there was no observed rock detachment that could be clearly associated with lichen occurrence. When observed under the microscope, all the three crustose species among the selected group of four were able to show evidences of medullar or hyphal inclusion of lithic fragments deriving from the disaggregation of the substratum. This kind of inclusion was never observed in rock samples colonized by the squamulose *Peltula euploca*.

The chemical action of the species on the colonized surfaces (parameter e) was previously studied using X-ray microdiffraction, FT-Raman spectroscopy and X-ray fluorescence to evaluate the production of neoformed minerals and element mobilization at the lichen-rock interface. The evaluation of the level of chemical alteration is based on the recovery of neogenesis minerals at the lichen–rock interface, relying on the principle that neoformed mineral production is the result of ion mobilization from the rock by lichens via the secretion of complexing agents, usually oxalic acid (Del Monte *et al.* 1987; Lisci *et al.* 2003). With the exception of *Aspicilia hoffmanniana*, crystals of calcium oxalate dehydrate (weddelite) were detected at the lichen-rock interface of the three remaining species.

The hyphal spread (parameter f) was evaluated through image analysis of the same cross-sections above described, allowing to accurately measure the volume occupied by lichen hyphae of each species into the rock interior. Lastly, for bioprotection assessment (parameter g), there were no reports of biocovering effects for the species studied.

To evaluate the possible relationship of LPBA value with the climatic conditions in the study, a set of variables (Appendix I, Table VI) was used to perform the analysis. Surface aspect was obtained from a digital elevation model (DEM) in ArcMap 10.1 (ESRI 2012) and only four classes were considered (North, South, East and West). To acquire micro-scale environmental data, eleven data loggers (Maxim Integrated, Sunnyvale (CA) USA) were spread throughout strategic vertical schist surfaces in the Côa valley with almost a four-year series (September 2010 to June 2014) of data collected concerning temperature (°C) and relative humidity (%) on an hourly or half-hourly recording frequency. Data loggers were placed to obtain measurements of all orientations (number of data loggers per orientation: north = 3; south = 6; east = 1; west = 1). For each data logger, the mean value of temperature and relative humidity was calculated and the results were paired according to aspect. Data obtained from these dataloggers reflects the micro-scale variation in temperature and relative humidity at the level of the vertical schist surfaces. For meso-scale environmental data, three weather stations located in the Côa river valley with a 4-year data series (March 2010 to June 2014) that collected, among other parameters, temperature and relative humidity. To obtain a surface with these variables, an interpolation method was performed (Inverse Distance Weighting) in ArcMap 10.1 (ESRI 2012) and the values were extracted to sampling surfaces for acquiring information at the valley level. Finally, macro-scale environmental variables, annual mean temperature (°C) and annual mean precipitation (mm) were obtained from the Iberian Climate Atlas (AEMET & IM 2011) and the values were extracted to sampling points and this broader perspective gives information at the regional level.

Differences in the LPBA related with surface aspect were tested by Analysis of Variance (ANOVA). Linear regressions were used to predict lichen-induced deterioration based on LPBA estimation. We tested three different sets of explanatory variables: (1) lichen cover only; (2) climatic variables only, (3) lichen cover and climatic variables. Variables to be included in the final model were selected by stepwise regression. A subset of three randomly chosen schist surfaces was kept for model validation. All statistical analysis were performed in R.

For a first attempt to spatialize LPBA results in the study area, an interpolation method (Inverse Distance Weighting) was performed in ArcMap 10.1 (ESRI 2012).

## Results and discussion

The estimated values of the Index of Lichen Potential Biodeteriogenic Activity (LPBA) for the studied schist surfaces in the Upper Douro Region ranged from 0 to

