

LIGHT, TEMPERATURE, AND NUTRIENT AVAILABILITY INFLUENCE MICROBIAL COLONIZATION OF LAVA CAVES

C. M. Phillips-Lander¹, Kayla Miller¹, Elisabeth M. Hausrath², Amanda M. Stockton³, Nina McCollum¹, and M.E. Elwood Madden¹ ¹School of Geology and Geophysics, University of Oklahoma, Norman, OK, USA, (charity.m.lander@ou.edu) ²Department of Geoscience, University of Nevada-Las Vegas, Las Vegas, NV, USA ³School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA, USA

Introduction: Lava tubes have been observed on Earth and in Hesperian to Amazonian terrains on Mars [1-5]. These lava caves have been identified as “special regions” [6,7] which may serve as past or current refugia on Mars because (a) they act as preferential conduits for water flow, even under semi-arid to arid conditions [8], (b) their insulating properties may maintain stable, habitable, environmental conditions over extended periods, which may be significantly different than surficial conditions [9], and (c) they provide UV protection, which would enhance the preservation of organic biosignatures [10]. Therefore, lava cave systems may preferentially preserve biosignatures indicative of past life on Mars.

Internal cave climates are controlled by the connection of the lava cave with the surface [9], therefore, a variety of lava cave climates, including ice caves, may have been present historically and currently in Mars’s subsurface. This internal climate variability (liquid water+rock or ice+rock conditions) is expected to produce distinct biosignatures in cave minerals on Mars [10]. Because cave climate conditions likely influence biosignature formation and expression, L  veill   and Datta [8] noted “a systematic study of basaltic caves...in different climates is required in order to elucidate the geomicrobiology and geochemistry of basaltic caves and cave mineral deposits. Specifically, a suite of robust ...geochemical and mineral biosignatures in basaltic cave deposits needs to be developed.”

This study documents and directly compares chemical and biological weathering and biosignature formation in 4 spatially co-located lava tubes of Craters of the Moon National Monument, Idaho (CROM) (43.2058   N, 113.5002   W). These caves, located within a 1.6 km area within the Grassy Flow (7,360  60 yr) [11] represent warm/dry (Screaming Jaws of Death Cave), warm/wet (Pond Cave), cold/wet (Ice Lake Cave) and cold/damp (Spongy Floor Cave) conditions respectively. Results from this study will define a range of weathering, mineralogical, and trace element signatures that may be encountered during cave exploration on Mars.

Methods: We utilized coupled field and laboratory methods to determine the degree of chemical and microbially induced weathering in different lava cave environments present at CROM.

Geochemistry Tiny Tag TGP-4500 temperature and humidity sensors were deployed within the caves. Data from these sensors were compared to Western

Regional Climate Center weatherstation at CROM to determine how internal cave climate was influenced by external temperature conditions.

Temperature and pH were measured in the field. We filtered water samples in the field using 0.2   m polystyrene syringe filters. Samples for cation analysis were acidified with trace metal grade nitric acid. We allowed frozen samples to melt before filtration. Upon collection and filtration, we placed all samples on ice in a cooler, where they remained at or below 4  C during transport to the laboratory. Major and trace elements were analyzed using inductively coupled plasma mass spectrometer at Oklahoma State University. Filtered, unacidified samples for anion analysis were analyzed using a Microdrill Ion Chromatograph at KU. Alkalinity was determined in the laboratory using filtered unacidified samples.

Microbial Colonization and Weathering We deployed field microcosm experiments [12-16] in order to differentiate in situ chemical and microbial weathering rates. Holes were drilled into HDPE boxes in order to allow for the passage of water into and out of the microcosm. Filters (0.45   m) were attached to the interiors of one half of microcosms to prevent microbial incursion into the experimental apparatus, while allowing for the passage of water. The other half of the microcosms had no filters, in order to allow microbial colonization and weathering. Each microcosm was loaded with a 2.5 cm² McKinney Basalt billet. Each billet and microcosm had been previously sterilized using UV and 70% ethanol and were assembled in a sterile, UV hood. One of each microcosm (filtered control chemical weathering and unfiltered microbial weathering) were deployed for 6 months to determine initial microbial colonization and weathering. Upon retrieval from the caves, basalt billets were immediately placed in sterile, wide-mouth HDPE bottles with 2.5% glutaraldehyde solution in order to fix samples and preserve fine-scale microbe-mineral interactions [17]. Billets were ethanol dehydrated and critically point dried prior to analysis with scanning electron microscopy (SEM).

We analyzed sample billets from microcosm experiments to determine the potential microbial influence on secondary weathering products. We determined primary and secondary mineralogy using a Rigaku Ultima IV X-ray diffractometer with MDI Jade(2010) and a Renishaw InVia Raman microscope with 532nm laser. Microbial associations with mineral

surfaces were determined using a Zeiss NEON SEM with energy dispersive X-ray spectroscopy [14,16].

