GEOMICROBIOLOGY AND HYDROLOGY OF POOL PRECIPATES IN THE GUADALUPE MOUNTAINS, NEW MEXICO, USA

ARA KOOSER[1], LAURA CROSSEY[1], DIANA NORTHUP[2], MIKE SPILDE[3], LESLIE MELIM[4]
[1] Earth and Planetary Science Department, Univ. of New Mexico, Albuquerque, NM 87131 USA
[2] Biology Department, Univ. of New Mexico, Albuquerque, NM 87131 USA
[3] Institute of Meteoritics, Univ. of New Mexico, Albuquerque, NM 87131 USA
[4] Department of Geology, Western Illinois University, Macomb, IL 61455 USA

The Guadalupe Mountains of southeastern New Mexico (USA) are home to hundreds of caves hosting a variety of speleothems. These arid-land caves, as semi-closed systems stripped of the influence of surface weathering, provide a particularly valuable window into the world of carbonate-precipitating microorganisms and the interaction of meteoric waters mixing with deep waters.

The cave pool precipitates, many of which are biogenic, record microbial influences, surface climate, and ecosystem changes. The cave pool precipitates researched in this study are pool fingers. Pool fingers are subaqueous pendant, finger-like speleothems. Previous work in Hidden and Cottonwood caves has shown microbial fossils associated with pool finger precipitates. This work expands on those observations by presenting data on biomarkers extracted from pool fingers. Preliminary analysis indicates that plant waxes (imported into the cave environment) dominate the organic residue in abiologic carbonates (pool spar), while evidence of hopenoids coupled with an absence of plant residues is found in carbonate deposits associated with biogenically-active environments ('moonmilk').

The connection between microbial communities and their chemical environment is especially strong in 'extreme' environments (extreme pH, salinity, temperature, presence/absence of light, low nutrient conditions, etc.). Modern bacterial communities are known to utilize chemical species that are present in the cave environments for metabolic processes. Through the use of available water data from the literature and calculations of Gibbs free energy available from equilibrium considerations we identify energetically-favored metabolic pathways. Study of the 'fossil' biomarkers combined with modern environments will lead to a better understanding of subsurface carbon cycling, characterization of microbial communities, and the input of deeply circulating waters.

1. Introduction

Fundamental questions remain regarding formation of secondary calcite and the possible role of microbial precipitation. An interdisciplinary approach to field and laboratory studies allows us to address some of these major geomicrobiological issues. Subsurface carbonate systems constitute a major arena of interaction between microorganisms, minerals, and water (Barton and Northup, 2007). The bio- and geochemical signatures identified in carbonates may be generalized to other biogenic carbonate occurrences such as travertine mounds (Crossey et al., 2006) and deep sea hydrothermal vents, and have potential use in identifying the presence of life on other planets.

This research focuses on a less well-studied speleothem that forms in subaqueous cave environments: pool fingers. In the Guadalupe Mountain (Fig. 1) cave pools host a wide variety of speolothems such as pool fingers, webulites, U-loops, and pool meringue (Davis et al. 1990; Hill & Forti 1997; Queen & Melim, 2006). Pool fingers are finger-like speleothems that hang down in cave pools. Pool fingers form entirely underwater and lack a central drip canal. The majority of pool fingers are 1-2 cm in diameter and 5-15 cm long (Davis et al. 1990). The giant pool fingers of Hidden Cave, NM are an exception at nearly 10 cm in diameter and well over 1 m in length. The internal layering of pool fingers indicates downward growth with lesser outward growth (Fig. 2). The current hypothesis is that microbial filaments, hanging down from submerged surfaces, act as nuclei for the growth pool fingers. A previous study of fossil pool fingers in Hidden Cave found evidence of alternating microbial and abiotic layers (Melim et al. 2001). Further work in other caves has found a number of potentially biogenic carbonate precipitates (Queen & Melim, 2006). These published studies suggest biogenic processes contributed to the formation of these cave paleo-pool carbonate deposits, and thus pool fingers provide a record of paleo-hydrology and paleomicrobiology.