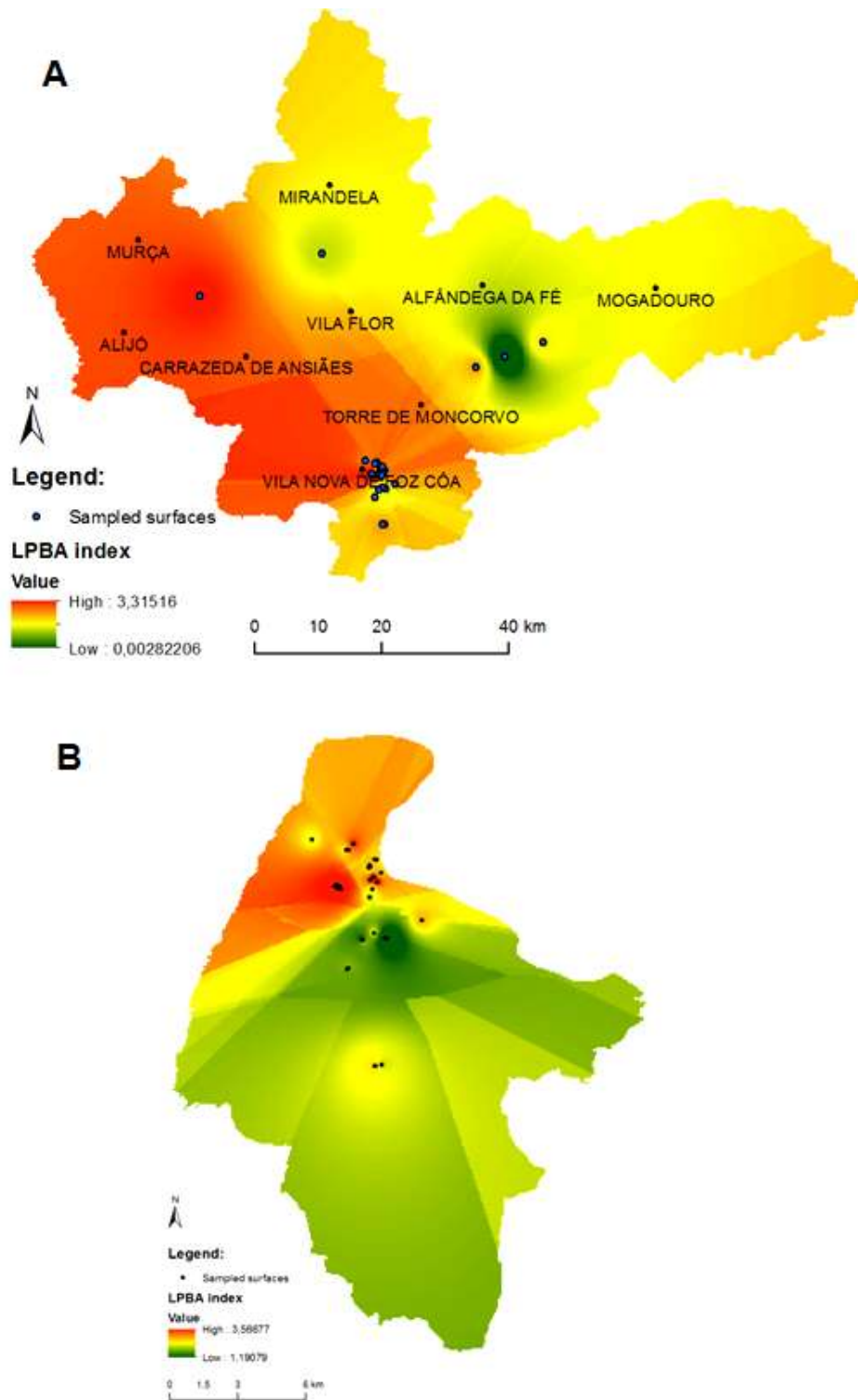
3,68 (Appendix I, Table V). In the original work, the highest reached value was achieved in sandstone substrate (LPBA value = 4,66). The spatial distribution of estimated LPBA values is depicted in Figure 11.

The cover of the target species in each analysed surface varied between 0% (absence of target species) and 70% (*Aspicilia hoffmanniana*: 0–50%, *Caloplaca subsoluta*: 0–40%, *Lecanora pseudistera*: 0–30% and *Peltula euploca*: 0–70%), and the mean cover of target lichens was 7,63% (*Aspicilia hoffmanniana*: 10,44%; *Caloplaca subsoluta*: 6,98%; *Lecanora pseudistera*: 3,09%; *Peltula euploca*: 11,34%). Only two surfaces were completely deprived of the target species (EP215 facing north-west, and CI13 facing south-east).

*Aspicilia hoffmanniana* was rarely fertile in the study area, producing only sterile apothecia and thus received the lowest value (1) for reproductive potency. *Caloplaca subsoluta* and *Lecanora pseudistera* were persistently fertile and were given the value 5 for this parameter. Although frequently fertile in the study area, *Peltula euploca* is typically sorediate therefore capable of releasing vegetative propagules which are considered to increase the reproductive potency of the species, and received the maximum value of 10 for this parameter (Appendix I, Table V).

Maximum attributed value for the parameter *physical action* was 3 since there was no observed rock detachment that could be clearly associated with lichen occurrence. When observed under the microscope, all the three crustose species among the selected group of four were able to show evidences of medullar or hyphal inclusion of lithic fragments deriving from the disaggregation of the substratum thus receiving a 3.

This kind of inclusion was never observed in rock samples colonized by the squamulose *Peltula euploca* which received a 1. The evaluation of the level of *chemical alteration* is based on the recovery of neogenesis minerals at the lichen–rock interface, relying on the principle that neoformed mineral production is the result of ion mobilization from the rock by lichens via the secretion of complexing agents. Oxalic acid is considered the most active among the complexing agents produced by lichens not only because it is an effective "solvent" of both clay materials and iron-oxides but also because it is able to dissolve the SiO<sub>2</sub> from soils (Ascaso & Galvan 1976). Species producing this acid are therefore considered more aggressive and supposed to receive the maximum value (5) for this parameter.