Results and Discussion: Temperature Surface and internal cave temperatures co-vary in caves where the connection to the surface is large and the cave is not deep. CROM daily high temperatures varied from 7.6 to 33.9°C between May and September 2016. SJD increased from 4 to 17°C and Pond Cave increased from 0 to 5.6°C. However, caves which had narrow, deep (~4.5 m) entrances maintained persistent 0°C temperatures (i.e Ice Lake and Spongy Floor). Therefore, there is potential to create computer modeling of cave openings [e.g. 9] to predict internal cave climate and habitability potential.

Aqueous Geochemistry indicates water in the four caves derive from a similar source, as major anions and cations are similar between the Spongy Floor, Ice Lake, and Pond. Phosphate limited conditions occur in Spongy Floor (4.8 μM), Pond (4.4 μM), and Ice Lake (2.44×10^{-2} μM) caves. No water sample was collected from Screaming Jaws of Death (SJD) due to low flow rates (damp wall conditions were observed).

Mineralogy Secondary clay minerals, iron oxides, and carbonates are present in Pond, Spongy Floor, and Ice Lake Cave. Amorphous silica, calcite, and Fe-oxides occur as alteration phases in SJD. Given the similarity in geochemistry, differences in secondary mineralogy may be related to microbial processes. Fe-oxides and amorphous silica are associated with microbial surfaces in SJD.

Microbial Colonization Microbial population size is greatest in the twilight zone of Screaming Jaws of Death where photosynthetic microorganisms are present and decreases in the dark zone. Decreasing temperatures and phosphate availability correlate with decreased microbial population sizes between Pond and Ice Lake caves. In Ice Lake cave, microorganisms occur as individuals attached to basalt surfaces by large networks of nanowires (Figure 1).

Implications for lava caves on Mars: A variety of different habitable environments may occur in spatially co-located lava caves. Differences in light availability, temperature, and nutrient availability influence microbial population size and community

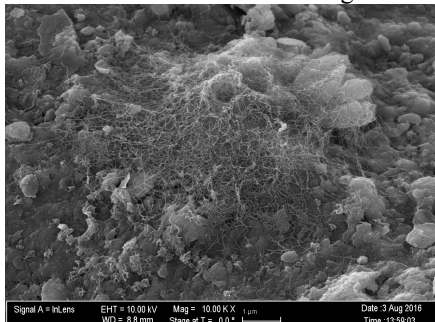


Figure 1: Ice Lake Cave. Nanowires attached to rock surface. Depressions are where cocci were detached during sample preparation.

structure, potentially leading to differences in biosignature formation.

Future mission site selection could be based on cave entrance geometry and inferred differences in light availability and temperature. Both light availability and temperature may be modeled [9], however, UV-radiation may degrade organic biosignatures making biosignature detection in these caves challenging. Moreover, microbial populations in shallow lava caves with larger openings may be more vulnerable to post-Noachian climatic changes that occurred on Mars.

While microbial population decreases with decreasing nutrient availability, previous studies have demonstrated nutrient limitation drives microbial weathering in basalts [18,19]. Microbially-induced dissolution may mobilize trace elements, which may act as a potential biosignature and enhanced organic preservation may occur in these deeper cave environments. These deeper caves, while more challenging to detect from satellite imaging, may preserve stable habitable environments over long time periods, even as the external climate changed.

Potential future cave missions should consider designs that maximize the potential to explore several co-located caves, as temperature and habitability conditions vary significantly between caves located in close proximity to one another. This plan would maximize potential biosignature detection and therefore science return.

References: [1] Hodges and Moore (1994) *USGS Prof. Paper 1534*. [2] Wyrick et al. (2004) *JGR E: Planets* 109. [3] Cushing et al. (2007) *Geophys. Res. Lett.* 34. [4] Keszthelyi et al. (2007) 7th Intl. Conf. on Mars, Abstract #3314. [5] Leone (2014) *J. of Volcano. & Geothermal Res.*, 277, 1-8. [6] Rettberg et al. (2016) *Astrobiol.*, 16, 119-125. [7] Rummel et al. (2014) *Astrobiol.*, 14, 887-968. [8] Léveillé and Data (2010) *Planet. and Space Sci.*, 58, 592-598. [9] Williams et al. (2010) *Icarus*, 209, 358-368. [10] Boston et al. (2001) *Astrobiol.*, 1, 25-55. [11] Kuntz et al. (2007) *USGS Sci. Invest. Map 2969*. [12] Rogers and Bennett (2004) *Chem. Geo.*, 203, 91-108. [13] Engel et al. (2004) *Geology*, 32, 369-372. [14] Bennett et al. (2006) *Geomicro J.* 18, 3-19. [15] Kandianis et al. (2008) *GSA Bull.*, 120, 442-450. [16] Phillips-Lander et al. (2014) *Geomicro. J.* 31, 23-41. [17] Gorby et al. (2006) *PNAS*, 103, 11358-11363. [18] Hausrath et al. (2011) *Chem. Geo.*, 290, 89-100. [19] Hausrath et al. (2009) *Am. J. of Sci.*, 309, 633-660.

Acknowledgements: Funding for this project was provided by a NAI/APS Lewis and Clark Astrobiology Field Scholarship. Laboratory assistance was provided by Preston Larson of the Sam Noble Microscopy Laboratory at OU.