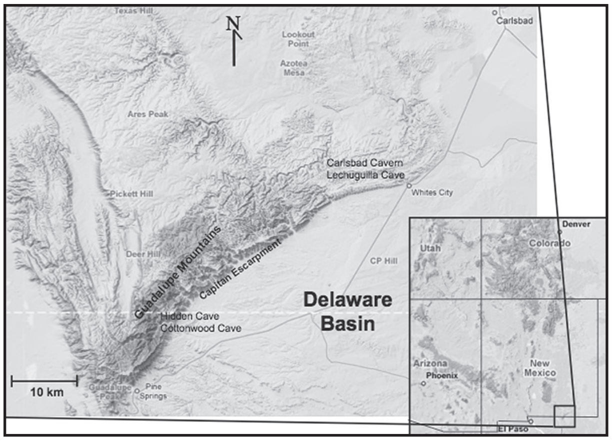

Figure 1: Regional and local map of the study area.

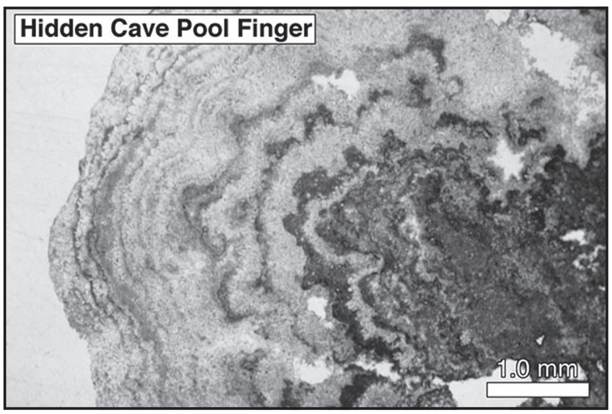

Figure 2: Cross section of pool finger from Hidden Cave. Photo by Dr. Leslie Melim.

Biomarker analysis is an important tool for understanding biogenic carbonates. Past and present bacterial communities utilize chemical species present in the cave environments for metabolic processes and may directly or indirectly contribute to carbonate production. The lithified communities leave behind fingerprints in the form of biomarkers. Biomarkers can include fossilized microbes, mineral fabrics, mineralogy, and preserved lipids. Lipid biomarkers left behind by microbes in the calcite can have a unique signature that can be used to identify different types of microorganisms. Several studies (Blyth et al., 2006, 2008) have shown that bacteria and plant biomarkers can be extracted from cave stalagmites.

Earlier studies (Thraikill 1971; Forbes 2000; Turin & Plummer 2000, Palmer and Palmer, 2001) have examined the chemistry of pool waters or compared the chemistry to pool precipitates, but none have considered the role of microbes in modifying either the pool chemistry, pool speleothems, or linked the geochemistry, mineralogy, and microbiology of the cave pools. Bacterial communities utilize chemical species present in the cave environments for metabolic processes. Using available water data from the literature and calculations of Gibbs free energy available from equilibrium considerations, we identified the energetically favored metabolic pathways. Most metabolically important reactions involve oxidation-reduction reactions among chemical (gas, aqueous and mineral) forms of hydrogen, nitrogen, carbon, sulfur, iron and manganese. The combination of thermodynamic analysis and field observations (minerals forming in cave environments and identified microbial communities) further refine the list of potential metabolic reactions. These predictions guide further investigation into the ways in which microbial species participate in the formation of cave precipitates.

2. Methods

Samples were collected from Hidden Cave and Carlsbad Cavern. A pool finger from Hidden Cave and pool spar were processed for lipid biomarker analysis. The biomarkers were extracted using a series of solvent washes in a Soxhlet Extractor; the products of each wash were analyzed using gas chromatography followed by gas chromatography/mass spectroscopy. Detecting other types of biomakers requires using various microscopy techniques. We used a on a JEOL 5800 scanning electron microscope (SEM) equipped with an Oxford (Link) Isis energy dispersive x-ray analyzer (EDX) to identify mineral fabrics and fossilized microbes; x-ray mapping provided mineral composition and on occasion identification of mineral via crystal habits, distribution of elements, and targets for the microprobe; back-scattered emission (BSE) supported the identification of chemical layering; cathode luminescence gave information about different trace element variations among layers. Analysis on a JEOL 8200 electron microprobe provided quantitative elemental composition, and x-ray diffraction (XRD) was used for confirmation of mineral phases.