**Figure 11:** LPBA spatialization for the Upper Douro Region (A) and for Cõa Valley Archaeological Park (B).

However, calcium oxalate production at the lichen-rock interface had been shown to be related with slope aspect varying within the same species depending on the orientation of the surface where it comes from (north-west or south-east facing) (Marques *et al.* submitted). The minimum value for this parameter (0) in south-facing

surfaces was attributed to *Aspicilia hoffmanniana*, since no neoformed minerals were detected at the lichen-rock interface of samples colonized by this species whereas the maximum value (5) was attributed to the remaining three species due to the previous detection of calcium oxalate. When considering north-west facing surfaces, the values attributed where the minimum (0) for *Aspicilia hoffmanniana*, *Caloplaca subsoluta* and *Peltula euploca* while *Lecanora pseudistera*, received the intermediate value (3), for being a producer of atranorine, a lichen acid with mineral leaching effect (Ascaso & Galvan 1976).

The minimum value for hyphal spread (0,1) was attributed to *Aspicilia hoffmanniana*, *Caloplaca subsoluta* and *Peltula euploca* whereas the second lowest (0,3) was attributed to *Lecanora pseudistera*.

Finally, there were no evidences of biocovering effects by any of the target species and the neutral value (1) was attributed to all target species.

The correlations between the estimated LPBA values and the cover of each target species were rather weak (*Aspicilia hoffmanniana*:  $r^2 = 0,283784$ ; *Lecanora pseudistera*:  $r^2 = 0,3441$ ; *Caloplaca subsoluta*:  $r^2 = 0,415303$  and *Peltula euploca*  $r^2=0,529733$ ), enhancing the need to account for other deteriorating agents instead of considering only species abundance. Surprisingly, there was no effect of slope aspect on LPBA.

The three models found for the different combinations of groups of variables for explaining the LPBA are highly significant ( $P < 0.001$ , Table IV). The cover of dominant lichens is significantly related with the degree of lichen-induced deterioration, as expected. However, target species cover alone explains more than half of the total variation in LPBA values ( $R^2 \text{ adj.} = 0,52$ ), showing a certain dependence of LPBA values on species cover and suggesting the need for the calibration of some of the parameters.

**Table IV:** Summary of the results obtained in the model.

Response variable	Explanatory variable	$R^2$ adjusted	p-value
LPBA	Species cover	0,52	< 0,001
	Climatic variables	0,30	< 0,001
	Species cover and climatic variables	0,59	< 0,001

The model based only on the climatic variables had the lowest  $R^2$  adjusted ( $R^2 \text{ adj.} = 0,30$ ) of all models. The selected climatic variables were therefore weak predictors of lichen-induced deterioration in the study area. As micro-scale climatic



variables were also included in the model, the selection of climatic variables seems quite reasonable to characterize the climatic conditions at the scale of the schist surface and the only explanation available for this low performance is that lichen-induced deterioration is probably controlled by factors not correlated with climate such as intra-specific variability and rock bioreceptivity leading to greater environmental heterogeneity at the surface level.

Nevertheless, as hypothesized, the model based only on climatic variables was considerably improved by adding lichen species cover as an additional predictor. The model combining the cover of target species and the climatic variables performed better than both the lichen cover and the climate model ( $R^2_{adj.}=0.59$ ).

Starting the variable selection procedure with the cover of target species and the climatic variables, resulted in a model with a slightly better  $R^2_{adj.}$  value (0,60). The most important climate variables for LPBA estimation included both small- and meso-scale variables, including temperature and relative humidity at the surface level and temperature at the level of the main valley. Temperature at the micro-scale, relative humidity at the micro-scale and the cover of all target species were positively related to LPBA in the model with the highest  $R^2_{adj.}$  whereas temperature at the meso-scale was negatively correlated to the LPBA.

Furthermore, the cover of *Peltula euploca* turned out to be the most important variable in all models which included this variable (largest t-values).

The observed and predicted values of LPBA at the three surfaces left for model validation (CA4, VFN1, VFS3) are significantly correlated ( $r^2=0.97$ ; p-value=0.8163), indicating that the best model found may still be useful to forecast lichen-induced deterioration and help prioritise interventions.

## Conclusions

Field observations are indispensable if lichen-induced deterioration is to be estimated in the Upper Douro region. At least species cover and micro- and meso-scale environmental variables have to be assessed to predict lichen-induced deterioration with some reliability. Large-scale environmental variables, however, are of minor importance. To decrease the costs of surveys, it should be tested if archaeologists and conservators could be trained to recognize the target species and estimate their respective cover reliably in the field. We expect that our approach is feasible in many other rock-art sites and monuments. Rock conservation strategies ignoring the effect of these highly specialized organisms are likely to miss their principal aim.

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## Appendix I

**Table V:** LPBA calculation for dominant species in the Upper Douro Region. Aspect is related to surfaces orientation, facing north (N) or south (S). Parameters a-g from the LPBA index were calculated for each species separately and the final value is listed in the last column.

