Diana E. Northup, Clifford N. Dahm, Leslie A. Melim, Michael N. Spilde, Laura J. Crossey, Kathy H. Lavoie, Lawrence M. Mallory, Penelope J. Boston, Kimberley I. Cunningham and Susan M. Barns - Evidence for geomicrobiological interactions in Guadalupe caves. *Journal of Cave and Karst Studies 62*(2): 80-90.

EVIDENCE FOR GEOMICROBIOLOGICAL INTERACTIONS IN GUADALUPE CAVES

DIANA E. NORTHUP
Biology Department, University of New Mexico, Albuquerque, NM 87131 USA dnorthup@unm.edu
CLIFFORD N. DAHM
Biology Department, University of New Mexico, Albuquerque, NM 87131 USA
LESLIE A. MELIM
Department of Geology, Western Illinois University, Macomb, IL 61455 USA
MICHAEL N. SPILDE
Institute of Meteoritics, University of New Mexico, Albuquerque, NM 87131 USA
LAURA J. CROSSEY
Earth and Planetary Sciences, University of New Mexico, Albuquerque, NM 87131 USA
KATHLEEN H. LAVOIE
College of Arts and Sciences, SUNY-Plattsburgh, Plattsburgh, NY 12901 USA
LAWRENCE M. MALLORY
Biomes, Inc., Olney, MD 20832 USA
PENELOPE J. BOSTON
Complex Systems Research, Inc., Boulder CO 80503 USA
KIMBERLEY I. CUNNINGHAM
Geo-Microbial Technologies Inc., P.O. Box 132, Ochelata, OK 74051 USA
SUSAN M. BARNS
Environmental Molecular Biology, Los Alamos National Laboratory, Los Alamos, NM 87545 USA

Caves in the Guadalupe Mountains offer intriguing examples of possible past or present geomicrobiological interactions within features such as corrosion residues, pool fingers, webulites, u-loops, and moonmilk. Scanning electron microscopy, transmission electron microscopy, molecular biology techniques, enrichment cultures, bulk chemistry, and X-ray diffraction techniques have revealed the presence of iron- and manganese-oxidizing bacteria in corrosion residues, which supports the hypothesis that these organisms utilize reduced iron and manganese from the limestone, leaving behind oxidized iron and manganese. Metabolically active populations of bacteria are also found in "punk rock" beneath the corrosion residues. Microscopic examination of pool fingers demonstrates that microorganisms can be inadvertently caught and buried in pool fingers, or can be more active participants in their formation. Enrichment cultures of moonmilk demonstrate the presence of a variety of microorganisms. Humans can have a deleterious impact on microbial communities in Guadalupe caves.

The caves of the Guadalupe Mountains are located in southeastern New Mexico, U.S.A., near the city of Carlsbad (Fig. 1). Over 300 known caves exist in these mountains, the most famous being Carlsbad Cavern and Lechuguilla Cave. These caves are filled with secondary mineral deposits (speleothems), which have been described and classified by Hill (1987) and Hill & Forti (1997).

Microorganisms have been found in association with carbonate and silicate speleothems, sulfur compounds, iron and manganese oxides, and saltpeter (Northup *et al.* 1997). As in surface environments, cave microorganisms participate in precipitation of minerals either passively by acting as nucleation sites (Went 1969), or actively through the production of enzymes or substances that lead to precipitation by changing the microenvironment (Danielli & Edington 1983). Microorganisms can also cause the dissolution of cave features through acidic metabolic by-products. While geomicrobiological interaction studies in the outside world are rather common (Ehrlich 1996), such studies in caves are just beginning. Studies of geomicrobiological interactions in caves can shed light on basic mechanisms of dissolution and precipitation by microorganisms, and, thus on the origin of specific types of speleothems.

Guadalupe Mountain caves contain a number of examples of possible interactions between microorganisms and speleothems. In particular, Lechuguilla and Cottonwood caves contain speleothems that have been referred to as "biothems" by Cunningham *et al.* (1995)–features such as webulites and u-loops that appear to be calcified filamentous microorganisms. The discovery of "snottites" in Cueva de Villa Luz (Hose & Pisarowicz 1999; Hose *et al*. 2000), a cave with active hydrogen sulfide vents, allows researchers to speculate that such

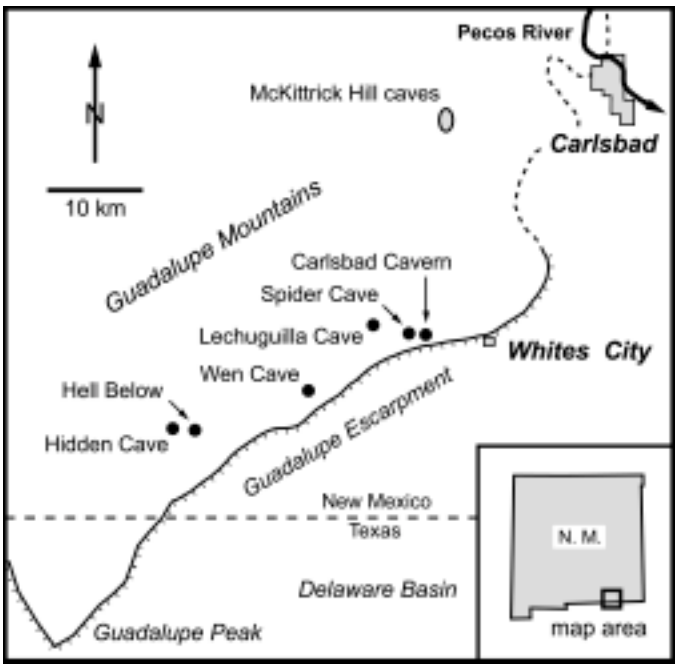

Figure 1. Location map of caves mentioned in the text. Modified from Palmer & Palmer (2000).

bacterial structures could become lithified later in the evolution of the cave, producing the u-loops that we see in Lechuguilla Cave today.

Other speleothems in Guadalupe caves also show microscopic or macroscopic evidence of possible bacterial interaction in their formation. Lechuguilla and Hidden caves contain pool fingers that show evidence of bacterial presence (Fig. 2). Also, a pool basin in Hell Below Cave contains unusual growths that resemble stiff meringue peaks. Manganese and iron oxide material in the form of "corrosion residues" from

Figure 2. Pool fingers in Hidden Cave. Note their "robust" nature and covering of popcorn-like crust. Photo by Kenneth Ingham.

Figure 3. Corrosion residues in EA survey in Lechuguilla Cave. Corrosion residues remain on the cave walls and ceiling after the carbonate fraction of the wall rock has been dissolved away. Field of view is ~5 cm. Photo by Robert Buecher.

Lechuguilla and Spider caves contain bacteria whose closest relatives are iron-oxidizing bacteria, a group of organisms that can contribute to corrosion (Fig. 3). Davis *et al.* (1990) demonstrated the presence of filaments in the cores of the "rusticles", an iron oxide speleothem in Lechuguilla Cave.

In this paper we investigate the nature of the relationship between the presence of bacteria in speleothems and bedrock residues, and the dissolution and precipitation of these deposits. We do this by presenting three case studies: corrosion residues, pool fingers, and moonmilk. We then discuss the impact that humans can have on microbiological and geologic interactions.

