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Composition and spectra of copper-carotenoid sediments from a pyrite mine stream in Spain

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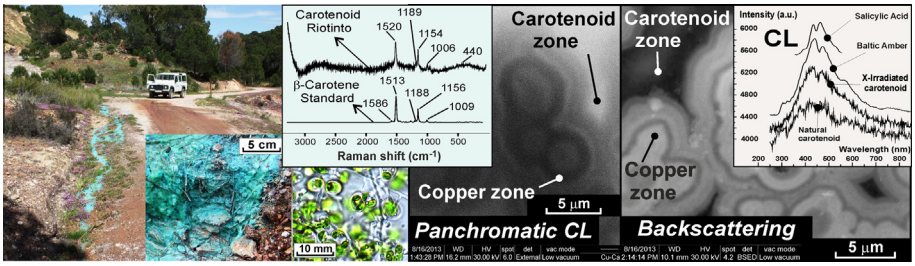
HIGHLIGHTS

- As received carotenoids in copper sulfate can be detected by Raman and Luminescence.
- Cu-sulfate-carotenoid sediments change by mineral crystallization and biological activity.
- Raman spectra of these samples exhibit major emissions at approximately 1006, 1154, and 1520 cm^{-1}.
- Pre-X-irradiation of carotenoids raise spectra CL peaks linked to hydroxyl and carboxyl groups.
- Cu-carotenoids are biomarkers and proxies for remote sensing and acid mine drainage.

GRAPHICAL ABSTRACT



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ABSTRACT

Mine drainages of La Poderosa (El Campillo, Huelva, Spain), located in the Rio Tinto Basin (Iberian Pyrite Belt) generate carotenoid complexes mixed with copper sulfates presenting good natural models for the production of carotenoids from microorganisms. The environmental conditions of Rio Tinto Basin include important environmental stresses to force the microorganisms to accumulate carotenoids. Here we show as carotenoid compounds in sediments can be analyzed directly in the solid state by Raman and Luminescence spectroscopy techniques to identify solid carotenoid, avoiding dissolution and pre-concentration treatments, since the hydrous copper-salted paragenesis do not mask the Raman emission of carotenoids. Raman spectra recorded from one of these specimens' exhibit major features at approximately 1006, 1154, and 1520 cm^{-1}. The bands at 1520 cm^{-1} and 1154 cm^{-1} can be assigned to in-phase C=C (γ^{-1}) and C—C stretching (γ^{-2}) vibrations of the polyene chain in carotenoids. The in-plane rocking deformations of CH_3 groups linked to this chain coupled with C—C bonds are observed in the 1006 cm^{-1} region. X-irradiation pretreatments enhance the cathodoluminescence spectra emission of carotenoids enough to distinguish organic compounds including hydroxyl and carboxyl groups. Carotenoids in copper-sulfates could be used

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as biomarkers and useful proxies for understanding remote mineral formations as well as for terrestrial environmental investigations related to mine drainage contamination including biological activity and photo-oxidation processes.

