

## Cave geomicrobiology in the Indian context

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Cave geomicrobiology is a new interdisciplinary subject involving the application of many natural sciences, including chemistry, biology and geology, to study microbial–mineral interactions in caves. In carrying out such studies, the field also draws on resources in materials science, medical microbiology, environmental science and engineering. Caves are locally abundant in India and allow easy human access, where they represent ideal experimental study systems to understand the subsurface geomicrobiological processes<sup>1,2</sup>. Caves are nutrient-limited environments, dark due to the absence of solar energy, are geologically isolated and contain low levels of available organic carbon to support diverse microbial communities<sup>3</sup>. Cave geomicrobiology therefore has the potential to provide invaluable information on subterranean, microbial chemolithotrophic and heterotrophic ecosystem processes<sup>4–10</sup>. Indeed, in the last 10 years, cave geomicrobiology has led to recognition of the role microorganisms can play in geological processes in caves and has altered our perception of the structure of cave ecosystems<sup>11</sup>.

Microbial life on earth had to evolve in an environment prior to photosynthesis when there was limited nitrogen and where the majority of organisms used minerals for sources of energy. As a result, cave environments offer the potential to study ancient evolutionary relationships, the use of alternative sources of energy and systems developed by microbes for scavenging scarce nutrients in such environments. Recent research on the microbial ecosystems surviving under starved cave conditions confirm that they can indeed induce a myriad of energy conserving reaction<sup>4,5,12,13</sup>. Such processes in caves are evidenced by the discovery of oxidized iron-manganese (ferromanganese) deposits, calcium carbonate moonmilk deposits, corrosion residues from the disintegration of the host rock, historic saltpeter (calcium nitrate) deposits and elemental sulphur<sup>14</sup>, which are the geochemical relics of microbial activity in these environments. Nonetheless, laboratory-based experiments are still critical to establish the

role microorganisms play in affecting such geochemical processes, which cause the dissolution and precipitation of such minerals. Within India, new caves continue to be discovered, which may provide potentially unique geochemical environments to be examined.

As an emerging science, cave geomicrobiology has only attracted scientific attention in the last 20 years, and significant research funding began only in the last decade<sup>11</sup>. As a result, the contributions of this science, both to themes within microbiology and applications to industry remain in their infancy. This, therefore represents an exciting, but uniquely open field, with the potential for significant contributions from Indian investigators. To date, the significant contributions of cave geomicrobiology include the following.

*Recognition of the contributions that microbial species make to mineral precipitation:* The nature of the cave environment, shielded from weathering and physically stable for potentially hundreds of thousands of years, means that erosional processes are limited. As a result, the subtle chemical changes made by microorganisms on surfaces are more apparent in caves. This has led to a better understanding of the role that microorganisms may play in transforming minerals, such as iron and manganese oxides and carbonates, in caves<sup>12–16</sup>. As a result, cave science has also aided researchers in their understanding of the pathways of microbial metabolism and biotransformation in geochemical environments<sup>12,17,18</sup>.

*A new niche to study subsurface ecosystems:* Emerging evidence suggests that a range of energetics are available within cave environments. From the entrance, where heterotrophic interactions based on surface-derived nutrient and energy sources predominate, to the depths where a subtle interplay of autotrophic reactions and carbon turnover allow subsistence under the most starved conditions<sup>19,20</sup>. While the complex geochemical environment of the host rock may be able to support energy production primarily;

autotrophic systems and hydrogen produced in the deep subsurface (from volcanism, serpentinization and radiolysis) may also provide sufficient energy for growth<sup>21–23</sup>. It remains to be determined if such energy sources are utilized in the most starved regions of deep caves.

*Microbial activity in cave formation:* Perhaps the biggest field that has been benefitted from cave geomicrobiology is geology. By understanding the metabolic processes carried out by microorganisms in the subsurface, it has been determined that such processes make significant contributions to cave formation (speleogenesis). This can be as subtle as the generation of CO<sub>2</sub>, which forms the carbonic acid that is responsible for the formation of the vast majority of the world's caves, to sulphuric acid from sulphate reducing bacteria, a mechanism that can form caves, hundreds of kilometres in length.

