THE SUITABLE CAROTENE AND XANTHOPHYLL IDENTIFICATION IN *LECANORA* LICHENS: RESONANCE RAMAN SPECTROSCOPIC STUDY.

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Introduction: Lecanora lichen species have been recognized as air pollution resistant due to their ability in acquiring new knowledge to protect themselves from high concentration of acid gases and metals [1, 2].

Carotenoids, ubiquitous compounds in lichens, are often classified as protective pigments because they dissipate the light energy used in photosynthesis and inhibit the formation of harmful reactive oxygenated species [3].

Raman Spectroscopy has been used widespread for characterization purposes of lichen metabolites, especially in the characterization of lichen acids and pigments such as carotenoids. But sometimes the Raman assignments are not clear, are into controversy and the majority of the authors do not reach agreement [4, 5].

The Raman spectra of carotenoids present three major bands in the following order: in the regions of 1500-1550 cm^{-1} and 1150-1170 cm^{-1} appears two strong bands specific to the conjugated polyene chain of the carotenoids. The first one (ν_1) belongs C=C bonds in main skeleton and the second one (ν_2) related the C–C bonds of the same skeleton. In the 1000-1020 cm^{-1} region another peak (ν_3) appear related to the -CH_3 groups attached to the polyene chain. For instance, low number of conjugated double bonds shifts ν_1 band to major wavenumbers and vice versa [6].

The selection of the incident laser wavelength is an important aspect that influences the absorption of the chromophore. When maximum absorption is achieved, the analyses are said to be done in resonance conditions, where the Raman peaks of the chromophoric compound are enhanced significantly respect to other compounds presented [5].

The aim of this work is to characterize by Resonance Raman Spectroscopy a *Lecanora* lichen colonies to make proper Raman asignments in the path of the differentiation of both carotenes and xanthophylls.

Experimental: The measurements were done by using a Renishaw InVia Raman spectrometer, joined to a Leica DMLM microscope. The spectra were acquired with the Leica 50x N Plan (0.75 aperture) lens. The spatial resolution for the 50x lens is 2 μm. The laser of 514 nm was used in all the measures to obtain the resonance effect of the carotenes and xanthophylls.

Results: Different areas of the Lecanora Muralis lichen were distinguished according to the colour provided by the main photosintethic pigment present. For instance, astaxanthin was the mayor pigment in brown areas, whereas zeaxanthin and β-carotene were the responsibles for the black colour. The figure below displays some of the Raman signatures obtained in the lichen colony. The main band ν_1 of the carotenoid allows to differentiate between astaxanthin (ast, 1507cm^{-1}) and β-carotene (beta, 1521 cm^{-1}). However, none of the three bands normally used for carotenoid identification are enough as to distinguish zeaxanthin from β-carotene. Therefore, Raman signatures between 3100-3500 cm^{-1} corresponding to O-H bonds that are observed under resonance conditions are needed. Moreover, those additional Raman bands can guarantee sometimes the correct carotenoid assignment when Raman features of other lichen compounds such as scytonemin are observed in the same spectrum.

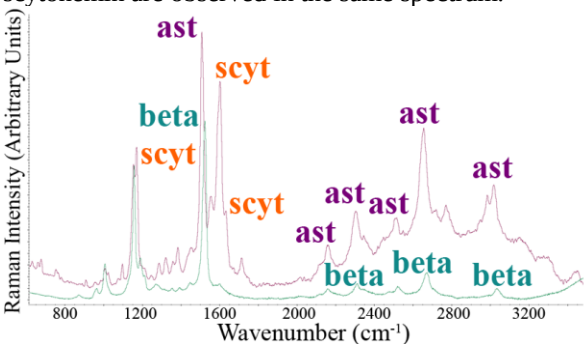

Fig. Resonance Raman spectra of Astaxanthin (ast) and scytonemin (scy) in comparison with β-carotene (beta) in black and brown areas, respectively, of the Lecanora Muralis lichen.

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