Introduction

Carotenoids and copper sulfate mixtures are scarcely studied by spectroscopic methods because of its poorly crystalline structure, unstable hydration states, carotenoids variety, unpredictable impurities and low intensity of the Raman emission from the copper sulfate masses. One of the world's richest regions of polymetallic sulfide deposits is the Iberian Pyrite Belt located in the southwest of the Iberian Peninsula. This area includes the Rio Tinto Basin, a natural highly acid rock drainage system due to the creation of highly acidic conditions via the weathering of rocks containing base metal sulfide minerals with abundance of pyrite and chalcopyrite. This basin contains an interesting extremophile biological activity [1–3]. Studies on these acid drainage materials of Rio Tinto Basin were performed to test analytical devices also operating onto Mars surface, such as robotic drills [4] or chromatography-mass spectrometers [5]. Miniaturized versions of Raman-Photoluminescence and X-ray diffraction spectrometers are operating in the Opportunity and Curiosity rovers with new analytical facilities for determining minerals phases on basis to their molecular and structural configurations in the short and long atomic orders. Carotenoids are particularly interesting in plants and algae absorbing light energy for use in photosynthesis and protecting chlorophyll from photo-damage [6–9]. *Rhodotorula mucilaginosa* is an example of a pigmented yeast, found in a copper mine in the province of Tucuman, Argentina, supporting high concentrations of Cu(II) and providing relationships among carotenoid production, copper bioremediation and oxidative stress associated to this yeast [10]. The separation of carotenoids by high performance liquid chromatography (HPLC) could provide: (i) carotenoids containing different end-groups; (ii) stereoisomers of carotenoids; (iii) geometrical isomers of carotenoids; (iv) configurational (optical) isomers of carotenoids. The choice of the specific HPLC column for separating stereo-isomers of carotenoids is critical, whereas the geometrical isomers of beta-carotene are best separated on an HPLC lime column; geometrical isomers of several other carotenoids abundant in fruits and vegetables can be better separated on a C_{18} reversed phase column [11]. Later, the separately purified carotenoids can be identified from their UV/visible and mass spectra and by comparison of their HPLC retention times and UV/visible absorption spectra with synthetic carotenoid patterns. Carotenoid synthesis is also induced by copper in the bacterium *Myxococcus xanthus*, the blue light was the only environmental agent known to induce carotenogenesis in this bacterium, since under blue light copper activates the transcription of the structural genes for carotenoid synthesis through the transcriptional activation of the carQRS operon [12]. Recent studies pointed to β-carotene detection by Raman spectroscopy as a possible biomarker in the Martian evaporite environment. [13–15]. Carotenoids can be identified using Raman micro-spectroscopy by the characteristic Raman spectral bands centered at 1518 cm^{-1} and 1156 cm^{-1} [16–19]. Raman spectroscopy is a very sensitive technique detecting carotenoids into ionic salts since they not exhibit Raman emission. Laboratory driven Raman measurements performed on different proportions of mixtures obtained β-carotene Raman signals at the 10 mg kg^{-1} concentration level in sulfates and halide matrices, interesting results that will aid in situ analyses on Mars. [20–22]. Unfortunately, common simple natural features such as hydration, small grain size of crystals, bacterial or fungi bioturbation, iron presence, photo-oxidation and chemical disorder generate near amorphous compounds with difficult determination by both Raman and X-ray diffraction techniques. The diode-pumped solid state laser light of our Raman microscope (532 nm) produces detectable photoluminescence emission (PL) in many hydrous basic copper compounds, such as carbonates, sulfates, phosphates, silicates and chlorides. Frequent publications focus on β-carotene Raman-PL spectroscopy in solution [23] but rarely on natural sediments with solid carotenoid. Solid carotenoids in copper ores collected in nature seem scarcely studied directly by luminescence techniques. Here we analyze organic–inorganic complexes naturally formed into an open mining environment of copper brines exposed to the environmental weathering of the Rio Tinto Basin including biological activity and sequential humidity-desiccation cycles. Accordingly, the collected Cu-carotenoid samples were analyzed by Environmental Scanning Electron Microscopy with energy dispersive spectrometry probe (ESEM-EDS), X-ray diffraction (XRD), X-ray fluorescence spectrometry (XRF), Raman-Photoluminescence spectrometry (RPL), Differential Thermal and Thermo-gravimetric Analyses (DTA–TG), Thermoluminescence (TL) and Cathodoluminescence (CL).

Site, samples and methods

In a field trip around the Iberian Pyrite Belt, on April 25, 2013, we observed on the surroundings of the abandoned La Poderosa mine, north of El Campillo (Huelva, Spain) a small, natural drainage stream with a light blue color and pH 6.4 (Fig. 1a). The stream which extended for only few tens of meters was not recent, as deducted from the mineral precipitation on the shores and in the end of the stream (Fig. 1a). Near the upwelling point formed blooms of a living alga identified as *Dictyosphaerium* sp. At some places, a tiny light blue mineral crust was observed on the water surface and, a dense green algal biofilm that enclosed numerous bubbles was evident upon removal of the crust. These bubbles were also previously described for cyanobacterial mats [24]. One month later, the stream was almost dry, but still retained its blue color, with very restricted green biofilm patches and extensive mixing of sediments and lysed algae (Fig. 1b). At this time the pH of the water/sediments was 7.5 and we collect dry samples of blue color to be analyzed. The taxonomical identification of the alga was supported by laboratory cultures and microscopic studies. The alga was characterized by oval cells surrounded by a mucilaginous envelope [25–27] (Fig. 1c and d). Species of the genus *Dictyosphaerium* grow in freshwaters where they commonly participate in the formation of green algal blooms. It has been reported that species of this genus are resistant to acidic waters and metal-rich waters [26]. In addition, the biofilm was formed by other microorganisms, which were also isolated and identified as *Hyaloraphidium curvatum* and *Stilbella fimetaria*. *H. curvatum* is a rare representative of freshwater nanoplankton, which was traditionally classified as a colorless green alga but now recognized as a lower fungus [27]. *S. fimetaria* has been recorded as endophytic fungus from marine algae [28] and it was also found in saline and acidic soils from a Czechian natural reserve [29]. Fungal DNA extraction, PCR amplification of DNA, clone libraries, sequencing