Cave pool water samples were collected and analyzed using inductively-coupled plasma optical emission spectroscopy (Perkin Elmer Optima 5300 DV ICP-OES) and anion chromatography (Dionex 500x) to provide major ion chemistry for the active hydrologic system. Alkalinity was performed by standard titration methods using sulfuric acid. Analyses were performed in accordance with standard quality assurance – quality control protocol. These data provided information for hydrochemical mixing models, microbial metabolism models, and evaluation of stability of several important minerals (including gypsum and carbonate). To understand trends in hydrochemistry, regional aquifer data were compiled from the literature and Piper diagrams were constructed to identify geologic units with chemically similar water and to define the evolution in water chemistry along potential flow paths. Chloride/bromide plots were constructed to determine salt sources, and external carbon was computed (Chiodini et al., 2000) to identify the presence of carbon sources above that due strictly from carbonate dissolution. To provide reference points for active speleothem formation, we incorporated data from two cave systems, caves in Alaska (Cataract Cave and Thrush Cave) with active moonmilk formation and unnamed caves in La Madera, NM with active pool fingers, were examined as well. Several water analyses were selected for further thermodynamic modeling. The initial modeling looked at five pools from Lechuguilla Cave: Lake of the Blue Giant, Sulfur Shores, Lake of the White Roses, Lake Chandelier, and Briny Pool. Waters that represent active biogenic formations as well as inactive waters were plotted and modeled to determine likely microbial metabolic pathways. The thermodynamic models were compared to each other to discuss differences/similarities in potential pathways.

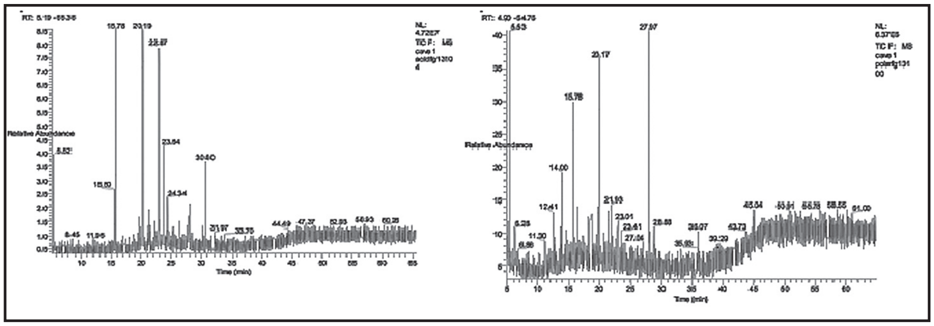

Figure 3: Preliminary biomarker data from moonmilk portion of a pool finger.

3. Results and Discussion

Biomarker analysis was done on a pool finger from Hidden Cave (Fig. 3) and a piece of pool spar from Carlsbad Cavern. The moonmilk portion of the large pool finger from Hidden Cave contained several short-chained fatty acids (C16-C22). The C16-C22 are considered generic lipid biomarkers. In the polar fraction unknown hopanes were detected. The presence of a hopanes with the short-chained fatty acids confirms the presence of bacterial biomarkers in the moonmilk portion of the pool finger. The pool spar sample (assumed to be abiotic) produced a different mass spectra pattern for the acid fraction and polar fraction. The acid fraction contains short chain fatty acids (C16-22), but there are no hopanes present in the other fractions. The polar fraction for the polar spar is dominated by plant biomarkers producing the "rainbow" spectra of C22 and higher chains. The initial x-ray map of one section of a pool finger showed spheres of magnesium carbonates (confirmed by EDS) (Fig. 4), but this is not conclusive evidence for microbial involvement and bears further investigation. The cathodoluminescence (CL) images of the pool finger showed very faint CL in the cores of two samples and plumes of brighter areas rimward (Fig. 5). The results from the CL imaging might be due to changes in trace elements or from re-precipitation (biotic or abiotic) of the calcite. Further investigation using high resolution imaging and microprobe data is planned.