Surfaces	Aspect	<i>Aspicilia hoffmanniana</i>								<i>Caloplaca subsoluta</i>								<i>Lecanora pseudistera</i>								<i>Peltula euploca</i>								LPBA value
		a	b	c	d	e	f	g		a	b	c	d	e	f	g		a	b	c	d	e	f	g		a	b	c	d	e	f	g		
AHFT23	N	0	1	10	3	0	0,1	1	0,0	1	5	10	3	0	0,1	1	15,0	0	5	10	3	3	0,3	1	0,0	5	10	10	1	0	0,1	1	50,0	1,81
CA1	N	0	1	10	3	0	0,1	1	0,0	10	5	10	3	0	0,1	1	150,0	0	5	10	3	3	0,3	1	0,0	5	10	10	1	0	0,1	1	50,0	2,30
CA-RT1	N	0	1	10	3	0	0,1	1	0,0	1	5	10	3	0	0,1	1	15,0	0	5	10	3	3	0,3	1	0,0	0	10	10	1	0	0,1	1	0,0	1,18
EP153	N	10	1	10	3	0	0,1	1	30,0	5	5	10	3	0	0,1	1	75,0	1	5	10	3	3	0,3	1	90,0	15	10	10	1	0	0,1	1	150,0	2,54
EP215	N	0	1	10	3	0	0,1	1	0,0	0	5	10	3	0	0,1	1	0,0	0	5	10	3	3	0,3	1	0,0	0	10	10	1	0	0,1	1	0,0	0,00
EP954	N	30	1	10	3	0	0,1	1	90,0	5	5	10	3	0	0,1	1	75,0	0	5	10	3	3	0,3	1	0,0	0	10	10	1	0	0,1	1	0,0	2,22
P3	N	5	1	10	3	0	0,1	1	15,0	5	5	10	3	0	0,1	1	75,0	1	5	10	3	3	0,3	1	90,0	5	10	10	1	0	0,1	1	50,0	3,13
QTN1	N	10	1	10	3	0	0,1	1	30,0	30	5	10	3	0	0,1	1	450,0	5	5	10	3	3	0,3	1	450,0	15	10	10	1	0	0,1	1	150,0	3,09
QTN2	N	0	1	10	3	0	0,1	1	0,0	40	5	10	3	0	0,1	1	600,0	15	5	10	3	3	0,3	1	1350,0	0	10	10	1	0	0,1	1	0,0	1,63
QTN3	N	0	1	10	3	0	0,1	1	0,0	30	5	10	3	0	0,1	1	450,0	20	5	10	3	3	0,3	1	1800,0	10	10	10	1	0	0,1	1	100,0	2,55
QTN4	N	0	1	10	3	0	0,1	1	0,0	5	5	10	3	0	0,1	1	75,0	10	5	10	3	3	0,3	1	900,0	10	10	10	1	0	0,1	1	100,0	3,15
VFN1	N	0	1	10	3	0	0,1	1	0,0	1	5	10	3	0	0,1	1	15,0	20	5	10	3	3	0,3	1	1800,0	0	10	10	1	0	0,1	1	0,0	3,14
VFN2	N	0	1	10	3	0	0,1	1	0,0	5	5	10	3	0	0,1	1	75,0	20	5	10	3	3	0,3	1	1800,0	0	10	10	1	0	0,1	1	0,0	0,00
VFN3	N	0	1	10	3	0	0,1	1	0,0	5	5	10	3	0	0,1	1	75,0	30	5	10	3	3	0,3	1	2700,0	0	10	10	1	0	0,1	1	0,0	2,54
VFN4	N	0	1	10	3	0	0,1	1	0,0	5	5	10	3	0	0,1	1	75,0	15	5	10	3	3	0,3	1	1350,0	0	10	10	1	0	0,1	1	0,0	2,61
VJEN1	N	15	1	10	3	0	0,1	1	45,0	1	5	10	3	0	0,1	1	15,0	5	5	10	3	3	0,3	1	450,0	0	10	10	1	0	0,1	1	0,0	2,81
VJEN2	N	0	1	10	3	0	0,1	1	0,0	0	5	10	3	0	0,1	1	0,0	1	5	10	3	3	0,3	1	90,0	0	10	10	1	0	0,1	1	0,0	2,48
VJEN3	N	5	1	10	3	0	0,1	1	15,0	5	5	10	3	0	0,1	1	75,0	5	5	10	3	3	0,3	1	450,0	0	10	10	1	0	0,1	1	0,0	2,66
VJEN4	N	1	1	10	3	0	0,1	1	3,0	1	5	10	3	0	0,1	1	15,0	1	5	10	3	3	0,3	1	90,0	0	10	10	1	0	0,1	1	0,0	3,13
AHFT5-1	S	30	1	10	3	0	0,1	1	270,0	30	5	10	3	5	0,1	1	1200,0	0	5	10	3	5	0,3	1	0,0	10	10	10	1	5	0,1	1	60,0	3,68
AHFT5-2	S	0	1	10	3	0	0,1	1	0,0	1	5	10	3	5	0,1	1	40,0	0	5	10	3	5	0,3	1	0,0	20	10	10	1	5	0,1	1	1200,0	2,96