METHODS

FIELD TECHNIQUES

Samples of corrosion residues were collected from the EA survey, Sanctuary, PHD Room, Ghostbusters Hall, Apricot Pit, Rainbow Room, the FN survey, and the Snowing Passage (FN27) in Lechuguilla Cave (Fig. 4) and from the Grand Canyon, H1X, and the Decision Room in Spider Cave (Fig. 5). Enrichments for iron- and manganese-oxidizing bacteria were inoculated using aseptic techniques on site in the PHD Room and Sanctuary in Lechuguilla Cave (Fig. 4), and at TM12 off the Decision Room and at H1X in Spider Cave (Fig. 5). Samples for DNA extraction were immediately placed on dry ice for transport to the lab and then stored in an ultra-cold freezer. Pool finger specimens were collected from Lechuguilla and Hidden caves (Fig. 1). Samples of moonmilk were collected from the Deep Point in Spider Cave (Fig. 5).

Corrosion residues and punk rock for metabolic activity studies were aseptically sampled using a flame-sterilized spat-

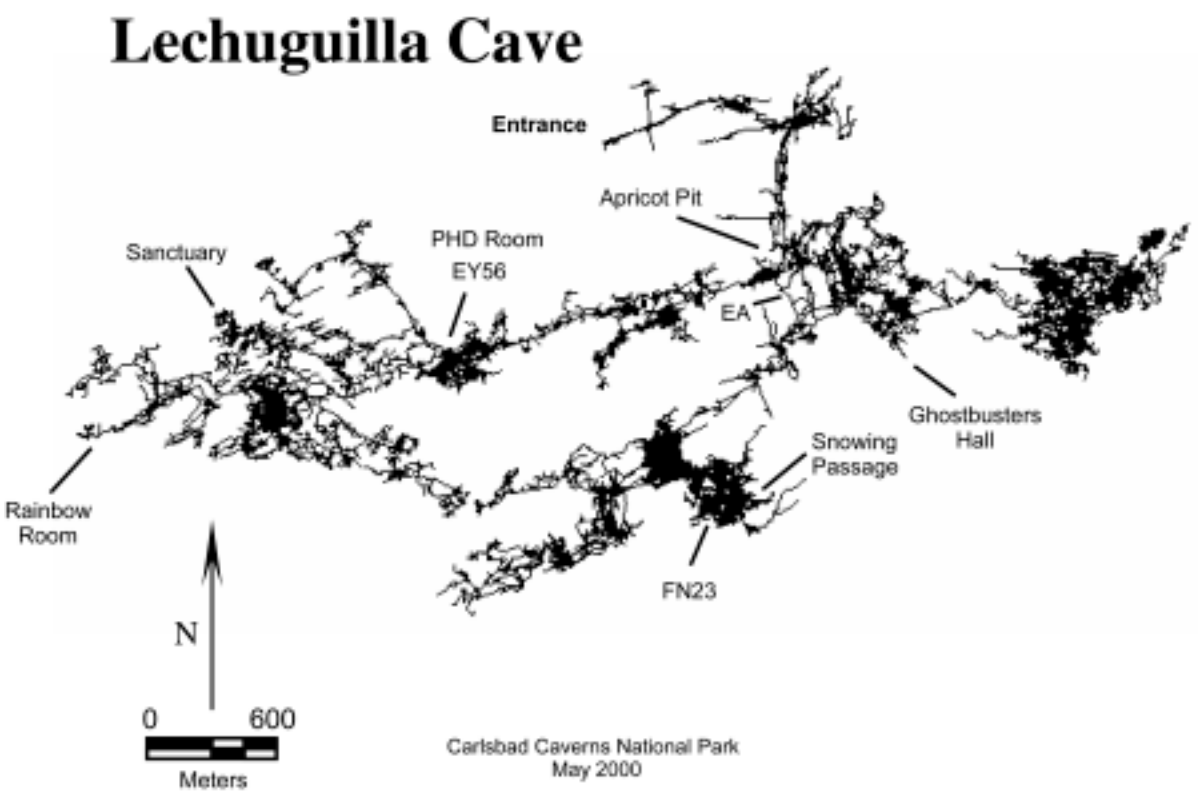

Figure 4. Sample sites in Lechuguila Cave. Map by Stan Allison and Paul Burger, U.S. National Park Service.

ula. Punk rock was sampled by carefully removing the surface corrosion material before sampling. Sample material was placed directly into sterile microcentrifuge tubes and controls were killed using formalin. A respiratory dye solution, [2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride]: INT, was added to all tubes and incubated for 4 hours, then formalin was added to the live tubes.

LABORATORY TECHNIQUES

Scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy dispersive X-ray spectrometry (EDX), and light microscopy. SEM, coupled with an EDX analyzer, was used to study the surfaces of samples for evidence of bacteria and their by-products. All samples were coated with gold-palladium. Freshly broken pool fingers were acid-etched before SEM examination. Samples for transmission electron microscopy (TEM) were ground in acetone, mounted in a liquid suspension on a porous carbon grid, and mounted in the microscope. This method is useful on crystalline material for imaging crystal lattice fringes and diffraction patterns. Amorphous and microcrystalline Fe and Mn oxyhydroxides, present in many of the corrosion residues, were also identified by transmission electron microscopy (TEM). Microbial populations were viewed with light microscopy using methylene blue, gram, acid fast, and sudan black stains (Clark 1973).

Chemical analyses. Chemical analyses were performed on whole rock samples of wall rock, punk rock, and corrosion residue. For major and most trace elements, samples were analyzed using X-ray fluorescence (XRF). Selected trace elements were also analyzed by atomic adsorption. Other analytical determinations included insoluble residue analysis by dissolution of bedrock samples, water content, and Fe^{+2}/Fe^{+3} (Husler & Connolly 1991; Kolthoff & Sandell 1952). Mineralogical compositions were determined by X-ray diffraction (XRD) and scanning electron microscopy.

Enrichments. Enrichment and isolation media for manganese- and iron-oxidizing bacteria were prepared such that reduced iron (Fe^{+2}) or manganese (Mn^{+2}) was suspended in a stable gel to allow O_2 to diffuse towards the reduced material.

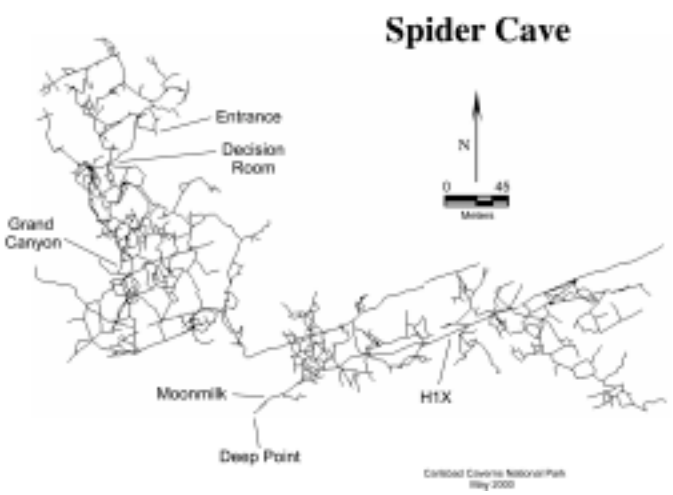

Figure 5. Sample sites in Spider Cave. Map by Stan Allison, U.S. National Park Service.

