

Briny SLiMEs in the Subsurface of Earth and Mars. Tullis C Onstott¹, Calvin Rusley¹, Renxing Liang¹, Zachary K. Garvin¹, Devan M. Nisson¹, Rachel Harris¹, John Higgins¹, Nicolas W. Slater¹, Esterhuizen Van Heerden², Brenda Freese², Bennie Leibenberg², Hiroshi Ogasawara³, Esta van Heerden⁴, Errol Cason⁵ Jan-G Vermeulen⁵, Barbara Sherwood Lollar⁶, Tom Kieft⁷, Thomas Wiersberg⁸, Martin Zimmer⁸, Cliff Walters⁹, B. Freifeld¹⁰ and Joseph R. Michalski¹¹

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Introduction: Earth's deep subsurface is perhaps the closest analogue for the study of Martian habitability, owing to the hypothesized similarity of each planet's subsurface geology and the irrelevance of atmospheric or radiative factors. As such, terrestrial subsurface lithoautotrophic microbial ecosystems (SLiMEs) are a promising area of astrobiological study. Hypersaline subsurface ecosystems are of special interest because of their likely occurrence on present day Mars [1]. During the formation of the cryosphere the fractional crystallization of ground water will form a residual brine. On Earth, salt-saturated brines are mostly associated with Phanerozoic salt deposits and saline ground waters and brines hosted in Precambrian crystalline rocks [2]. At Kidd Creek mine in Canada, brines discovered in ore deposits have been isolated from the surface for billions of years [3] in a setting with a relatively low thermal history (<100°C) for the past 2 billion years. Such brines provide terrestrial analogues to the past and present Martian subsurface, and potentially provide clues as to whether subsurface abiotic organosynthesis reactions lead to the emergence of life.

In early 2018 we discovered a brine reservoir in the Archean Kaapvaal Craton of South Africa. Samples were collected of high temperature brine from 3.0 to 3.1 km underground in the Moab Khotsong gold mine, South Africa (26.98°S, 26.78°E). The host shale, quartzite, conglomerate, and amygdaloidal lava are referred to as the West Rand Group (a part of the Witwatersrand Supergroup), and were deposited between 3.1 to 2.9 Ga and intruded by 2.7 Ga mafic sills. The brines are associated with the contact zones between the mafic intrusions and host rock and occur between 2.55 km to 3.4 km below ground. Brine temperatures range from 48 to 55°C and pressures are over 100 bars.

One sample collected at 3 km depth exhibited high concentrations of Cl⁻ (4.3 M), Na⁺ (1.3 M), and Ca²⁺ (1.5 M), with minor amounts of sulfate (0.8 mM), nitrate (14 µM), total Fe (2.8 mM), Mg (5.3 mM), acetate (30 µM), and formate (198 µM). Another sample at 3.1 kmbls contained high level of Cl⁻ (4.2M), lower concentration of sulfate (0.2 mM), nitrate (7.4 µM), but much greater acetate (259 µM), and formate (684 µM) concentrations. A gas sample collected at 3.1 km was mainly CH₄ (62 vol-%), followed by He (16 vol-%), N₂ (15 vol-%), H₂ (4.8 vol-%), Ar (1.6 vol-%), and higher hydrocarbon gases including alkylsulfides. The carbon isotope signatures of CH₄ and higher hydrocarbon gases suggest an abiogenic gas source produced by water-rock reactions. Epifluorescence microscopy and SEM imaging revealed microbial cells in one borehole at 10⁴ cells/mL. This is noteworthy given the energetic tax imposed by high salinity environments. Moab Khotsong is the only location where subsurface brines have been encountered in the Witwatersrand Basin. The composition of these brines suggests they have likely been isolated from the surface since the Proterozoic, providing a terrestrial analogue to the Martian subsurface. Furthermore, the discovery of living biomass from such hypersaline, deep, and presumably old water, extends the abiotic fringe and could provide clues to the limits of habitable subsurface environments on Mars.

References: [1] Huang, J. et al. 2018. *Astrobiology* 18. [2] Warr et al., 2018 *GCA*. [3] Holland, G. et al. 2013. *Nature* 497, 357–360. [3] Michalski et al. 2018, *Nature Geoscience*, 11, 21–26.