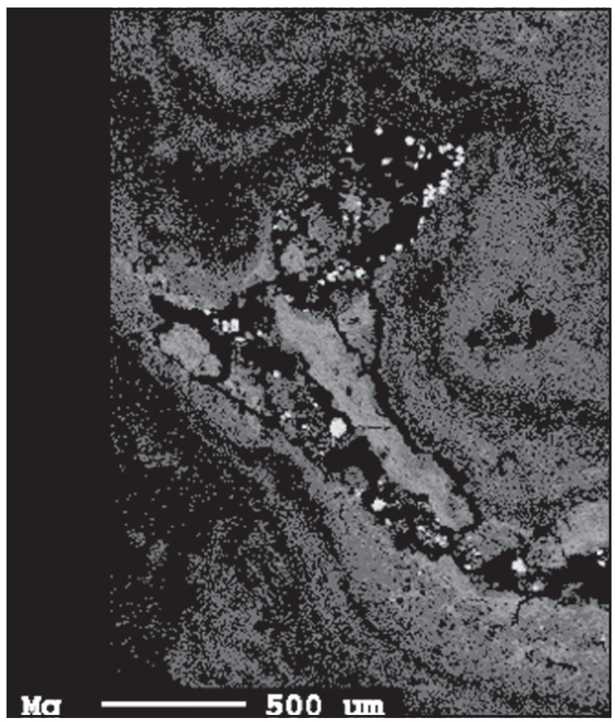

Figure 4: Microprobe x-ray map of magnesium in pool finger. The light grey spheres are a magnesium carbonate.

Fig. 5

Geochemical analysis was done on waters from three different cave systems: the caves in Alaska (in the Tongass National Forest); the La Madera waters (travertine mounds with caves); and finally, the cave pools in the Guadalupe Mountains The Piper Diagram shows a clear trend for the different cave systems (Fig. 6). The Alaskan caves, which have actively precipitating moonmilk, are clustered in the lower-left corner. The La Madera waters, which originate

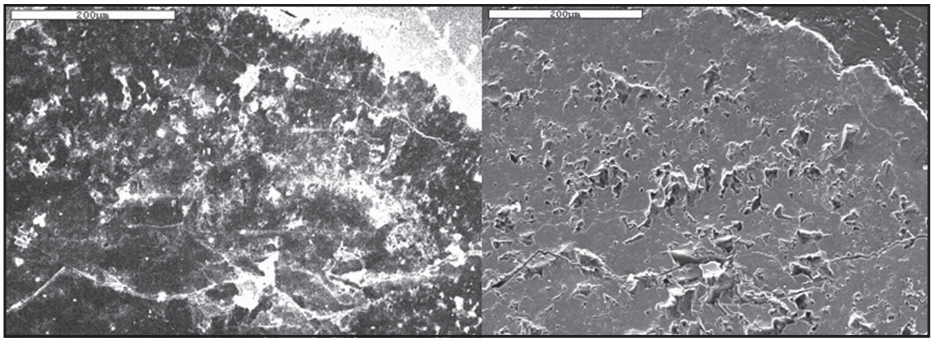

Figure 5: CL (left) and BSE (right) image of outer portion of pool finger.

from CO_2 springs with other deeply sourced gases (Newell et al.,), plot roughly in the center, whereas the Guadalupe Mountain cave pool waters trend to the far left side. The sulfate + bicarbonate plotted against calcium + magnesium shows a clear trend of the Guadalupe Mountain waters trending on the 1:1 line with the Alaskan and La Madera waters are off to the right of the line. There are a few exceptions for the Guadalupe Waters that do not follow the 1:1 line. The 1:1 line represents carbon sources from water-rock, soil, and atmosphere input (Crossey et. al., in press). The waters that plot off this line have a higher then expected carbon signal. The chloride/bromide plot shows that most of the salt present in the cave waters is from water-rock interactions. The preliminary thermodynamic modeling (using reaction quotient computations from activities calculated by the speciation program PHREEQc (Parkhurst, 1995) and methods of Meyer-Dombard et al., 2005 for five cave pools from Lechuguilla Cave showed that microbial communities would potentially use oxygen as the primary electron acceptor, followed by nitrate, goethite, hematite, and sulfur. One pool, Sulfur Shores, was predicted to have communities that utilize goethite and hematite before elemental sulfur.