Hanert's modification of Wolfe's FeS medium was used for iron-oxidizing strains, and steel carpet tacks were added to the bottom of the media as a source of iron (Hanert 1992). Samples of moonmilk from Spider were inoculated on site into a very low nutrient medium developed for microbes using low molecular weight organic carbon sources (C_1-C_3) compounds and into an organic-rich medium suitable for various actinomycetes and fungi (Hose *et al.* 2000).

Molecular phylogeny. To avoid the limitations of culture-based techniques (Amann *et al*. 1995; Ward *et al*. 1992), molecular phylogenetic methodology that uses genetic sequence data from the small subunit ribosomal RNA (SSU rRNA) was utilized (Barns *et al*. 1994; Reysenbach *et al*. 1994).

Using techniques developed at Los Alamos National Laboratory for soil, we extracted DNA from Lechuguilla Cave corrosion residue samples using bead mill homogenization, followed by nucleic acid purification (Kuske *et al.* 1997). We then performed the polymerase chain reaction (PCR) employing primers specific for rRNA genes (rDNAs) to produce copies of the rDNAs in the extracted DNA. Amplification products were sorted by cloning into plasmid vectors. PCR products from eighty-two clones with the correct size insert were purified with a QIAprep plasmid miniprep kit (Qiagen, Inc., Chatsworth, Calif.). Purified DNA was used as a template in cycle sequencing. Sequences were tested for the presence of PCR-produced chimeric artifacts known to occur during this type of natural population analysis (Liesack *et al.* 1991; Kopczynski *et al.* 1994).

Bacterial metabolic activity. Direct estimates of total and respiring cells in both corrosion material and "punk rock" were made using microscopic techniques (Rusterholtz & Mallory 1994). Total cells were determined by counting cells stained with acridine orange, a dye that intercalates into DNA and is detected using epifluorescence microscopy. Acridine orange-stained cells fluoresce a bright green. Respiring cells are detected by staining cells with a respiratory dye ([2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride]: INT), added on site in the cave. This chemical is taken up and reduced by actively respiring cells. The reduced dye is seen as a distinct red crystal using bright-field microscopy. Killed controls and previously live samples were stained with a 0.1% acridine orange solution in the laboratory.

CASE STUDIES

CASE STUDY I: CORROSION RESIDUES

One cave environment having a high potential for geomicrobiological interactions is that of the "corrosion residues" (Fig. 3). Lechuguilla Cave is notably rich in this material, while lesser amounts exist in Spider Cave, Carlsbad Cavern, other Guadalupe caves, and also in caves in other parts of the world. Researchers have often assumed that corrosion residues are essentially insoluble components remaining after acid dissolution of cave bedrock that formed by abiological, dominantly inorganic, processes. For example, Queen (1994) hypothesized that corrosion residues are the long-term result of upwelling corrosive air where Rayleigh-Benard convection may induce air circulation that cools upon reaching the ceiling of the cave, resulting in the condensation of water droplets that absorb CO_2, forming carbonic acid that corrodes the bedrock.

Discovery of bacterial and fungal communities in the corrosion residues (Cunningham 1991a,b; Cunningham *et al*. 1995; Northup *et al*. 1995) has led to another explanation: that microbes could be active participants in the production of corrosion residues, in conjunction with abiological processes. Potentially, microorganisms could oxidize reduced compounds from the atmosphere or wall rock, producing acidity as a by-product. Extracellular polymers from bacteria are usually acidic and contain functional groups that readily bind metal ions and contribute to corrosion of metals and the breakdown of carbonates and silicates (Ford & Mitchell 1990; Little *et al.* 1986ab). Seminal studies by Costerton *et al.* (1987) have shown that microbial biofilms can greatly affect the substrates on which they grow by altering the microenvironments under the films to enhance corrosion and degradation of many materials.

Energy sources in corrosion residues, "punk rock", and wall rock. Bulk chemical and XRD analyses of host carbonates, punk rock, and corrosion residues have allowed us to track the mineral transformation from the interior of the rock out to the corrosion residues and identify potential energy sources for microorganisms. Corrosion residue in Guadalupe caves occurs as coatings up to 2 cm thick, overlying a layer of altered carbonate host rock on cave walls, floors and ceilings and even on loose boulders (Fig. 3). The host rock can be altered to a depth of up to 10 cm or more, an occurrence that was first termed "punk rock" by Hill (1987). Our studies indicate that host carbonates (dolomite/limestone) of the Permian Capitan Reef Complex contain up to 2.3% insoluble residue of quartz and clays (illite, kaolinite, dickite, and smectite) (see also Polyak & Güven 2000).

Cunningham's (1991a,b) analysis of corrosion residues from Lechuguilla Cave using SEM and EDX techniques identified several iron and manganese phases: todorokite [$(Mn^{2+},Ca,Mg)\ Mn_3^{4+}O_7 \cdot H_2O$] and poorly ordered iron oxides and hydroxides (mainly hematite and goethite), which are potential end products of bacterial metabolism. Mineral analyses of corrosion residues show a very high (50-80% by weight) manganese mineral component (Cunningham *et al.* 1995). DuChene's (1997) mineralogical inventory of corrosion residues in Lechuguilla Cave also showed the presence of ankerite, endellite, gibbsite, hydrated halloysite (endellite), hematite, goethite, griegite, nordstrandite, rancieite, and todorokite.

Our study of corrosion residues in Lechuguilla and Spider caves supports the variability of their mineralogical composition. Carbonates and quartz are either absent or limited. Kaolinite, dickite, and endellite (10-Angstrom hydrated halloysite [$Al_2Si_2O_5(OH)_4 \cdot 2H_2O$]) clays may be present, along with well-ordered aluminum hydroxide (mainly gibbsite).

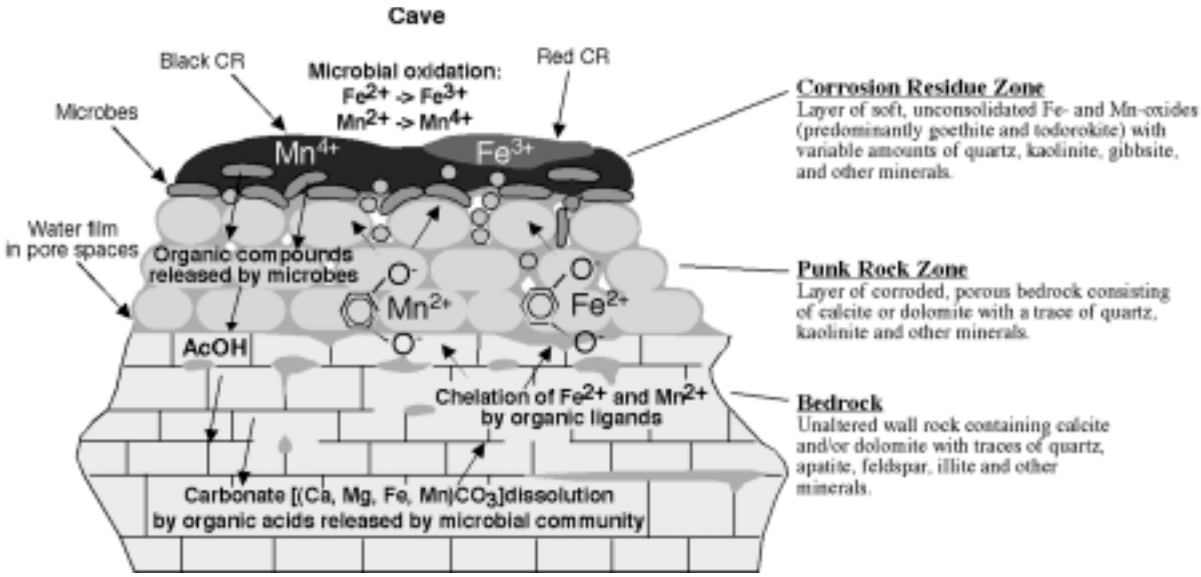

Figure 6.

