THE ROLE OF PHOSPHOROUS IN MICROBIAL COLONIZATION OF LAVA TUBE CAVES  
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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.

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. Fresh, sterilized, high (0.5-0.75 wt.% P) and low (0.2 wt.% P) glasses were incubated in each cave for 6 months. 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 microbiologically 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 µm 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 We deployed field microcosm experiments, a modification of the buried slide technique [12-16] to determine the role of mineral-bound P in microbial colonization. We incubated 2.5 cm² billets of McKinney Basalt (0.5-0.75 wt% P) and Hawaii basalt (0.2 wt% P). Each billet was sterilized using UV and 70% ethanol in a sterile, UV hood. One of each type of billet was deployed in for 6 months in each cave 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 with SEM to determine the potential microbial influence on colonization, weathering, and secondary products.

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. Caves which had narrow, deep (~4.5 m) entrances maintained persistent 0°C temperatures (i.e. Ice Lake and Spongy Floor).

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 (2.44x10⁻² µM) caves. No water sample was collected from Screaming Jaws of Death (SJD) due to low flow rates (damp wall conditions were observed).

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).
Mars Caves Mission Selection Criteria:

Cave Structure as a Habitability Selection Criteria
Because internal climate and water availability are influenced by lava tube depth and structure, potential future lava tube cave missions should consider designs that maximize the potential to explore several co-located caves. This would increase our ability to assess the variation in habitability and increase our understanding of the subsurface geology of Mars.

Phosphate as Astrobiological Selection Criteria
To reduce risk associated with astrobiologically focused exploration of the martian subsurface, we must develop methods to assess the habitability potential of the subsurface. While significant effort has been devoted to engineering selection criteria [18], significantly less work has been done to assess the geology of the Tharsis region to determine its habitability potential. Surficial analysis of different flow units associated with cave features could constrain the P content of lava flows in this region, which would provide insight into the best lava tube cave sites to examine for signs of present/past life.


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.