

Organics Detection in Acid Mine Drainage Sediments, with Implications for Organics Preservation in Iron-Rich Acid and Saline Environments on Mars. Chance Sturup¹, M. Rogers², and A. J. Williams¹, ¹Department of Geological Sciences, University of Florida (sturupc@ufl.edu), ²Environmental Science and Studies Program, Towson University

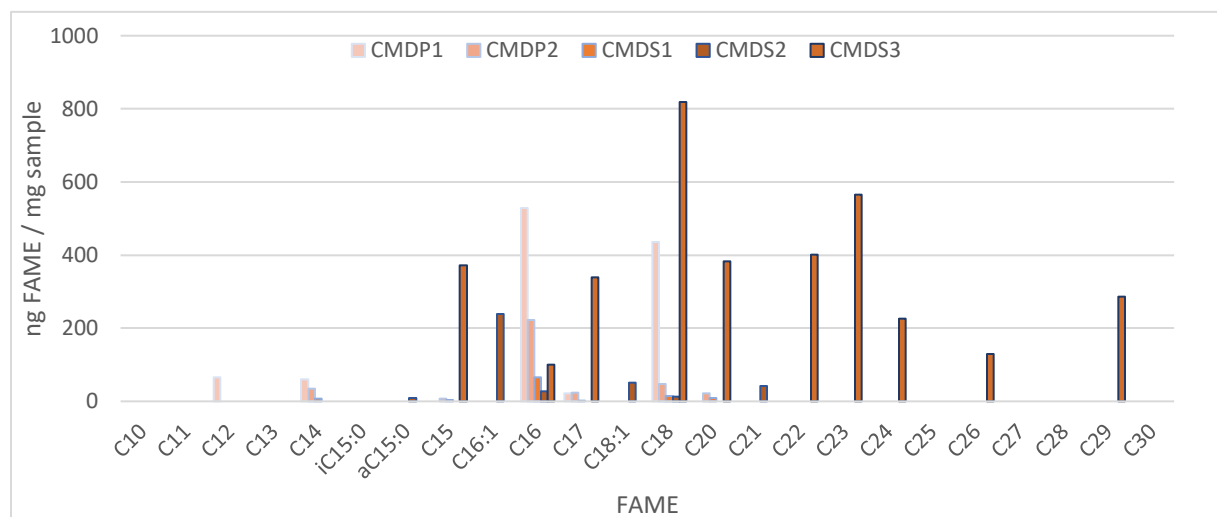


Figure 1. FAME in ng/ mg of sample from the five Centralia sample sites analyzed with TMAH. Increasingly darker legend colors darkness indicates increased distance away from primary AMD source.

Introduction: Discovering whether life exists on Mars, or did so in the past, is one of the most gripping questions of modern astrobiology. The sheer distance between Earth and Mars makes direct exploration for signs of life difficult, a hurdle which the upcoming NASA *Perseverance* and ESA Rosalind Franklin rovers aim to clear. Both missions will explore Mars for evidence of past or present microbial life [1][2]. Despite its seemingly alien nature, the Martian surface has several analogous environments on Earth, one of which is iron-rich acid mine drainage (AMD). AMD sites tend to carry high concentrations of heavy metals and precipitate sulfate-bearing ferric flocculent sediment. The mineralogy of these sediments are similar to some iron-rich environments on Mars such as the Burns Formation [3].

AMD conditions are inhospitable to many forms of life though some extremophiles have evolved to thrive in these environments. By exploring the preservation of organic matter from these extremophiles in terrestrial AMD, it is possible to extrapolate the degree of organic preservation possible for select acid-saline environments on Mars.

AMD samples for this project were collected from Centralia, Pennsylvania. Centralia hosts a coal mine system abandoned after the mine caught fire in the 1960s. The Centralia Mine Drainage Tunnel, originally drilled to lower the water table and facilitate coal extraction, currently drains 3.3 million gallons of pH 3.7 AMD daily into the surrounding streams. The

severe acidity of the AMD drastically alters the pH of the downstream Mahanoy Creek, causing it to fluctuate down to a pH of 4.0. The Centralia site also features an increased concentration of dissolved trace metals, most notably sulfate, aluminum, and iron. The mineralogy of the flocculent that precipitates from this system is primarily composed of hydrated iron minerals such as schwertmannite, goethite, and ferrihydrite. A similar mineralogy is present on Mars within rocks of the Burns Formation at Meridiani Planum, notably the high presence of oxidized iron-sulfur minerals such as those found in Centralia.

