Aqueous extract of a Mars analogue regolith that mimics the Phoenix landing site does not inhibit spore germination or growth of model spacecraft contaminants *Bacillus subtilis* 168 and *B. pumilus* SAFR-032

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Because Mars is a primary target for life detection and habitability assessment missions, its exploration is also by necessity a Planetary Protection issue. The recent finding of significant levels of perchlorate ($\text{ClO}_4^-$) in regolith sampled from the Phoenix landing site raises the question of its potential biotoxicity to: (i) putative indigenous martian life, (ii) microbial forward contaminants from Earth, or (iii) future human visitors. Perchlorate is toxic to the human thyroid in the ppm range, but is much more benign to microbes. At least 40 distinct species of terrestrial bacteria have been identified that can reduce perchlorate sequentially via chlorate and chlorite to chloride, and such bacteria have been considered as good candidates for bioremediation of perchlorate-contaminated sites. It has even been suggested that microbes in martian regolith environments might actually take advantage of perchlorate and reduced iron as a redox couple from which energy could be derived. Thus it is possible that areas such as the Phoenix landing site contain two important prerequisites for life: liquid water and an energy source.

To address this issue, an analogue regolith was constructed based on regolith chemistry data from the Phoenix landing site. A Mars Aqueous Regolith Extract (MARE) was prepared from the Phoenix analogue regolith and analyzed by ion chromatography. The MARE contained (mg/L) the cations Na$^+$ (1411 ± 181), Mg$^{2+}$ (1051 ± 160), Ca$^{2+}$ (832 ± 125), and K$^+$ (261 ± 29), and the anions SO$_4^{2-}$ (5911 ± 993), ClO$_4^-$ (5316 ± 1767), Cl$^-$ (171 ± 25) and F$^-$ (2.0 ± 0.4). Nitrogen-containing species NO$_3^-$ (773 ± 113) and NO$_2^-$ (6.9 ± 2.3) were also present as a result of regolith preparation procedures, but their relevance to Mars is at present unknown. The MARE was tested for potential toxic effects on two model spacecraft contaminants, the spore-forming bacteria *Bacillus subtilis* strain 168 and *Bacillus pumilus* strain SAFR-032. In *B. subtilis*, spore germination and initial vegetative growth (up to ~5 h) was not inhibited in a rich complex medium prepared with the MARE, but growth after 5 h was significantly suppressed in medium prepared using the MARE. Both *B. subtilis* and *B. pumilus* exhibited significantly higher rates of spore germination and growth in the MARE vs. distilled water with no additions (likely due to endogenous spore nutrients), but germination and growth was further stimulated by addition of glucose and a combination of buffered inorganic salts (K$_2$HPO$_4$, KH$_2$PO$_4$, (NH$_4$)$_2$SO$_4$, and MgSO$_4$). The data indicate that the aqueous environment in the regolith from the Phoenix landing site containing high levels of perchlorate does not pose a significant barrier to growth of putative forward contaminants such as *B. subtilis* and *B. pumilus* under Earth laboratory conditions. For details, see references [1, 2].

References:

Thanks to Krystal Kerney and Lashelle McCoy for valuable technical assistance. Funded in part by grants from the NASA Planetary Protection office (NNZ08AQ81A and NNH07ZDA001N).