CA2	S	1	1	10	3	0	0,1	1	9,0	1	5	10	3	5	0,1	1	40,0	0	5	10	3	5	0,3	1	0,0	0	10	10	1	5	0,1	1	0,0	3,42
CA4	S	5	1	10	3	0	0,1	1	45,0	1	5	10	3	5	0,1	1	40,0	0	5	10	3	5	0,3	1	0,0	5	10	10	1	5	0,1	1	300,0	3,63
CA-RT4	S	1	1	10	3	0	0,1	1	9,0	5	5	10	3	5	0,1	1	200,0	0	5	10	3	5	0,3	1	0,0	20	10	10	1	5	0,1	1	1200,0	2,35
CI1	S	5	1	10	3	0	0,1	1	45,0	1	5	10	3	5	0,1	1	40,0	1	5	10	3	5	0,3	1	120,0	20	10	10	1	5	0,1	1	1200,0	2,36
CI13	S	0	1	10	3	0	0,1	1	0,0	0	5	10	3	5	0,1	1	0,0	0	5	10	3	5	0,3	1	0,0	0	10	10	1	5	0,1	1	0,0	2,58
CI14	S	10	1	10	3	0	0,1	1	90,0	5	5	10	3	5	0,1	1	200,0	1	5	10	3	5	0,3	1	120,0	0	10	10	1	5	0,1	1	0,0	2,77
CI3	S	10	1	10	3	0	0,1	1	90,0	5	5	10	3	5	0,1	1	200,0	1	5	10	3	5	0,3	1	120,0	1	10	10	1	5	0,1	1	60,0	3,03
CM1	S	0	1	10	3	0	0,1	1	0,0	1	5	10	3	5	0,1	1	40,0	5	5	10	3	5	0,3	1	600,0	0	10	10	1	5	0,1	1	0,0	3,29
CM-RA2	S	0	1	10	3	0	0,1	1	0,0	0	5	10	3	5	0,1	1	0,0	0	5	10	3	5	0,3	1	0,0	5	10	10	1	5	0,1	1	300,0	3,37
FC16	S	0	1	10	3	0	0,1	1	0,0	1	5	10	3	5	0,1	1	40,0	1	5	10	3	5	0,3	1	120,0	5	10	10	1	5	0,1	1	300,0	3,03
FC93	S	1	1	10	3	0	0,1	1	9,0	1	5	10	3	5	0,1	1	40,0	1	5	10	3	5	0,3	1	120,0	20	10	10	1	5	0,1	1	1200,0	2,40
FCS1	S	15	1	10	3	0	0,1	1	135,0	10	5	10	3	5	0,1	1	400,0	1	5	10	3	5	0,3	1	120,0	70	10	10	1	5	0,1	1	4200,0	2,21
FCS2	S	30	1	10	3	0	0,1	1	270,0	10	5	10	3	5	0,1	1	400,0	1	5	10	3	5	0,3	1	120,0	5	10	10	1	5	0,1	1	300,0	2,61
FCS3	S	10	1	10	3	0	0,1	1	90,0	5	5	10	3	5	0,1	1	200,0	5	5	10	3	5	0,3	1	600,0	30	10	10	1	5	0,1	1	1800,0	2,89
FCS4	S	10	1	10	3	0	0,1	1	90,0	30	5	10	3	5	0,1	1	1200,0	10	5	10	3	5	0,3	1	1200,0	30	10	10	1	5	0,1	1	1800,0	3,33
MC7	S	1	1	10	3	0	0,1	1	9,0	1	5	10	3	5	0,1	1	40,0	1	5	10	3	5	0,3	1	120,0	1	10	10	1	5	0,1	1	60,0	3,33
QB3	S	0	1	10	3	0	0,1	1	0,0	5	5	10	3	5	0,1	1	200,0	1	5	10	3	5	0,3	1	120,0	1	10	10	1	5	0,1	1	60,0	3,21
QB-RA1	S	5	1	10	3	0	0,1	1	45,0	10	5	10	3	5	0,1	1	400,0	1	5	10	3	5	0,3	1	120,0	1	10	10	1	5	0,1	1	60,0	2,98
TD1	S	10	1	10	3	0	0,1	1	90,0	1	5	10	3	5	0,1	1	40,0	1	5	10	3	5	0,3	1	120,0	1	10	10	1	5	0,1	1	60,0	3,09
V1	S	1	1	10	3	0	0,1	1	9,0	1	5	10	3	5	0,1	1	40,0	1	5	10	3	5	0,3	1	120,0	00	10	10	1	5	0,1	1	0,0	1,63
V2	S	10	1	10	3	0	0,1	1	90,0	5	5	10	3	5	0,1	1	200,0	1	5	10	3	5	0,3	1	120,0	1	10	10	1	5	0,1	1	60,0	3,00
V3	S	5	1	10	3	0	0,1	1	45,0	1	5	10	3	5	0,1	1	40,0	1	5	10	3	5	0,3	1	120,0	10	10	10	1	5	0,1	1	600,0	1,63
VC1	S	0	1	10	3	0	0,1	1	0,0	20	5	10	3	5	0,1	1	800,0	1	5	10	3	5	0,3	1	120,0	20	10	10	1	5	0,1	1	1200,0	2,55
VC4	S	5	1	10	3	0	0,1	1	45,0	5	5	10	3	5	0,1	1	200,0	1	5	10	3	5	0,3	1	120,0	30	10	10	1	5	0,1	1	1800,0	2,41
VC5	S	10	1	10	3	0	0,1	1	90,0	10	5	10	3	5	0,1	1	400,0	5	5	10	3	5	0,3	1	600,0	10	10	10	1	5	0,1	1	600,0	2,16
VC6	S	5	1	10	3	0	0,1	1	45,0	1	5	10	3	5	0,1	1	40,0	0	5	10	3	5	0,3	1	0,0	15	10	10	1	5	0,1	1	900,0	3,26
VC-RT1	S	1	1	10	3	0	0,1	1	9,0	20	5	10	3	5	0,1	1	800,0	1	5	10	3	5	0,3	1	120,0	5	10	10	1	5	0,1	1	300,0	3,27