4. Conclusions

The initial lipid biomarker analysis shows potential bacterial biomarkers in the outer portion of the pool finger from Hidden Cave. The spar sample (assumed to be abiotic) does not have bacterial lipid biomarkers, but instead there are preserved plant biomarkers, potentially confirming its abiogenic nature. These results suggest a bacterial role in precipitation of the outer portion of the pool finger. The x-ray maps from the microprobe and the CL images from the SEM showed areas on the pool finger sample that will be targeted for analysis.

The geochemical analysis shows that most of the cave pool water chemistry in the Guadalupe Mountains is derived from water-rock interactions. The Alaskan caves and La Madera waters are a combination of water-rock interactions and other processes. There are several (nine pools) exceptions in the Guadalupe Mountains. The nine pools in the Guadalupe Mountains, along with the Alaskan and La Madera have an external source of carbon above what would be expected from water-rock, soil, and atmosphere interaction (Crossey et. al., 2003, 2006). Usually, this signal is accompanied by other gases such as H_2 or H_2S. In the case of La Madera, these gases are from deep sources (Newell et al., 2005). In the Guadalupe waters this might arise from active microbial communities. We will target these nine pools for further study. The thermodynamic profiles predict that the five pools that were modeled would have bacterial communities that use similar metabolic pathways except for Sulfur Shores. The thermodynamic profiles are not complete until fieldwork has been done to confirm mineral phases and microbial communities that may be present.

The pool finger communities in the Guadalupe Mountains do not appear to be currently active based on the water chemistry and field observations. However there are nine pools in the Guadalupe Mountains that need to be investigated based on their geochemistry to determine if there are active pool finger communities. The Alaskan caves and La Madera provide a speculative glance at what geochemical conditions would support a microbial role in pool finger formation. Evidence from previous studies by Melim et. al. (2001) and current work with lipid biomarkers provides increasing evidence of a biogenic origin for pool fingers.

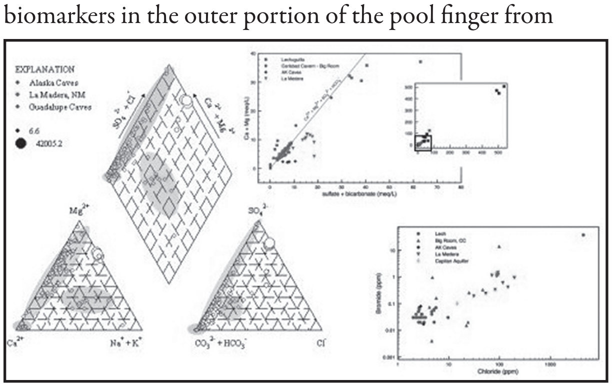

Figure 6: Geochemical analysis including Piper Diagram, Chloride/Bromide Plot, and External Carbon Plot.

Acknowledgements
We thank John Craig for analytical work; Paul Burger, Stan Allison, Pat Cicero, Aaron Stockton for gathering water and providing much needed data; Megan Curry for the AK water geochemistry; Kristen Mullen for the x-ray maps; Amy Williams for water chemistry help; Dr. Richard Pancost for the use of his lab; and Dr. Fiona Gill for helping with running the lipid biomarker analysis. This work would not have been possible without support from the Geosciences Directorate of the National Science Foundation, grants EAR 0719507 and EAR643364 .

References
Blyth, A.J., P. Farrimond, M. Jones, (2006), An optimized method for the extraction and analysis of lipid biomarkers from stalagmites, *Organic Geochemistry*, vol 37, pp 882–890.

Blyth, A.J., A. Baker, M.J. Collins, K.E.H. Penkman, M.A. J.S. Gilmour, Moss, D. Genty, R.N. Drysdale, (2008), Molecular organic matter in speleothems and its potential as an environmental proxy, *Quaternary Science Reviews*, vol 27, pp 905–921.