Schematic depiction of a cross-section from the aerobic cave environment to more reducing conditions encountered within the wall. Modified from L. Mallory (unpublished). CR = corrosion residue.

Todorokite, goethite and hematite most likely represent oxidized by-products of reduced Mn and Fe present in trace or minor amounts in the original carbonate minerals.

Chemical analyses confirm that high concentrations of manganese and iron exist in corrosion residues, with a good correlation between color and chemical composition. Iron is associated with red, red-brown, brown and black (40, 50, 80, and 25 wt% oxide, respectively), and manganese is strongly associated with the black residues, which have yielded as high as 21.6 wt% Mn-oxide. Therefore, the corrosion residue shows a strong enrichment of manganese and iron relative to whole rock composition.

The corrosion residues do not form simply through dissolution of the host carbonate. They are mineralogically and chemically distinct from whole rock insoluble residue as shown by comparison of elemental ratios. Using the insoluble constituent aluminum for normalization, the ratio of SiO_2/Al_2O_3 is about 5.5 in the insoluble residue, but is closer to 1 in most corrosion residue analyses. Iron and manganese are highly enriched in almost all corrosion residues. Melim (1991) found that Mn^{+2} ranged from 30-220 ppm in the bedrock of Capitan Formation (reef and forereef) and from 20-200 ppm in the bedrock of backreef (Tansill and Yates formations), while Fe^{+2} ranged from 50-2863 ppm in the reef and forereef and from 64-2428 ppm in the backreef. Utilizing both these published ranges for manganese concentrations of 20-200 ppm in host rock Mn (our unpublished SIMS analyses for selected host carbonates yield concentrations from 18-50 ppm), and ~11% MnO in corrosion residues, mass balance calculations indicate a concentration factor of about 50 times by weight for Mn. However, the low density of corrosion residue translates to a thickness of 2.2 cm of host rock required to generate 1 cm of residue. Other elements, including Al, Fe, Si, and Mg, have been leached from punk rock. Ca, Mg, and Si have also probably been partially removed through aqueous leaching, although only trace amounts of liquid water are present.

Figure 6 schematically depicts a hypothesized cross-section from the aerobic cave environment to more reducing conditions encountered within the wall rock itself. Carbonate removal is proposed to be accomplished through microbially assisted dissolution of dolomite and limestone within the punk-rock zone. The presence in corrosion residues of bacteria whose closest identified relatives are bacteria known to utilize Fe or Mn oxidation as a metabolic pathway (*Leptothrix*, *Pedomicrobium manganicum*, and unidentified iron-oxidizing bacteria) lends support to this hypothesis. Quartz and illite in the wall rock react to form more acid-stable clays such as kaolinite; excess aluminum is incorporated into gibbsite in corrosion residues. As iron and manganese are oxidized, todorokite and amorphous/poorly crystalline ferric phases form, later aging to goethite and hematite.

Microscopic evidence of bacteria associated with corrosion residues. The morphology of putative bacteria in corrosion residues, punk rock, and wall rock includes coccoids, filaments, rods, stalked bacteria and more unusually shaped forms (Fig. 7a&b). Interwoven fabrics of filaments found in corrosion residues hanging and falling from the ceiling of Snowing Passage in Lechuguilla Cave (Fig. 8a&b) appear to be mineral. EDX studies show these filaments to be manganese oxides (todorokite), which knit together to form a mineral surface. Studies underway will establish whether there is any microbial involvement in their initial formation.

Enrichments for manganese- and iron-oxidizing bacteria. Enrichment cultures targeting iron-oxidizing bacteria, which utilize reduced iron in the media, produced visible growth in 72% (26/36) of tubes. Those targeting manganese-oxidizing bacteria produced growth in 90% (28/31) of tubes. In most instances, growth occurred as a band between 2-9 mm below the surface of the medium; occasionally growth at greater depths was observed. Visible growth ranged in color from white to yellow/orange (sometimes with black speckles) to black/brown. More black/brown growth was observed in the

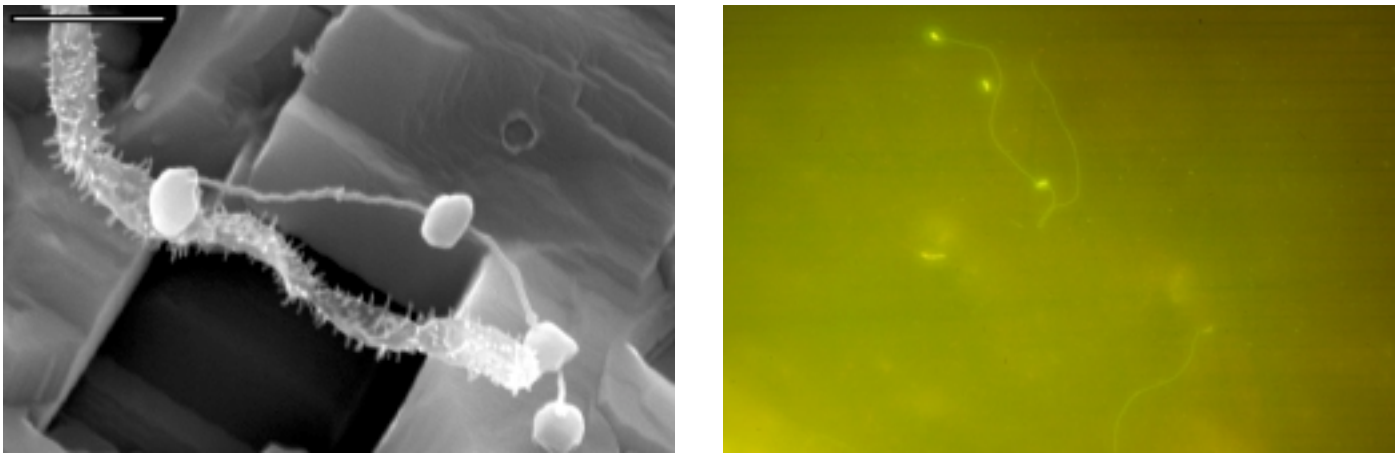

Figure 7. (a - Left) Bacteria found on corroded speleothem in Spider Cave. Note probable attachment structures. Scale bar = 2 μm. (b - Right) Stalked bacteria and rods stained with acridine orange. Field of view is ~200 μm. Photo by Rachel Schelble.

manganese enrichments. Examination of culture material with SEM/EDX revealed the presence of iron and manganese, presumably in the form of oxy-hydroxides. Such deposits were not part of the original media and demonstrate the ability of the cultured organisms to produce iron and manganese oxides. Molecular phylogenetic analysis of one culture showed the closest relatives to be actinomycetes. Some iron and manganese bacteria produce acidity as a product of their metabolic reactions (Ehrlich 1996). Therefore, these organisms are possible candidates for promoting dissolution of the wall rock and formation of corrosion residue.

