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## **Genesis of the moonmilk deposits at Sahastradhara cave, India: geochemical, mineralogical and microbiological observations**

Baskar, R.<sup>1</sup> and Baskar, S.<sup>2</sup>

<sup>1</sup>Department of Environmental Science and Engineering, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, \*Corresponding author e-mail: rbaskargjuhisar@yahoo.com

<sup>2</sup>Environmental Studies, School of Agriculture, Indira Gandhi National Open University (IGNOU), New Delhi

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Genesis of the moonmilk deposits formed on the cave walls of the Sahastradhara cave, Dehradun, India are discussed on the basis of the mineralogical, chemical and microbiological observations.

Geochemical analyses of the drip waters indicated concentrations of: Ca 85 mg/L, Fe 1.02 mg/L, K 2.74 mg/L, Mg 55 mg/L, Mn 6 mg/L, Si 2.70 mg/L, Sr 540 mg/L. The in situ pH of the drip waters ranged from 7.4–7.6, and conductivity was around 1700 mS. The sampled moonmilk deposits had high water content (72–74%), and the total organic carbon content ranged from 3.0 to 3.5 wt% (moonmilk) and 1.25 wt% (stalactite). FTIR of the moonmilk indicates a number of organic compounds. X-ray diffraction analysis indicated that the moonmilk were mainly composed of calcite. Isotopic analysis performed on the moonmilk indicated  $\delta^{13}\text{C}$  values from -4.45‰ to -4.44‰ and  $\delta^{18}\text{O}$  values from -6.64‰ to -6.63‰. The stalactite indicated  $\delta^{13}\text{C}$  value from -2.20‰ to -2.50‰ and  $\delta^{18}\text{O}$  value from -4.84‰ to -4.90‰ (relative to VPDB). The drip waters had an average  $\delta^{18}\text{O}$  value of -6.73‰ to -6.84‰ (relative to VSMOW).

Scanning electron microscopy (SEM) of moonmilk showed: i) spiral filaments (diameter  $\approx$ 1  $\mu\text{m}$ ); ii) long stalk like spiral and tubular filaments; iii) calcite minerals closely associated with filamentous structures; iv) bacterial cells; v) empty filamentous organic structures; vi) calcite formed in the vicinity of Exopolysaccharides, and vii) fiber calcites. ESEM-EDAX analyses of these calcite filaments indicated Ca. ESEM showed numerous calcite fibers (length 0.5 $\mu\text{m}$  to 1.5  $\mu\text{m}$ ; diameter 0.1  $\mu\text{m}$  to 0.25  $\mu\text{m}$ ; fibres primarily consisted of Ca, O, C, traces of Mg and S).

Light microscopy revealed a large number of diverse indigenous bacteria, similar to Cyanobacteria spp. and Spirulina spp. The total number of microbial cells using SYBR Gold is  $6.5 \times 10^5$  cells, g  $\text{sed}^{-1}$  and FISH showed that approximately  $3.5 \times 10^5$  cells, g  $\text{sed}^{-1}$  belong to Eubacteria. Further enumeration in different media indicated different cellular abundances for moonmilk and the different metabolic pathways followed by the bacterial species. Experimental geomicrobiology *in vitro* using strains isolated from moonmilk show that the strains had the capability to precipitate biominerals and the dominant crystal precipitating strains belonged to *Bacillus* spp. Evidences based on the mineralogy, geochemistry and microbiology indicate a biogenic input in the formations. The diverse microbial community and the capability of the isolated strains to induce biomineralization *in vitro* support the possible biogenicity of the Sahastradhara moonmilk.

