

Biominerals Resulting from Biodeterioration Promoted by Different *Saxicolous* Lichens

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INTRODUCTION

The original mineralogical composition of the stone based materials used in rural and urban architecture changes with years due to deterioration process they can suffer when they are exposed to environment. Above all when they belong to facades of buildings located in areas with high concentrations of acid gases like CO₂, NO_x and SO_x and/or particulate matter. Nowadays, another type of deterioration is being taken into consideration.

Lichens are common organisms all around the Earth and they have the capacity to colonize a great diversity of stones including building materials such as limestones, marbles, sandstones, mortars and concrete. The bio-weathering activity of lichens is a mixture of physical and chemical deterioration of the materials. For example, crustose lichens can affect the properties of the stone where they are living, due to biodeterioration processes that can alter and even disintegrate the surface of the stone. The mechanism that lichens use to attach to the rock substrata is composed by long and branching filamentous structures called hyphae which penetrate into the rock substrata and allow the attachment of the biofilms. The penetration of hyphae has several destructive consequences in the integrity of the rock structure due to the mechanical, physical and chemical processes involved: (a) the growth of these structures promotes the biophysical degradation (weathering and cracking) of stones and related materials, (b) biochemical deterioration can also occur through the reactions promoted by the excretion of their metabolic compounds, called commonly as lichen acids or secondary metabolites (Chem et al., 2000).

The best well known and more studied

acid excreted by lichens is the oxalic acid, produced by the lithobiont part of the lichen symbiosis. If this acid is excreted in a substrate with a great amount of calcium carbonate, a neutralization acid-base reaction takes place, appearing the calcium oxalates, either as the monohydrated whewellite (CaC₂O₄·H₂O) or as the dihydrated form, weddellite (CaC₂O₄·2H₂O). These compounds formed in-situ as a consequence of the biological activity are known as biominerals. When the new biominerals are involved in a certain degradation process Raman Spectroscopy supported with SEM images and XRF elemental analyses are the analytical techniques of choice to characterize the original and new created mineral phases. Raman Spectroscopy is sensitive and selective to a lot of inorganic and organic molecules, which can unambiguously be identified without any manipulation process of the sample. In fact, the size of the sample is not a problem and thus, it is possible to perform the analyses in a non destructive way.

In this study we have characterized the products involved in a stone lichen-mediated decaying process (biodecaying) occurred not only in samples of carbonated stones but also in non carbonated ones. We have also determined, following the previous experience in this topic, the deterioration mechanisms that these materials have suffered.

MATERIALS AND METHODS

The ultramobile (handheld) Raman spectrometer (InnoRam B&WTEK_{INC}) was used for field measurements. The equipment works with a 785 nm excitation laser and different optical lens (4x, 20x and 50x). It has a variable range to control thermal decomposition of the measured surfaces. The laboratory Raman analyses were performed using a

Renishaw Raman spectrometer operating with 785nm excitation laser wavelength with charge-coupled device (CCD) detector and lens of 20x giving a footprint of 50µm. All the spectra were collected between 100-2200 cm⁻¹ (20 accumulations of 10 second duration spectra).

The elemental distribution was carried out by µ-ED-XRF, using the ArtTax model of Rontec (nowadays Bruker AXS Berlin, Germany) that incorporates an X-ray tube with molybdenum anode working at a maximum 50 kV voltage and a maximum 600 µA current. The X-rays are collimated by a tantalum collimator (diameter of 0.65 mm). EVO@40 Scanning Electron Microscope (Carl Zeiss NTS GmbH, Germany) coupled to an XMax Energy-Dispersive X-ray spectroscopy equipment (Oxford Instruments, Abingdon, Oxfordshire, United Kingdom) was used for electron image acquisitions and elemental composition determinations. The SEM images were obtained at high vacuum employing an acceleration voltage of 20 KV and a 6–13 mm working distance. Different magnifications (reaching up to 10,000x) were used to take the images by a secondary electron detector.

Colonized sandstones, limestones and marbles with bright orange lichens belonging to *Caloplaca* genera were collected in the industrial-urban area of Getxo. Also *Lecanoraceae* genera lichens were collected with their substrate along the geography of the Northern Iberian Peninsula.

RESULTS AND DISCUSSION

Biominerals in Lichens Attached to Carbonated Stones

The lichen belonging to the *Caloplaca* genera has different zones with different orange brightness. Each part of the

lichen was characterized with Raman spectrometry including the areas of the stone that are not colonized. The sandstone was composed mainly of quartz grains (main Raman band at 464 cm^{-1}) with calcite (1085 cm^{-1}) acting as cement. In the orange brightest areas weddellite was found as a major compound with its Raman bands at $1475, 911\text{ cm}^{-1}$. The white central part of the same lichen is composed mainly by whewellite, with Raman bands at $1626, 1488, 1462, 896, 518$ and 497 cm^{-1} . The lichen's colony grows in circles, spreading out the excreted components related to its metabolism. In the forefront of the colony, weddellite is formed due to the chemical reaction between oxalic acid excreted by the lichen and calcium carbonate presented in the stone. When the lichen is leaving a certain area of the stone surface, weddellite transforms in whewellite because the biochemical activity fall down.