Molecular evidence of manganese- and iron oxidizing bacteria from corrosion residues. Red-brown corrosion residues at the EA site have yielded diverse groups of organisms using molecular phylogenetic techniques from analysis of 46 clones. The closest known relatives of these microbes include low temperature archaea (the dominant clones at 56.5%), actinomycetes, nitrite-oxidizing bacteria, gram-positive bacteria, and Proteobacteria in the beta, gamma, and alpha subdivisions. Of particular interest for geomicrobiological studies is the presence of clones whose closest relatives are unidentified iron-oxidizing bacteria, *Leptothrix*, and *Variovorax paradoxus* in the beta subdivision of the Proteobacteria. *Leptothrix* is a manganese-oxidizing bacterium and *Variovorax paradoxus* is capable of lithoautotrophic growth using H_2 as an energy source. One clone's closest relative in the alpha subdivision is *Pedomicrobium manganicum*, a budding bacterium that accumulates iron and manganese oxides (Bergey 1989). Several sequences show less than 0.5 similarity to any other known 16S rDNA sequence, indicating they are novel organisms.

Studies of bacterial metabolic activity. Preliminary results show moderately large and active populations of bacteria in both corrosion material and punk rock. Respiring cell counts for corrosion residues examined in the EA survey indicate 1.6 x 10^7 cells per cm^3 of material representing 30% of total cells. Cell densities in punk rock were more varied. Respiring cell counts for punk rock examined in the EA survey ranged from 1 x 10^6 to 8.6 x 10^6 cells per cm^3 of material, representing 15-29% of total cells. The highest counts to date were found in the Sanctuary where 2 x 10^7 actively respiring cells per cm^3 of corrosion material were detected. These cells represent 37% of the total cells detected.

Summary. The presence of reduced iron and manganese compounds in wall rock, the existence of bacterial morphologies in corrosion residues and wall rock, culturing of putative iron- and manganese-oxidizing bacteria from corrosion residues, identification of organisms whose close relatives are iron- and manganese-oxidizing bacteria using molecular phylogenetic techniques, and bacterial metabolic activity in the punk rock region provide preliminary support for the hypothesis that microorganisms participate in the dissolution of the wall rock to form corrosion residues. We propose that the corrosion residues have built up over long time periods through microbially assisted dissolution and leaching of underlying host carbonate rock.

CASE STUDY II: POOL FINGERS

Davis *et al.* (1990: 80) described pool fingers as "slightly wiggly, slender calcite fingers up to 30 cm long and about 1.5-6 mm in diameter". Pool fingers are found in many dry and some active pools in Lechuguilla Cave, and also in Carlsbad Cavern and Hidden Cave. Pool fingers show evidence of bacterial presence, although the degree to which bacteria are involved in their formation has not been established.

Hidden Cave pool fingers. These pool fingers occur in a dry cave pool below the former water level and range from 1 to 5 cm in diameter and up to 20 cm long. They are more "robust" than those in Lechuguilla, having a rough outer surface with knobby protrusions up to 1 cm in diameter that extend up to 5 mm out from the side of each finger.

The core of each sampled pool finger consists of micritic

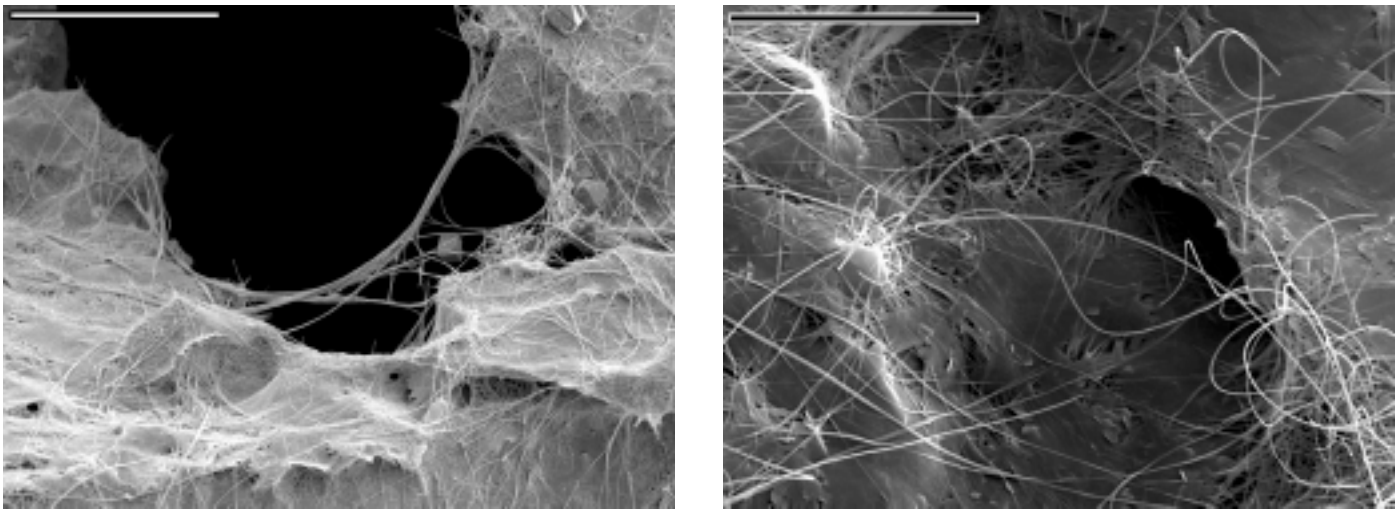

Figure 8. (a - Left) Interwoven fabric of filaments found hanging and falling from the ceiling of Snowing Passage in Lechuguilla Cave. The material may be mineralized microbial filaments. Scale bar = 20 μm. (b - Right) Higher magnification image of possible mineralized microbial filaments. Scale bar = 10 μm.

calcite, usually with dark-brown, 30-50 μm spherical lumps (Fig. 9). The thickness of the core ranges from 1-3 mm and varies within and between pool fingers. The core is not solid but rather has irregular, rounded pores up to 500 μm in diameter that are either open or filled later with brown clay and, rarely, hydromagnesite. These pores are much larger than the micritic material and are similar in appearance to fenestral pores found in marine limestones.

The outer part of each pool finger is composed of alternating layers of dark micritic calcite and bladed to dogtooth calcite spar (Fig. 9). The ratio of micrite to spar is highly variable, both between samples and within individual samples. For example, HC-3 is almost 50% micrite while HC-4 is only about 20% micrite. Micritic layers are usually <1 mm thick but reach up to 3 mm in thickness. The micrite varies from vaguely laminated to clotted with dark-brown, 30-100 μm blebs. Individual laminae thicken and thin dramatically along the length of each pool finger, often expanding into distinct clotted lumps up to 1 mm high made up of coalescing micrite. The protrusions seen on the macroscopic scale are underlain by clotted lumps of micrite on the microscopic scale that have been later coated and expanded by layers of calcite spar.

Spar layers are irregular and composed of crystals 50-100 μm wide and up to 2 mm long, often radiating out from dense micritic cores (Fig. 9). The distinction between spar and micrite is often gradational over ~1 mm, from micrite to microspar to coarse bladed calcite. Inclusions elongate and parallel to the long axis of the crystal are common. Some of these appear empty; others appear to be filled with micrite.