The practical applications of cave microbiology are wide and varied. These include preservation of historical monuments and sculptures, through the identification of microbial species that can precipitate protective calcite coatings<sup>24,25</sup>, and the potential to harbour unique antibiotics due to the isolation and presumably the chemical warfare that may occur between microbial species under such nutrient limitation. Due to unique adaptations to starvation, these species also have the potential to carry out bioremediation, although the ability of cave microorganisms to carry out such activities is only now beginning to be explored. For example, bacterial cave species have been found that can degrade complex hazardous aromatic compounds, such as the plasticizers benzothiazole and benzenesulphonic acid, even though plastics are not naturally found in caves. Within eukaryotic species, fungal isolates from the cave cricket *Troglophilus neglectus*<sup>26,27</sup> demonstrated high chitinolytic, lipolytic and proteolytic activities<sup>28</sup>.

The geomicrobiology of caves reveals some of the diversity and resilience of microbial life, including the nutritional limits for survival. Microbial metabolism

under such starvation and its active influence on geochemistry can be used to aid in the recognition of biomarkers for subsurface life on other planetary bodies<sup>29–33</sup>. Such ideas are an extraordinary application of cave geomicrobiology. National Aeronautics and Space Administration (NASA) has undergone a dramatic refocus by gearing its activities to the exploration of Mars and finding traces of past life, where any science that plays a role in identifying the subtle geochemical changes of life can play an important role<sup>34</sup>. While there are no known carbonate deposits elsewhere in our solar system, cave geomicrobiology nonetheless can provide information on the preserved microfossils and geochemical evidence that can be used to differentiate between biotic and abiotic signatures.

Among the more than 1545 caves<sup>35</sup> that are known to exist in the Indian subcontinent, no detailed and systematic geomicrobiological explorations for most of these caves have been carried out, except in the Sahastradhara<sup>36,37</sup>, Borra<sup>38,39</sup> and Meghalaya<sup>40</sup> caves. This lack of progress probably reflects cave geomicrobiology as an emerging field and a lack of formal coordinated programmes by geologists and biologists<sup>41</sup> in the subcontinent. Recent studies in India have been focussing on geochemical, mineralogical, traditional and molecular microbiological approaches to understand the extent of microbial involvement in secondary cave formations. This includes the documentation and recognition of mineralized microbes, including microfossils, microfossils and large scale structures, such as microbialites, preserved in rocks, either through the presence of DNA or geochemical gradients that are indicative of life. The goal of such work is to continue to locate and identify microorganisms which inhabit different niches in caves, towards understanding the role microbes play in such geochemical processes.

The Department of Science and Technology (DST), New Delhi, in its vision paper on earth sciences<sup>42</sup> has emphasized interactive geoscientific studies, which 'increased understanding of the earth processes as well as emerging newer concepts and methodologies that requires interactive research programmes involving geoscientists, physicists, chemists, biologists and mathematicians'. The Earth System Science (ESS) division of the DST has identified a national programme on 'Science of shallow subsurface stu-

dies'. It is proposed that cave geomicrobiology shall be specifically included as a thrust area, as there is an urgent need to initiate such studies, given the potential of this field and our lack of understanding of the role of subsurface microbes in human health, such as the role such species play in maintaining the quality of subsurface water in aquifers. Such studies have applications to understand the retention/mobility of certain metals which affect drinking water quality. Therefore, cave geomicrobiology ought to be strongly supported as one of the new, revolutionary areas of knowledge at the boundary of geology and microbiology. We conclude that cave geomicrobiology research can generate fundamental knowledge about the subtle interplay between mutualism/competition and heterotrophy/autotrophy in terrestrial subterranean systems, which has numerous practical applications within medicine, human health and industry, and can aid in our ability to understand the possibility of life elsewhere in our solar system.

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