Organics Detection in Acid Mine Drainage Sediments, with Implications for Organics Preservation in Iron-Rich Acid and Saline Environments on Mars.

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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 Mars 2020 and ESA ExoMars 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 (Fig. 1). The mineralogy of these sediments is 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 (Fig 2). 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) is particularly important due to their role in microbial membranes. As such determining how FAs 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.

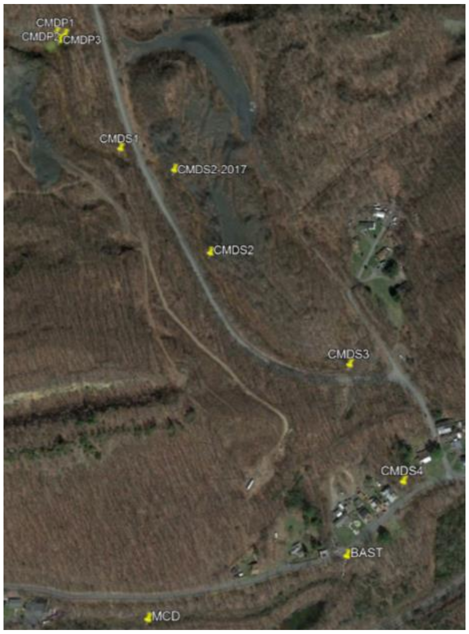

Figure 1. Map of the Centralia AMD site with the location of each sample marked for distance from the primary source.

Methods: AMD flocculent sediment samples were collected using organically clean techniques (e.g. [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). C_{19} 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. 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. FAMEs from C_4 to C_{30} were detected, including monounsaturated fatty acids (Fig. 3). C_{16} and C_{18} are the dominant FAMEs due to the abundance of these lengths in both bacterial and eukaryotic cellular membranes. FAMEs longer than C_{18} are likely wax esters derived from higher plants. The even-over-odd chain length preference in FAME distribution is characteristic of a modern microbial community within the AMD flocculent. FAME abundances were highest in the CMDP1 (Centralia AMD pool) and CMDS3 (Centralia stream site 3) samples. The elevated wax ester concentrations in the CMDS3 site is generated by abundant deciduous plant matter that was entrained in the AMD flocculent. The wax esters in the other samples derived from the same plant matter source.

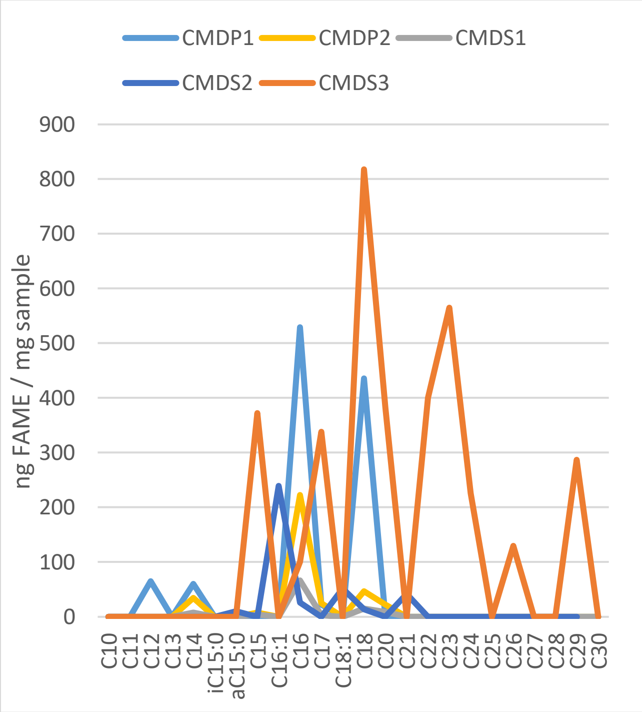

Figure 2. Plot of FAME concentrations of Centralia samples

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. As such the results obtained looking at Earth-based AMD can reasonably be extrapolated to apply to Martian soils. Analysis of terrestrial AMD sediments revealed that FAMEs were well preserved and easily detectable via TMAH thermochemolysis. It would therefore be capable of detecting any similar microbial communities existing on Mars using the aforementioned instruments on Martian rovers. However, it is important to note that current data is limited to recent microbial communities as it is still unclear how readily FAME molecules breakdown over spans of geologic time. This limits TMAH thermochemolysis to only being able to determine the presence of active or freshly preserved microbial communities.

Conclusions: TMAH thermochemolysis using GC-MS instruments is a proven effective method of identifying the presence of microbial communities in AMD sites. Despite the iron-rich and low pH of these environments FAMEs are able to be preserved and detected using this method. The success of this technique on Earth suggests an application for analogous iron-rich sites on Mars, namely those 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 past or current microbial communities.

Ongoing and Future Work: Samples from Girardville and Packer Five will be analyzed in contrast to those from Centralia. The samples from Girardville and Packer Five are net alkaline and a comparison to Centralia will elaborate on the effect extreme acidic conditions have on FAME preservation and detection.

References: [1] Mustard J. F., et al. (2013) *Report of the Mars 2020 Science Definition Team by the Mars Exploration Program Analysis Group (MEPAG)*., [2] Vago J. L., Westall F., et al. (2017) Habitability on Early Mars and the Search for Biosignatures with the ExoMars Rover. *Astrobiology* **17**, 471–510. [3] Squyres S. W. (2004) In Situ Evidence for an Ancient Aqueous Environment at Meridiani Planum, Mars. *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*)* Production and Early Preservation of Lipid Biomarkers in Iron Hot Springs. *Astrobiology* **14**, 502–521. [6] Tan J., Lewis J. M. T. and Sephton M. A. (2018) The Fate of Lipid Biosignatures in a Mars-Analogue Sulfur Stream. *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.