Preliminary SEM results document evidence of bacteria within the "robust" pool fingers of Hidden Cave. Rods, filaments, and more complex diamond, cross-hatched forms were observed on both freshly broken and etched samples (10% HCl after polishing). Some of these rods are hollow (Fig. 10). The dark, micritic cores of the Hidden Cave pool fingers are very similar to pelmicritic fabrics from marine environments that have been shown by Chafetz (1986) to be bacterial in origin. The laminated to clotted fabrics look like microbialites described from modern lacustrine (e.g., Braithwaite & Zedef 1996), hot spring (e.g., Chafetz & Folk 1984), and marine (e.g., Burne & Moore 1987; Chafetz & Buczynski 1992) environments. Based on the similarity of fabrics and the SEM documented occurrence of fossil bacteria, it is possible that bacteria contributed to the precipitation of the "robust" pool fingers.

Lechuguilla Cave pool fingers. The Lechuguilla Cave pool fingers sampled (1-1.5 cm across) are only slightly larger than those described by Davis *et al.* (1990). They are composed of radiating crystals of bladed calcite exhibiting a sweeping

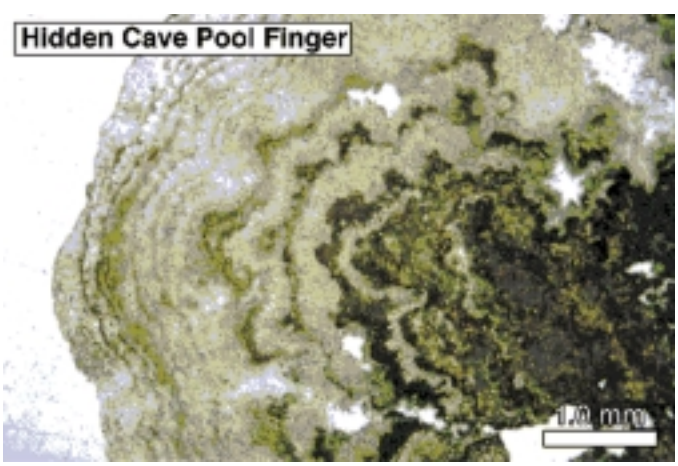

Figure 9. Photomicrograph of a cross-sectioned Hidden Cave pool finger showing alternating layers of dense micrite and light-colored spar. Note the distinct clumping of micrite in some layers.

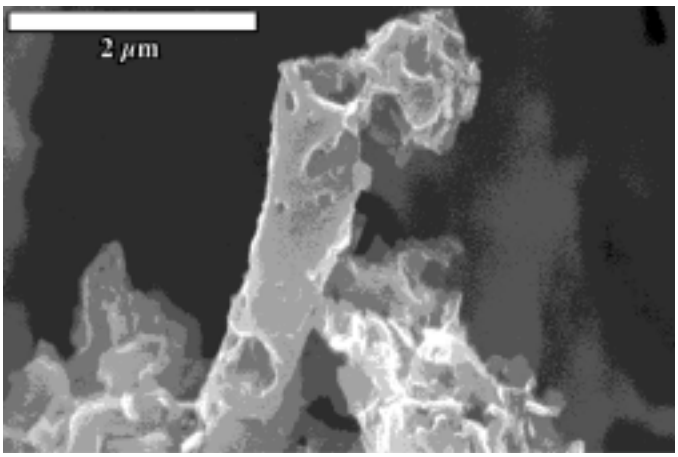

Figure 10. Scanning electron photomicrograph of hollow tube interpreted as bacterial filament found in Hidden Cave pool finger, etched sample.

extinction pattern and dogtooth terminations. The pool fingers do not have a distinct core. Individual crystals usually extend from the center of the finger all the way to the outer edge (5-7 mm), with the width increasing as the diameter of the finger increases. The crystals are limpid for the first 2-3 mm and then show faint-yellow, inclusion-rich banding. The bands are 100-200 μm thick inclusion-rich layers that form ghost outlines of dogtooth crystal terminations.

Like the spar in the Hidden Cave pool fingers, elongate inclusions are common in the Lechuguilla samples; however, micritic layers are absent. Filamentous inclusions occur irregularly, usually associated with patches of elongate or equant inclusions (Fig. 11a). The filaments are 1 μm in width and 50-100 μm long and intertwine like tangled hairs (Fig. 11b). The filaments do not appear to branch, but the way they intertwine makes it difficult to be sure. The filaments are most common in the clear portions and are nearly absent in the yellow, inclusion-rich bands of ghost crystals. These filaments are probably bacteria, but they do not appear to have been responsible for mineral precipitation because of their irregular distribution. Chafetz & Folk (1984) documented similar fabrics and considered them passive colonies engulfed by growing crystals.

Summary. Pool fingers are elongate, pendant speleothems that form underwater. They have an irregular, knobby external appearance underlain by laminations of dark, clotted micrite and/or clear, inclusion-rich calcite spar. In some samples, the inclusions are obviously bacterial. The external and internal fabrics of Hidden Cave pool fingers strongly resemble well-described microbialites from surface environments. This similarity, combined with SEM identification of fossil bacteria in etched samples, suggests bacterial involvement in the formation of the pool fingers.

CASE STUDY III: MOONMILK

Moonmilk is a generic term for a pasty, semi-fluid material consisting of a suspension of micrometer-sized crystals of calcite, hydromagnesite, gypsum, or other minerals (Hill & Forti 1997). Some moonmilk is thought to be the result of chemical precipitation, particularly in the case of gypsum paste residue from the reaction of limestone with sulfuric acid. However, other types of moonmilk may have a microbial origin, either by direct precipitation of calcite by microorganisms (Northup *et al.* 1997*;* Castanier *et al*. 1999), or by forming a nucleation surface on which minerals precipitate (Pentecost & Bauld 1988; Jones & Kahle 1993).

Although moonmilk is present in many caves in the Guadalupe Mountains, it rarely occurs as extensive deposits. In Spider Cave, however, a spectacular type of moonmilk coats the walls and ceilings of the lowest portions of the cave. This moonmilk resembles curds of cottage cheese with a greasy texture and is slippery under foot. When examined by SEM, this material exhibits a filamentous habit (Fig. 12), and resembles a felted mat of fibers. The individual fibers have a curved morphology unlike most crystalline minerals. EDX analysis in the

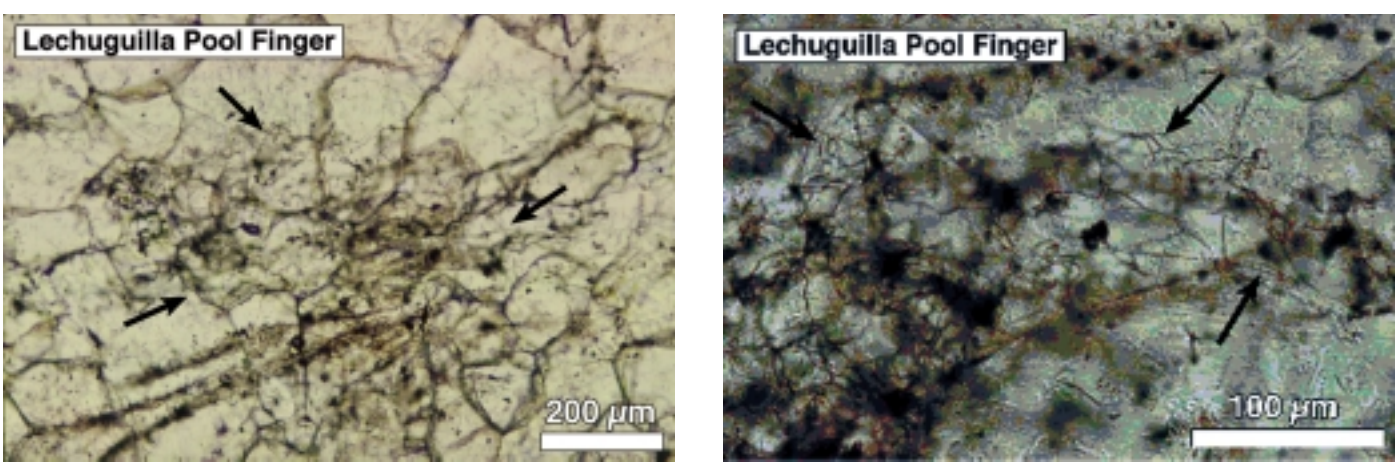

Figure 11. (a - Left) Lechuguilla Cave pool finger showing fine filaments (arrows) in clear spar associated with inclusions (dark areas). (b - Right) Higher magnification image of filaments (arrows).