Chiodini, G., F. Frondini, C. Cardellini, F. Parello, and L. Peruzzi, (2000) Rate of diffuse carbon dioxide Earth degassing estimated from carbon balance of regional aquifers: The case of central Apennine, Italy, *Journal of Geophysical Research*, vol 105, pp 8423–8434.

Crossey, L.J., T. Fischer, K.E. Karlstrom, J. Patchett, D. Hilton, W. Sharp, and A. Reynolds, (2003), Springs, gases and travertines of the Colorado Plateau: Xenowhiffs, links to tectonism, and view of a deeply-dissected hydrologic system: *Geological Society of America Abstracts with Programs*: vol 35, no. 6, p 200.

Crossey, L.J., T.B. Fischer, P.J. Patchett, K.E. Karlstrom, D.R. Hilton, D.L. Newell, P. Huntoon, and A.C. Reynolds, (2006), Dissected hydrologic system at Grand Canyon: Interaction between upper world and lower world waters in modern springs and travertine: *Geology*, vol 34, pp 25–28.

Davis, D.G., M.V. Palmer, and A.N. Palmer, (1990) Extraordinary subaqueous speleothems in Lechuguilla Cave, New Mexico: *National Speleological Society Bulletin*, vol 52, pp 70–86.

Forbes, J. R., (2000) Geochemistry of Carlsbad Cavern pool waters, Guadalupe Mountains, New Mexico: *Journal of Cave and Karst Studies*, vol 62, pp 127–134.

Hill, C.A. and P. Forti, (1997), *Cave Minerals of the World, 2nd Edition*: National Speleological Society, Huntsville, 463 pp.

Melim, L.A., K.M. Shinglman, P.J. Boston, D.E. Northup, M.N. Spilde, and J.M. Queen, (2001), Evidence of microbial involvement in pool finger precipitation, Hidden Cave, New Mexico. *Geomicrobiology Journal*. vol 18, pp 311–330.

Meyer-Dombard, D.R., E.L Shock, and J.P. Amend, (2005). Archaeal and bacterial communities in geochemically diverse hot springs of Yellowstone National Park, USA. *Geobiology*, vol 3, pp 211–227.

Newell, D.L., D. Koning, K.E. Karlstrom, L.J. Crossey, and M. Dillon, (2004) Plio- Pleistocene incision history of the Rio Ojo Caliente, northern Espanola Basin, and an overview of the Rio Grande system in northern New Mexico, New Mexico Geological Society Guidebook 55: Geology of the Taos region, pp 121–134.

Newell, D.L., L.J. Crossey, K. Karlstrom, T. Fischer, D. Hilton, (2005) Continental-scale links between the mantle and groundwater systems of the western United States: Evidence from travertine springs and regional He isotope data, *GSA Today*, vol 15, no. 12, pp 4–10.

Northup, D.E. and K. Lavoie, (2001), Geomicrobiology of Caves: A Review. *Geomicrobiology Journal,* vol 18(3), pp 199–222.

Palmer, A.N. and M.V. Palmer, (2000), Hydrochemical interpretation of cave patterns in the Guadalupe Mountains, New Mexico. *Journal of Cave and Karst Studies*, vol 62(2), pp 91–108.

Parkhurst, D.L., (1995), User's guide to PHREEQC—A computer program for speciation, reaction-path, advective transport, and inverse geochemical calculations: U.S. Geological Survey Water Resources Investigations Report 95-4227, 143 pp.

Thrailkill, J., (1971), Carbonate deposition in Carlsbad Caverns: *Journal of Geology*, vol 79, pp 683–695.

Turin, H.J. and M.A. Plummer, (2000), Lechuguilla cave pool chemistry. 1986-1999: *Journal of Cave and Karst Studies*, vo; 62, pp 135–143.

Queen, J.M. and L.A. Melim, (2006), Biothems: Biologically influenced speleothems in caves of the Guadalupe Mountains, New Mexico, USA, in Raatz, W., Land, L., and Boston, P., editors, *Caves and Karst of Southeastern New Mexico, New Mexico Geological Society Guidebook, 57th Field Conference*, pp 167–174.