VC-RT6	S	1	1	10	3	0	0,1	1	9,0	1	5	10	3	5	0,1	1	40,0	0	5	10	3	5	0,3	1	0,0	0	10	10	1	5	0,1	1	0,0	3,44
VF9	S	20	1	10	3	0	0,1	1	180,0	1	5	10	3	5	0,1	1	40,0	0	5	10	3	5	0,3	1	0,0	15	10	10	1	5	0,1	1	900,0	3,15
VFIG-R10	S	1	1	10	3	0	0,1	1	9,0	1	5	10	3	5	0,1	1	40,0	0	5	10	3	5	0,3	1	0,0		10	10	1	5	0,1	1	0,0	3,39
VFIG-R11	S	5	1	10	3	0	0,1	1	45,0	1	5	10	3	5	0,1	1	40,0	0	5	10	3	5	0,3	1	0,0	5	10	10	1	5	0,1	1	300,0	3,05
VFIG-R12	S	0	1	10	3	0	0,1	1	0,0	5	5	10	3	5	0,1	1	200,0	0	5	10	3	5	0,3	1	0,0	1	10	10	1	5	0,1	1	60,0	3,43
VFII-RT5	S	15	1	10	3	0	0,1	1	135,0	1	5	10	3	5	0,1	1	40,0	0	5	10	3	5	0,3	1	0,0	1	10	10	1	5	0,1	1	60,0	3,45
VF-RA4	S	10	1	10	3	0	0,1	1	90,0	1	5	10	3	5	0,1	1	40,0	0	5	10	3	5	0,3	1	0,0	40	10	10	1	5	0,1	1	2400,0	3,27
VFS1	S	1	1	10	3	0	0,1	1	9,0	10	5	10	3	5	0,1	1	400,0	1	5	10	3	5	0,3	1	120,0	10	10	10	1	5	0,1	1	600,0	2,37
VFS2	S	30	1	10	3	0	0,1	1	270,0	5	5	10	3	5	0,1	1	200,0	0	5	10	3	5	0,3	1	0,0	40	10	10	1	5	0,1	1	2400,0	2,71
VFS3	S	10	1	10	3	0	0,1	1	90,0	10	5	10	3	5	0,1	1	400,0	0	5	10	3	5	0,3	1	0,0	40	10	10	1	5	0,1	1	2400,0	1,95
VFS4	S	5	1	10	3	0	0,1	1	45,0	1	5	10	3	5	0,1	1	40,0	0	5	10	3	5	0,3	1	0,0	30	10	10	1	5	0,1	1	1800,0	2,73
VJE17	S	5	1	10	3	0	0,1	1	45,0	1	5	10	3	5	0,1	1	40,0	1	5	10	3	5	0,3	1	120,0	1	10	10	1	5	0,1	1	60,0	2,03
VJES1	S	20	1	10	3	0	0,1	1	180,0	5	5	10	3	5	0,1	1	200,0	0	5	10	3	5	0,3	1	0,0	5	10	10	1	5	0,1	1	300,0	2,75
VJES2	S	10	1	10	3	0	0,1	1	90,0	5	5	10	3	5	0,1	1	200,0	1	5	10	3	5	0,3	1	120,0	20	10	10	1	5	0,1	1	1200,0	3,19
VJES3	S	10	1	10	3	0	0,1	1	90,0	15	5	10	3	5	0,1	1	600,0	1	5	10	3	5	0,3	1	120,0	15	10	10	1	5	0,1	1	900,0	3,22
VJES4	S	50	1	10	3	0	0,1	1	450,0	20	5	10	3	5	0,1	1	800,0	5	5	10	3	5	0,3	1	600,0	15	10	10	1	5	0,1	1	900,0	3,39
V-RA1	S	20	1	10	3	0	0,1	1	180,0	1	5	10	3	5	0,1	1	40,0	1	5	10	3	5	0,3	1	120,0	5	10	10	1	5	0,1	1	300,0	2,72

**Table VI:** Data set used in the correlation between LPBA values, species abundance and climatic variables. Aspect is related to surfaces orientation, facing north (N) or south (S). Maximum species abundance in the sampled surfaces (AH: *Aspicilia hoffmanniana*; CS: *Caloplaca subsoluta*; LP: *Lecanora pseudistera*; PE: *Peltula euploca*). Three-scale climatic variables divided in micro-, meso- and macro-scale (temperature (°C), relative humidity (%) and precipitation (mm)).