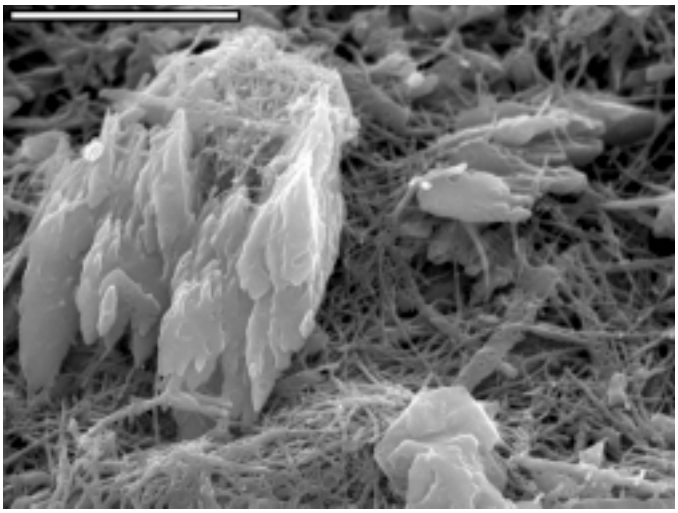

Figure 12. Fibrous calcite from Spider Cave. SEM micrograph of filamentous calcite moonmilk. Both fibers and corroded calcite crystals are present. Scale bar = 5 μm.

SEM shows the presence of Ca, O and C peaks; X-ray diffraction patterns indicate that only calcite makes up the bulk material. When the curds are dissolved in dilute hydrochloric acid, a gelatinous mass remains. This fact, coupled with its unusual filamentous fabric, suggests that the material may consist of microbial filaments coated or mineralized with calcite. However, when samples are viewed under TEM the resulting images show straight, needle-like fibers of crystalline calcite. Since mineral texture alone did not seem sufficient to determine origin, biological culturing experiments were also conducted on this moonmilk.

Viable cultures of mixed cellular types were obtained on three different versions of low nutrient media. Coccoid, ovoid, and stubby rod-shaped cells (~1-2 μm) were present upon inspection with a number of standard light microscopy techniques. In addition, fibers of a bimodal size distribution were present. Large fibers ranged from 1-2 μm in diameter and ranged from 10-50 μm in length; small fibers range from 0.05-0.1 μm in diameter and <2 μm long.

Only a few of the coccoid cells stained with simple methylene blue stain. Interestingly, and surprisingly, some of the fibers also stained with methylene blue. There were mixed gram negative and gram positive cells of all shapes. There were no acid-fast cells, but some of the small fibers did show positive acid-fast staining. No cells or fibers took up the sudan black into their interiors. Acridine orange (AO) stained many of the cellular bodies and also many of the fibers, especially the large fibers. Different concentrations of AO were used, and the length of time the samples took to be stained varied. In culture, the number of visually distinguishable cell types diminished, although cellular densities remained high over 3 to 6 months. Fibers grew in the initial cultures, but after the third transfer few were left.

The nature of the fibers (mineral, biological or both) is unclear. Some of the fibers may be composed of exterior mineral coatings overlying living interiors. Such a possibility would explain why some fibers took up biological dyes. The fibers also may be non-living mineral by-products of cell metabolism. Alternatively, the fibers may be unrelated mineral phases that occur by means of purely mineralogical processes, with the resulting material simply being a suitable habitat for microbial colonization. Future experiments will include the extraction of DNA directly from moonmilk for molecular phylogenetic studies, *in situ* experiments looking for metabolic and enzymatic activity in the moonmilk, and further SEM studies to view the matrix of the material.

Summary. SEM studies of the extensive moonmilk deposits in Spider Cave revealed a felted mat of fibers composed of calcite. The images of the filaments suggest a microbial origin, but TEM studies suggest they are crystalline calcite. Enrichment cultures reveal a diverse microbial community, including filamentous bacteria, associated with the moonmilk. These conflicting results support the need for additional studies.

HUMAN IMPACT ON MICROBIOLOGICAL/GEOLOGIC INTERACTIONS

Rich and unique microbial communities in caves can be harmed by human visitation. Humans are great reservoirs of organic matter that can be shed in the form of hair, skin fragments, and clothing lint. Many microorganisms important in geomicrobiological interactions do not thrive in environments rich in organic matter, such as those created by the dumping of urine or feces. Dumped urine represents a very rich source of nitrogen-rich organic compounds that can support the growth of both bacteria and fungi alien to low-nutrient cave environments, eliminating native species and reducing biodiversity.

To assess the extent of harm of urine dumping cases, we studied total heterotrophic bacteria and fungi population changes through time in response to additions of urine or water to soil samples collected from Lechuguilla Cave. The loss of urea was measured as an indication of recovery time. Overall, population levels in all samples started out relatively low ($<10^4$ colony forming units/g) and stayed low when water was added to soils. Bacteria and fungi responded to the addition of large or small amounts of urine with an increase in the number of colonies by a factor of 100-1000 times. Concentrations of urea in the samples gradually decreased over the course of a month. When urine was re-added to the soils, the response of the microbes was much quicker, showing an accumulation and persistence of urine-utilizing bacteria in the soil. The build-up of microbes caused changes in the texture and structure of the soils. The addition of water to the soils that had first been treated with urine showed only minor changes in populations, demonstrating the importance of the added nutrients in urine to support microbial growth. These results show that urination in caves can cause drastic changes on population levels of microbes in soils, and that these changes persist after urine-

derived organic compounds have been removed by biological action. The effects appear to be additive, and responses of the microbial community to multiple applications are rapid.

Poor sanitation conditions while camping underground can lead to fecal contamination of drinking sources and cave soils (Boston 1999). Red Lake, in Lechuguilla Cave, has been used as an in-cave water source for many years, but fecal contamination was suspected after an expedition using the lake developed gastro-intestinal problems. Upon analysis, coliform bacteria were found in puddles and on surfaces near Red Lake, but not in the main pool itself. In an emergency response, the water from puddles was collected and hauled off to urine dump sites, and the Red Lake area closed to human traffic. Follow-up sampling showed the persistence of coliforms in all contaminated sites after 6 and 14 months.

Summary. Input of organic material can have a detrimental effect on oligotrophic microbial communities, resulting in changes in population and community structure. Bacteria that utilize inorganic energy sources, such as those reported in corrosion residues and sulfur deposits, also are negatively impacted by organic enrichment. Urine deposition and other sources of organic carbon enrichment can seriously impact indigenous populations of microbes, and must be minimized if indigenous microbial communities are to be preserved. We strongly recommend that to reduce human impact in Guadalupe caves, a filtration system be devised or that urine be removed from the cave.

