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# Traces of life in Río Tinto (Spain): Decoupling of morphological and geochemical biosignatures

Yu Pei<sup>‡</sup>, Philip Werner<sup>‡</sup>, Ricardo Amils<sup>§</sup>, Eric Runge<sup>I</sup>, Andreas Kappler<sup>‡</sup>, Jan-Peter Duda<sup>I</sup>, Muammar Mansor<sup>‡</sup>

‡ University of Tuebingen, Tuebingen, Germany

§ Universidad Autónoma de Madrid, Madrid, Spain

| University of Göttingen, Göttingen, Germany

Corresponding author: Muammar Mansor (muammar.muammar-bin-mansor@uni-tuebingen.de)

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#### Abstract

The Río Tinto system located in southern Spain is a 100-km long acidic (pH  $\approx$  2.3) river rich in dissolved iron, sulfate and heavy metals. The red-tinted river, formed as a product of natural acid rock drainage that has been exacerbated over the years by anthropogenic mining activities, hosts a variety of extremophilic microorganisms that are potential analogues to those found on early Earth and ancient Mars. Here we detailed the investigation of potential biosignatures in the forms of microbialites and sediments deposited along the river. The 20-50 cm thick microbialites (estimated age at most 100 years) are composed of layered structures of Fe(III) minerals with alternating porosities. High resolution imaging and elemental mapping coupled to mineralogical analysis suggest the transformation of K-jarosite (KFe<sub>3</sub>(SO<sub>4</sub>)<sub>2</sub>(OH)<sub>6</sub>; V<sub>m</sub> = 153 cm<sup>3</sup>/mol) to goethite ( $\alpha$  -FeOOH;  $V_m = 21 \text{ cm}^3/\text{mol}$ ), generating porosities in the process due to a reduction in the molar volume Vm. Meanwhile, riverbed sediments are composed of mixtures of schwertmannite (Fe <sub>8</sub> O<sub>8</sub>(OH)<sub>6</sub>(SO<sub>4</sub>) · nH<sub>2</sub>O), jarosite, goethite and other minor minerals (ferrihydrite, lepidocrocite, hematite). Culture experiments indicate that crystalline jarosite is formed via transformation from poorly-crystalline schwertmannite (Fe 8 O<sub>8</sub>(OH)<sub>6</sub>(SO<sub>4</sub>) · nH<sub>2</sub>O), but that the presence of Acidithiobacillus thiooxidans actually inhibits this transformation, possibly due to organic matter stabilization of schwertmannite. Fe isotopic analyses show no clear variations between layers in the microbialites ( $\delta^{56}$ Fe = -0.85 ±

0.10‰), while the riverbed sediments indicate progressively more negative values downstream over a 5-km distance ( $\delta^{56}$ Fe = +0.4 to -0.8‰). The spatial isotopic pattern of the riverbed sediments is consistent with rapid microbial oxidation of Fe<sup>2+</sup> and inconsistent with the much slower abiotic Fe<sup>2+</sup> oxidation rate. Taken together, our results show that while the microbialites' structure is suggestive of microbially-induced precipitation, its geochemical features are not. Meanwhile, the inconspicuous-looking riverbed sediments provide clear evidence of biogenicity from their isotopic compositions. This highlights the challenge in detecting life especially when decoupling of morphological and geochemical biosignatures is to be expected.

### Keywords

Río Tinto, microbialites, Fe isotopes, spatial biosignature, acid rock drainage, early Earth, Mars analogue

## Presenting author

Muammar Mansor

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# **Conflicts of interest**

The authors have declared that no competing interests exist.