**Introduction:** One of the main astrobiological goals of the Mars exploration program is to fully understand the extent of habitable environments on the red planet, which includes determining all the potential energy sources that could support microbial ecosystems. Yet, there has been sparse analog study of one of the most abundant and energetic metabolic martian resource: perchlorate. This is because, until recently, there has been no documented evidence of naturally occurring perchlorate (NOP) and perchlorate-reducing microorganisms (PRMs) co-existing in a relevant Mars analog environment. Recent work has now demonstrated the coexistence of PRMs with (NOP) in the Pilot Valley Basin Mars analog environment and more detailed study is underway to understand the characteristics of microbial (per)chlorate reduction in this relevant Mars analog environment as a guide to elucidating the possible influence of chlorine oxyanions on habitability and life on the red planet [1].

Perchlorate was first discovered on Mars during the 2008 Phoenix lander mission [2] and was subsequently inferred to have been present in soils examined by both Viking landers in 1976 [3]. Recent analyses by the Sample Analysis at Mars (SAM) instrument onboard the Curiosity rover provided the most detailed information about perchlorate presence on Mars. The SAM analysis detected 0.05-1 wt% perchlorate in the samples drilled in Gale crater. The detected perchlorate likely exists as salts physically mixed with minerals and/or adsorbed on mineral surfaces. Beside perchlorate, chloride and chlorate are also present in Gale crater[4]. Laboratory analysis of analog samples run under SAM-like conditions indicated that chlorate salts mixed with Fe(III) oxides and phyllosilicates can exist in Gale crater [5, 6]. The presence of chlorides in Gale crater has been inferred from an observation that chlorine content estimated from the SAM measurements of perchlorate and/or chlorate is always lower than the total chlorine measured by the Alpha Particle X-ray Spectrometer [7]. Collectively, these observations revealed that chlorine in all three oxidation states is present in Gale crater. Further, (per)chlorates have also been identified in both chondritic and Martian meteorites as well as lunar samples, thereby indicating the potential ubiquity of (per)chlorate salts in the solar system [8, 9].

The presence of oxychlorine and chloride species can therefore indicate that a complex chlorine biogeochemical cycle was once active on Mars. In particular, (per)chlorate may have helped support life during the planet’s wet past as a terminal electron acceptor, and possibly could still support life in the shallow subsurface (1m to 10m).

To assess the possibility of perchlorate-supported ecosystems on Mars, it is vital to understand these systems on Earth. Hence, an interdisciplinary study is underway to explore whether and how a (per)chlorate-based microbial chlorine cycle is driven in the basin sediments of Pilot Valley and could serve as a biogeochemical model for Mars.

**Geochemical Modeling:** The goal of the geochemical modeling portion of the study was to investigate the energetic potential for active biological perchlorate reduction in the Pilot Valley Mars analog and also under Mars environmental conditions using the PHREEQC geochemical modeling software package.

Pilot Valley is a paleolake basin analogous to those found on Mars in terms of aqueous mineral composition, and has special astrobiological relevance because like Mars, it contains naturally occurring perchlorate salts. PV is also home to the first reported instance of perchlorate reducing microbes (PRM) co-occurring with natural sources of perchlorate. Results of modelling in Pilot Valley indicate high favorability for active perchlorate reduction using multiple electron donors (Figure 1).

![Figure 1: The estimated real-world favorability of microbial respiratory pathways under simulated geochemical conditions of Pilot Valley, Utah using native electron donors.](image)

**References:**
[1] Lynch, K.L., Simpson, A., Machineni, S., Santiago-Vazquez, L., Goodale, J., Lopez, J. 1Lunar and Planetary Institute (USRA), Houston, TX 77058, 2Jet Propulsion Laboratory, Pasadena, CA 91109, 3Department of Biology & Biotechnology, University of Houston Clear Lake, Houston TX, 77058, 4The Evergreen State College, Olympia, WA 98505
For a thermodynamic analysis of biological perchlorate reduction feasibility on Mars, Gale Crater was chosen as a study environment for a potentially habitable subsurface aquifer. The Mars curiosity Rover has traversed and studied 400 m of elevation with stratigraphically distinct rock assemblages that have been characterized using a variety of instruments, making Gale Crater the best-studied depositional environment on Mars. There is strong evidence for perchlorate enrichments in Gale Crater sediments that indicate there could have also been dissolved perchlorate salts in subsurface fluids, much like Pilot Valley brines.

The preliminary work presented here focuses on estimating the thermodynamic favorability of known biological perchlorate reduction pathways within a simulated Gale Crater groundwater environment. Figures 2 & 3 shows initial results of thermodynamic favorability with three different electron donors for perchlorate reduction at the Cumberland sample site in Gale Crater.

**Microbial Ecology:** Our 2019 published experimental results show that perchlorate reducing bacteria co-exist with the naturally occurring perchlorate (NOP) present in the basin. Our results also provide strong evidence of active microbial reduction of the NOP throughout the basin. To understand what pathways are used for microbial (per)chlorate reduction in Pilot Valley, and which microorganisms are the dominant users of (per)chlorates, sediments have been cultured and isolated with (per)chlorates as the sole terminal electron acceptor for use in more detailed molecular and genomic studies which will be discussed further in our presentation.

![Figure 4: In Situ perchlorate reduction field experiment.](image)

**Acknowledgments:** This research has been partially supported by LPI Summer Intern Program, University of Houston Clear Lake, and USRA.