Surface	Aspect	LPBA	Species richness	Climatic variables									
				Species abundance				Micro-scale		Meso-scale		Macro-scale	
				AH	CS	LP	PE	Temperature	Relative humidity	Temperature	Relative humidity	Temperature	Precipitation
AHFT23	N	1,81	16	0	1	0	5	17,18	66,78	16,03	41,05	14,60	610,30
CA1	N	2,30	16	0	10	0	5	16,06	72,37	16,46	41,06	14,70	618,20
CA-RT1	N	1,18	21	0	1	0	0	16,06	72,37	16,46	41,06	14,70	618,20
EP153	N	2,54	15	10	5	1	15	17,18	66,78	16,03	41,05	15,10	601,80
EP215	N	0,00	9	0	0	0	0	17,18	66,78	16,03	41,05	15,40	542,20
EP954	N	2,22	10	30	5	0	0	17,18	66,78	16,03	41,05	15,20	598,20
P3	N	3,13	8	5	5	1	5	17,18	66,78	15,76	41,00	15,40	596,30
QTN1	N	3,09	29	10	30	5	15	16,06	72,37	15,76	41,08	16,10	641,50
QTN2	N	1,63	14	0	40	15	0	16,06	72,37	15,76	41,08	16,10	641,50
QTN3	N	2,55	20	0	30	20	10	16,06	72,37	15,76	41,08	16,10	641,50
QTN4	N	3,15	8	0	5	10	10	16,06	72,37	15,76	41,08	16,10	641,50
VFN1	N	3,14	17	0	1	20	0	16,06	72,37	15,95	41,07	15,40	587,40
VFN2	N	0,00	16	0	5	20	0	16,06	72,37	15,92	41,07	15,40	587,40
VFN3	N	2,54	20	0	5	30	0	16,06	72,37	15,95	41,07	15,40	616,10
VFN4	N	2,61	19	0	5	15	0	16,06	72,37	15,92	41,07	15,40	587,40
VJEN1	N	2,81	12	15	1	5	0	16,06	72,37	15,74	41,08	16,10	570,20
VJEN2	N	2,48	17	0	0	1	0	16,06	72,37	15,74	41,08	16,10	570,20
VJEN3	N	2,66	27	5	5	5	0	16,06	72,37	15,74	41,08	16,10	570,20
VJEN4	N	3,13	25	1	1	1	0	20,52	62,68	15,74	41,08	16,10	570,20
AHFT5-1	S	3,68	15	30	30	0	1	20,52	62,68	16,03	41,05	14,90	748,30
AHFT5-2	S	2,96	11	0	1	0	20	20,52	62,68	16,03	41,05	14,90	748,30