Regarding the specimens belonging to *Lecanoraceae* genera, several Raman analyses were collected in different target points of the surface of the lichen thallus. This lichen thallus was composed mainly by a white coloured vegetal layer with black apothecia. Enlightening Raman results were achieved only in the whitest part of the thallus, where very marked presence of calcium oxalates was detected. The black apothecia were not composed by calcium oxalate. In this case, the calcium oxalates did not follow a tidy distribution and these oxalates appeared often together in the same area, that is, they are not distributed in areas as in the case of the *Caloplaca* genera.

Other biominerals characterized during this study were different type of carbonates on *Lecanoraceae* lichens which are colonizing calcium carbonate stones. The lichen was not separated from the stone and a cross section of the stone with the lichen crusted was carried out to ascertain the diverse distribution of different layers. SEM images were first taken to view the microscopic details of these layers (Fig. 1). According to the Raman analyses, the support was composed by calcite ($282, 715$ and 1086 cm^{-1}) and vitrified carbon (1300 and 1600 cm^{-1}). The analyses on the first red-brown colour layer revealed the presence of magnesite (MgCO_3) identified by means of Raman features at $330, 738$ and 1094 cm^{-1} . The white layer was composed mainly by calcium oxalate in

the two hydration forms.

Another specimen belonging to the same specie was selected in an attempt to explain the occurrence of magnesium carbonates in a calcium carbonate based stone. In this case, the Raman spectrum revealed the presence of dolomite $\text{CaMg}(\text{CO}_3)_2$ identified by its main Raman band at 1098 cm^{-1} .

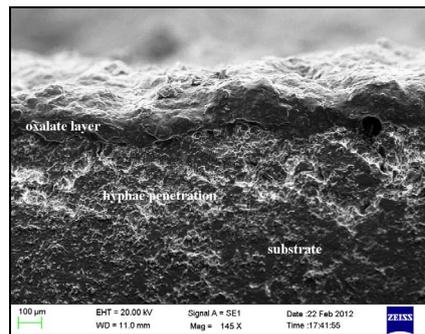


fig 1. SEM image of a *Lecanoraceae* lichen showing the layer distribution on the lichen surface and its substratum.

Neoformation of calcium oxalate minerals in lichens attached to non-carbonated sandstones

Usually, the occurrence of calcium oxalate minerals in the thallial surface of the lichens is associated to the biodeterioration of lime based sub-strates and the presence of calcium oxalates in non-carbonated stones has not been described in depth. For this purpose, several sandstones samples were taken in diverse polluted and non polluted emplacements to establish whether there is a relation between the occurrence of these calcium oxalate layers and the environmental conditions under which lichens are exposed.

Raman spectroscopy was used to characterize the studied non-carbonated stone supports. Minerals such as albite ($\text{NaAlSi}_3\text{O}_8$) were identified thanks to Raman bands at $164, 186, 209, 254, 273, 292, 331, 480, 508, 764$ and 815 cm^{-1} , together with quartz (SiO_2) as the cementing mineral due to Raman features at $128, 207, 265, 356, 395$ and 465 cm^{-1} . Any carbonate compound was found in these stone supports. However, the lichen thallus in all the samples was composed mainly by calcium oxalate dihydrate or by calcium oxalate monohydrate. To support the Raman data, μ -ED-XRF elemental analyses were done in the thallial surface of the lichens and in the non colonized surface of the sandstones; it was noticeable the high content of calcium observed in the thallus of the lichen compared with the absence

of this element in the stone substrate.

CONCLUSIONS

Raman Spectroscopy supported with SEM images and XRF elemental analyses can be considered as an adequate tool to investigate the formation of biominerals in lichens. These analyses serve as examples of the occurrence of biomineralization processes in several lichen genera despite of the composition of the stone support where the colony grows. Thanks to the Raman results obtained for each lichen genera it was possible to establish a different pathway in the oxalate formation into each lichen genera.

The calcium oxalates were found not only in colonies living on calcium carbonate based stones, but also in the not carbonated sandstones where calcium was absence. Although the capability of lichens to trap environmental particles has been already cited, this fact has not been demonstrated by Raman evidences up to now. Moreover, the fact that carbonates (magnesite, dolomite and calcite) are placed always in the lichen thallus let us to suggest that the presence of these compounds is somehow needed to achieve a good anchorage within pores and cracks of rocks (Garty et al., 2002). This discovery opens a discussion on the atmospheric intake of calcite particles in diverse lichen genera.

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