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Influence of (per)chlorate salts and regolith depth on the survival of microorganisms in simulated Mars-like conditions

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Liquid water, an essential compound for life on Earth, is not stable on the Martian surface, but the presence of hygroscopic salts like chlorates and perchlorates in the regolith could enable the formation of, at least temporarily, stable liquid brines[1]. In the near-subsurface, a thin regolith layer could prevent water sublimation, thus extending the lifetime of the brine [2]. Regolith layers can also shield microbes from harmful UV radiation, creating possibly favorable habitats for potential microbial life in the shallow subsurface. Our study aimed to understand how microbial survival in Mars-like conditions is influenced by (per)chlorate salts, water scarcity, UV irradiation, and regolith thickness. To achieve this, we conducted simulations using the Mars Environmental Simulation Chamber (MESCH) described by Jensen et al. (2008) [3]. The model organisms used in our experiments were *Debaryomyces hansenii* (a halotolerant yeast), *Planococcus halocryophilus* (a halotolerant and psychrophilic bacterium), and *Aspergillus niger* (a fungal spore former). Vegetative cells of *D. hansenii* and *P. halocryophilus*, as well as spores of *A. niger*, were suspended in their respective growth mediums with or without sodium (per)chlorate. Subsequently, 3 grams of the cell culture or spore solution, containing either 0.5 mol/kg $NaClO4$, 0.5 mol/kg $NaClO3$, or no additional salt, were inoculated into 30 grams of MGS-1 Mars Global Simulant and then placed in glass tubes with a depth of 15 cm. These glass tubes were transferred to the MESCH and exposed to simulated Mars-like conditions (constant temperatures of about -10 ℃,6 mbar CO2 atmosphere, and 2 hours of daily UV irradiation at 200-250 nm). Before and after exposure periods of three and seven days, the number of colony forming units (CFU) per gram of inoculated regolith and the water content at different regolith depths (0-0.5 cm, 1-3 cm, and 10-12 cm) were measured. CFUs were normalized to dry weight by subtracting the determined water content, allowing us to calculate the survival rates of the microorganisms. The low pressure conditions led to water evaporation from the top layers of the regolith. However, moisture was retained in the regolith at depths of 5 cm and below, with a residual water content of up to 95% (SD=15%) at 10-12 cm. This resulted in the crystallization of water ice at a depth of 10-12 cm, creating concentrated brines in samples containing sodium chlorate and sodium perchlorate, resembling potential brine conditions in the shallow subsurface of Mars. Interestingly, the survival rates of the three tested organisms were significantly higher in the NaClO3 sample compared to the NaClO4 sample. For instance, at a depth of 10-12 cm, *P. halocryophilus* displayed a survival rate of 95% (SD=13%) in the NaClO3 sample, while no survival was observed in the NaClO4 sample at 10-12 cm depth. These higher survival rates in chlorate salts underscore their critical role in the habitability of the Martian near-surface environment. Additionally, the regolith demonstrated its ability to shield against UV irradiation. The survival rates at 0-0.5 cm were at least 10% lower for all tested organisms than those at 1-3 and 10-12 cm.

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