The similar conditions of these environments is used as a proxy to explore organic molecule preservation in acidic iron-rich mineralogies, with the assumption that organics in the rock record would decay similarly between the two locales. Iron oxides are generally assumed to be detrimental to organics preservation [4] but recent research indicates that select microenvironments in iron oxides may facilitate organics preservation on shorter geologic timescales [5][6][7]. The preservation of fatty acids (FA) and alkanes are particularly important due to their role in microbial membranes. FAs can be synthesized biotically and abiotically and are expected to be ubiquitous with extraterrestrial life given the need of cells to compartmentalize themselves. As such determining how FAs and alkanes are preserved in AMD environments on Earth is paramount to

understanding their preservation on Mars and what the current and future rover missions can uncover.

Methods: AMD flocculent sediment samples were collected using organically clean techniques [8] from the Centralia, Girardville, and Packer five mine drainage sites, and stored refrigerated in the lab. Samples were dried out in an oven over a 24-hour period to remove excess moisture and subsequently homogenized into a fine powder using a solvent washed and ashed (at 500°C for 8 hours) mortar and pestle.

GC-MS of samples. A solvent-washed spatula was used to load 3-5mg of ground sample into a cup. Samples were analyzed on an Agilent GC-MS coupled to a Frontier pyrolyzer. Samples analyzed for FA were subject to TMAH (tetramethylammonium hydroxide) thermochemolysis at a ratio of 1μL TMAH to 1mg sample to convert FA to FAMES (fatty acid methyl esters). C₁₉ was used as the internal standard. Samples were pyrolyzed at 600°C for 0.5 min. The oven program ramped from 50°C to 300°C at 20°C/min with a 10-minute hold. Samples analyzed for alkanes were subjected to flash pyrolysis at 600°C and ramped pyrolysis at 35°C/min. Samples run in these methods had no additional reagents added. Molecules were identified using ChemStation software and known retention times from the Supelco FAME standard.

Results: In each sample analyzed from the Centralia AMD FAMES were well-preserved. C₁₆ is the most abundant FAME in most of the samples, though its concentration is significantly lower in CMD53. This specific data point is located further away from the AMD source (Fig.1) and has overall higher preservation of FAMES. Flash pyrolysis analyses did not yield any identifiable alkanes; however, preliminary analysis of ramped pyrolysis data revealed alkanes ranging from C₈-C₁₇ in the Packer-5 samples.

Discussion: The use of TMAH thermochemolysis GC-MS on Earth analog samples ties directly into the instrumental capability of current Mars rovers. Both the Sample Analysis at Mars (SAM) onboard the Curiosity rover and the Mars Organic Molecule Analyzer (MOMA) onboard the ExoMars mission are capable of performing such experiments. Studying how organics are preserved in analog samples from mineralogically similar, low-biomass, terrestrial AMD environments can provide powerful insight into how these biomarkers could be preserved on Mars. Results of these analyses can help inform sampling strategies for SAM, MOMA, and other future rovers. Analysis of terrestrial AMD sediments revealed that FAMES were well preserved and easily detectable via TMAH thermochemolysis. It would therefore be able to liberate fatty acids from Terran-similar modern

microbial communities existing on Mars, if present, using the aforementioned instruments on Martian rovers. However, FAMES readily degrade over spans of geologic time, so submodern microbial communities would be much more difficult to detect using this technique. Flash pyrolysis also does not appear to be able to detect alkanes with the same efficiency as FAMES, though future analyses will aim to fully validate this difference.

Conclusions: TMAH thermochemolysis using GC-MS is a proven effective method of identifying the presence of microbial communities in AMD sites. Despite the iron-rich nature and low pH of these environments, our investigation shows that FAMES are preserved in these environments and detectable with SAM-like methods. The success of this technique on Earth suggests an application for analogous iron-rich sites on Mars, e.g. within the Burns Formation. Current Martian rovers possess the ability to conduct similar experiments directly on Martian soils and could be used to detect FAMES preserved in the rock record from recent or current microbial communities.

References:

- [1] Mustard J. F., et al. (2013), [2] Vago J. L., Westall F., et al. (2017) *Astrobiology* **17**, 471–510. [3] Squyres S. W. (2004) *Science* (80-.). **306**, 1709–1714. [4] Sumner D. Y. (2004) *J. Geophys. Res. E Planets* 109, 1-8. [5] Parenteau M. N., et al. (2014) *Astrobiology* **14**, 502–521.
- [6] Tan J., Lewis J. M. T. and Sephton M. A. (2018) *Nat. Sci. Reports* **8**, 7586. [7] Williams A. J., et al. (2019). *Astrobiology* **19**, 522–546. [8] Wilhelm M. B., et al. (2017) *Org. Geochem.* 103, 97–104.