CA2	S	3,42	12	1	1	0	0	20,52	62,68	16,46	41,06	14,70	618,20
CA4	S	3,63	19	5	1	0	5	17,48	70,46	16,52	41,06	15,10	648,10
CA-RT4	S	2,35	20	1	5	0	20	17,48	70,46	16,52	41,06	15,10	648,10
CI1	S	2,36	5	5	1	1	20	20,52	62,68	16,58	41,06	15,10	620,10
CI13	S	2,58	2	0	0	0	0	17,48	70,46	16,58	41,06	15,10	620,10
CI14	S	2,77	7	10	5	1	0	20,52	62,68	16,58	41,06	15,10	620,10
CI3	S	3,03	4	10	5	1	1	17,48	70,46	16,58	41,06	15,10	620,10
CM1	S	3,29	10	0	1	5	0	17,48	70,46	16,18	41,06	15,10	609,80
CM-RA2	S	3,37	7	0	0	0	5	17,48	70,46	16,18	41,06	15,10	609,80
FC16	S	3,03	7	0	1	1	5	17,48	70,46	15,76	41,08	15,90	516,60
FC93	S	2,40	20	1	1	1	20	20,52	62,68	15,75	41,08	16,10	633,10
FCS1	S	2,21	21	15	10	1	70	17,48	70,46	15,74	41,08	16,20	678,40
FCS2	S	2,61	14	30	10	1	5	17,48	70,46	15,74	41,08	16,20	678,40
FCS3	S	2,89	14	10	5	5	30	17,48	70,46	15,74	41,08	16,20	678,40
FCS4	S	3,33	15	10	30	10	30	17,48	70,46	15,74	41,08	16,20	678,40
MC7	S	3,33	6	1	1	1	1	17,48	70,46	15,95	41,07	15,60	638,40
QB3	S	3,21	6		5	1	1	20,52	62,68	15,77	41,01	15,60	682,90
QB-RA1	S	2,98	24	5	10	1	1	20,52	62,68	15,77	41,01	15,60	682,90
TD1	S	3,09	14	10	1	1	1	20,52	62,68	15,94	41,07	15,20	596,80
V1	S	1,63	15	1	1	1	0	20,52	62,68	15,76	41,08	15,70	544,30
V2	S	3,00	22	10	5	1	1	20,52	62,68	15,76	41,08	15,70	544,30
V3	S	1,63	13	5	1	1	10	20,52	62,68	15,76	41,08	15,70	544,30
VC1	S	2,55	10	0	20	1	20	17,48	70,46	15,83	41,08	16,20	625,30
VC4	S	2,41	8	5	5	1	30	20,52	62,68	15,83	41,08	15,90	599,60
VC5	S	2,16	15	10	10	5	10	17,48	70,46	15,83	41,08	15,60	590,70
VC6	S	3,26	14	5	1	0	15	17,48	70,46	15,83	41,08	15,60	590,70
VC-RT1	S	3,27	12	1	20	1	5	17,48	70,46	15,83	41,08	16,20	625,30
VC-RT6	S	3,44	10	1	1	0	0	17,48	70,46	15,83	41,08	15,60	590,70
VF9	S	3,15	17	20	1	0	15	20,52	62,68	15,95	41,07	15,40	616,10

VFIG-R10	S	3,39	6	1	1	0	0	20,52	62,68	16,42	41,05	15,50	632,80
VFIG-R11	S	3,05	12	5	1	0	5	17,48	70,46	16,42	41,05	15,50	632,80
VFIG-R12	S	3,43	11	0	5	0	1	17,48	70,46	16,46	41,05	15,50	632,80
VFII-RT5	S	3,45	6	15	1	0	1	17,48	70,46	15,79	41,08	15,70	647,90
VF-RA4	S	3,27	16	10	1	0	40	20,52	62,68	15,95	41,07	15,40	616,10
VFS1	S	2,37	13	1	10	1	10	20,52	62,68	15,95	41,07	15,40	616,10
VFS2	S	2,71	18	30	5	0	40	20,52	62,68	15,91	41,07	15,40	615,30
VFS3	S	1,95	14	10	10	0	40	20,52	62,68	15,91	41,07	15,40	615,30
VFS4	S	2,73	17	5	1	0	30	20,52	62,68	15,91	41,07	15,40	615,30
VJE17	S	2,03	5	5	1	1	1	16,06	72,37	15,74	41,08	16,10	570,20
VJES1	S	2,75	13	20	5	0	5	20,52	62,68	15,74	41,08	16,00	574,50
VJES2	S	3,19	11	10	5	1	20	20,52	62,68	15,74	41,08	16,10	570,20
VJES3	S	3,22	15	10	15	1	15	20,52	62,68	15,74	41,08	16,10	570,20
VJES4	S	3,39	20	50	20	5	15	20,52	62,68	15,74	41,08	16,10	570,20
V-RA1	S	2,72	18	20	1	1	5	20,52	62,68	15,76	41,08	15,70	544,30



## Chapter 6 |

### General conclusions

Lichens are the most abundant colonizers of the schist surfaces in the Upper Douro region. For a practical evaluation of lichen-induced changes in the colonized surfaces, a subset of dominant species determined by species frequency and abundance at opposite slopes and three crustose species (*Aspicilia hoffmanianna*, *Caloplaca subsoluta* and *Lecanora pseudistera*) and one squamulose (*Peltula euploca*) were selected and used in the various assessments of lichen activity on schist. Lichen species distribution patterns on the schist surfaces in the Upper Douro region and their relationship with climatic variables was evaluated. The results demonstrated that, for total species assemblages, small to large scale variables (temperature, relative humidity and precipitation) were relevant to explain the variations perceived in the field with aspect-related dominance of species and, for dominant species, only variables at the surface level (micro-scale) were significant.

Elemental mobilization reflects an aspect-related pattern and the most intense activity was detected in the south-facing surfaces, corroborate prior knowledge about the stronger chemical activity in those surfaces whereas physical action is more evident in the north-facing surfaces.

LPBA results enhanced a strong dependence of cover (species abundance) in the final value and the addition of complementary parameters seems reasonable and climatic variables could be used as proxy of lichenic physical and chemical activity. LPBA index does not reflect the aspect-related differences in deterioration observed in the field, corroborated with elemental changes in colonized opposite surfaces.

However, a calibrated index is still needed for the schist surfaces. The approach developed in this work intended to provide a useful tool to archaeologist and conservators of open-air rock-art in the Upper Douro Region and clarify some questions around the role of lichenological colonization of engraved surfaces.

