

# Tracing Biosignatures from a Martian Analogue site of Puga Hot Spring Ladakh, India: Insights from Crystallography and Biomarkers

DR. AMRITPAL SINGH CHADDHA, PHD<sup>1,2</sup>, DR. SUNIL KUMAR SHUKLA<sup>2</sup>, DR. KAMLESH KUMAR<sup>1,2</sup>, DR. ANUPAM SHARMA<sup>2,3</sup>, PROF. MAHESH G. THAKKAR<sup>2</sup>, DR. DEVENDRA KUMAR PATEL<sup>4</sup> AND DR. G.N.V. SATYANARAYANA<sup>4</sup>

<sup>1</sup>Geochemistry Lab, Birbal Sahni Institute of Palaeosciences, Lucknow-226007, India

<sup>2</sup>Earth and Planetary Exploration Group (EPEG), Birbal Sahni Institute of Palaeosciences, Lucknow-226007, India

<sup>3</sup>Birbal Sahni Institute of Palaeosciences (DST, Govt. of India), Lucknow-226007, Uttar Pradesh, India

<sup>4</sup>Analytical Chemistry, Regulatory Toxicology Group, CSIR-Indian Institute of Toxicology Research, Lucknow – 226 001, India

- [1] Cayol, J. L. et al. (2015), *Environmental Microbiology: Fundamentals and Applications: Microbial Ecology*, 353-394.
- [2] Brock, T.D. (1967), *Science* 158, 1012-1019.
- [3] Sarkar, S. et al. (2022), *JGR Planets*.
- [4] Shukla, S.K. et al. (2023), *Authorea Preprints*.
- [5] Chaddha, A. S. et al. (2024), *Research Square preprints*.
- [6] Pokroy, B. et al. (2004), *Nature Mater* 3, 900–902.

Our understanding of habitability has expanded considerably over the past decades due to the growing evidence of microbial life thriving in Earth's extreme environments [1]. However, establishing the methods which can effectively detect the biosignatures and clear demarcation between true and false biosignatures within geological contexts are crucial for evaluating a planet's potential for the sustenance of life. Hot springs harbour diverse organisms surviving in specific water temperature and mineral compositions making hot springs valuable analogues for astrobiological studies [2,3].

Recent studies from the Puga hot spring based on diatoms and geochemical investigations using calc-sinter suggested the role of cold water for the interaction of biotic and abiotic components rather than the “hot water” of the gushed from the hot spring's vent [4,5]. To complement the previous studies, we attempted first time X-ray powder diffraction analysis and lattice parameter calculations for the calcitic sinter from the Puga hot spring, which reveals the abundance of biogenic calcite crystals with anisotropic lattice distortion [5,6]. Additionally, GC-MS-MS analysis of calc-sinter reveals organics such as  $\beta$ -alanine derivatives, pyran-2-thione, formamide TMS derivatives, cyclo-octane S<sub>8</sub> sulphur, and fatty acid methyl esters that indicate towards the interplay of a complex biogenic-abiotic mechanism. Our results suggest that calcite crystals were possibly formed through biologically controlled biomineralization. The presence of biomarkers and the biogenic calcite whereas the absence of aragonite phase collectively indicates that microorganisms developed in cooler waters, aligning with previous biotic and abiotic studies [4,5]. Therefore, we suggest that the biotic composition found in the Puga hot spring should be considered as “false biosignature” and interpreted with caution when correlating with the survival of life forms in extreme “hot” conditions of the hot spring found in colder environments, such as in Ladakh, India and Present-day Martian environment.