CONCLUSIONS

Metabolically active microorganisms are present on corroded formations in Spider Cave, and within punk rock and corrosion residues in Lechuguilla and Spider caves. Targeted enrichments and molecular phylogenetic studies confirm that at least some of these bacteria are iron- or manganese-oxidizing bacteria that may contribute to carbonate dissolution. Bulk chemistry and XRD studies have shown that the corrosion residues are not merely the product of dissolution. These results begin to build a case for microbially assisted dissolution and leaching of underlying host carbonate rock. Hidden Cave pool fingers show a dense micritic core, resembling pelmicritic fabrics of probable bacterial origin and laminated fabrics resembling microbialites. Lechuguilla Cave pool fingers show none of these features; filaments do occur, but are irregularly distributed. Thus, pool fingers show differing degrees of microbial involvement. Spider Cave moonmilk shows extensive filamentous deposits upon SEM examination, but TEM studies could not confirm a microbial component. Microbial enrichments of these deposits demonstrate the presence of a diverse microbial community, including filamentous bacteria. Thus, the evidence is equivocal.

Studies of the impact of urine dumping in low nutrient caves demonstrate that the addition of organic carbon can be highly detrimental to indigenous microbial populations. Therefore, limiting organic carbon addition is critical.

Ongoing studies of pool fingers, corrosion residues, and moonmilk will further elucidate the extent of bacterial-rock interactions. Other speleothems, such as cave pearls, show great promise of microbial involvement in their formation and await investigation. The study of geomicrobiological interactions in Guadalupe Mountain caves is only beginning.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Dale Pate, Stan Allison, Jason Richards, and Paul Burger of the National Park Service and Ransom Turner, Kevin Glover, and Susan Herpin of the U.S. Forest Service for their support of our field studies. Collection of all samples was by permit from Carlsbad Caverns National Park and the U.S. Forest Service. This work was supported by National Science Foundation grant number DEB-9809096, the National Speleological Society, and the Biology Department and the Institute of Meteoritics, University of New Mexico. Laura Bean, Rachel Schelble, Kathy Dotson, Andi Hunter, Cynthia Connolly, Marian Skupski, Don Natvig, Megan Armstrong, and Kristen Shinglman provided valuable lab assistance. Anna-Louise Reysenbach and Norm Pace provided critical technical guidance and lab support for the molecular work. We thank Gregor Eberli and Peter Swart for their support during the project. J. Michael Queen provided seminal discussions and field assistance. The manuscript benefited greatly from careful review by Carol Hill and Norman Pace.

REFERENCES

Amann, R.I., Ludwig, W. & Schleifer, K.-H. (1995). Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiological Reviews 59*: 143-169.

Barns, S.M., Fundyga, R.E., Jeffries, M.W. & Pace, N.R. (1994). Remarkable archaeal diversity detected in a Yellowstone National Park hot spring environment. *Proceedings of the National Academy of Sciences USA 91*: 1609-1613.

Bergey, D. H. (1989). *Bergey's manual of systematic bacteriology*, v. 3. Williams and Wilkins, Baltimore, MD: 1601-2298.

Boston, P.J. (1999). Red Lake contamination. Unpublished technical report to Carlsbad Caverns National Park: 6 pp.

Braithwaite, C.J.R. & Zedef, V. (1996). Hydromagnesite stromatolites and sediments in an alkaline lake, Salda Gölü, Turkey. *Journal of Sedimentary Research 66*: 991-1002.

Burne, R.V. & Moore, L.S. (1987). Microbialites: Organosedimentary deposits of benthic microbial communities. *Palaios 2*: 241-255.

Castanier, S., Metayer-Levrel, G.L. & Pertuisot, J.P. (1999). Ca-carbonate precipitation and limestone genesis-The microbiogeologist point of view. *Sedimentary Geology 126*: 9-23.

Chafetz, H.S. (1986). Marine peloids: A product of bacterially induced precipitation of calcite. *Journal of Sedimentary Petrology 56*: 812-817.

Chafetz, H.S. & Buczynski, C. (1992). Bacterially induced lithification of microbial mats. *Palaios 7*: 277-293.

Chafetz, H.S. & Folk, R.L. (1984). Travertines: Depositional morphology and the bacterially constructed constituents. *Journal of Sedimentary Petrology 54*: 289-316.

Clark, G. (1973). *Staining procedures used by the biological stain commission*. *3rd ed*. The Williams & Wilkins Co., Baltimore, Maryland: 418 pp.

Costerton, J.W., Cheng, K.-J., Geesey, G.G., Ladd, T.I., Nickel, J.C., Dasgupta, M. & Marrie, T.J. (1987). Bacterial biofilms in nature and disease. *Annual Review of Microbiology 41*: 435-464.

Cunningham, K.I. (1991a). Organic and inorganic composition of colored corrosion residues: Lechuguilla Cave: Preliminary report. *NSS News 49*: 252,254.

Cunningham, K.I. (1991b). News scrapbook. *NSS News 49*: 325.

Cunningham, K.I., Northup, D.E., Pollastro, R.M., Wright, W.G. & La Rock, E.J. (1995). Bacteria, fungi and biokarst in Lechuguilla Cave, Carlsbad Caverns National Park, New Mexico. *Environmental Geology 25*: 2-8.

Danielli, H.M.C. & Edington, M.A. (1983). Bacterial calcification in limestone caves. *Geomicrobiology Journal 3*: 1-16.

Davis, D.G., Palmer, M.V. & Palmer, A.N. (1990). Extraordinary subaqueous speleothems in Lechuguilla Cave, New Mexico. *NSS Bulletin 52*: 70-86.

DuChene, H.R. (1997). Lechuguilla Cave, New Mexico, U.S.A. In Hill, C.A. & Forti, P. (eds.) *Cave minerals of the world. 2nd ed*. Huntsville, AL. National Speleological Society: 343-350.

Ehrlich, H.L. (1996). *Geomicrobiology*. New York, Marcel Dekker: 719 pp.

Ford, T. & Mitchell, R. (1990). The ecology of microbial corrosion. In Marshall, K.C. (ed.). *Advances in microbial ecology II*. Plenum Press, New York, NY: 231-262.

Hanert, H.H. (1992). The genus *Gallionella*. In Balows, A. (ed.) *The prokaryotes: A handbook on the biology of bacteria: Ecophysiology, isolation, identification, applications*. Springer-Verlag, New York, NY: 509-515.

Hill, C.A. (1987). *Geology of Carlsbad Cavern and other caves in the Guadalupe Mountains, New Mexico and Texas*. New Mexico Bureau of Mines and Mineral Resources Bulletin 117: 150 pp.

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

Hose, L.D., Palmer, A.N., Palmer, M.V., Northup, D.E., Boston, P.J. & DuChene, H.R. (2000). Effects of geomicrobiological processes in a hydrogen sulphide-rich karst environment. *Chemical Geology 169*: 399-423.

Hose, L.D. & Pisarowicz, J.A. (1999). Cueva de Villa Luz, Tabasco, Mexico: Reconnaissance study of an active sulfur spring cave and ecosystem. *Journal of Cave and Karst Studies 61*: 13-21.

Husler, J. & Connolly, J. (1991*). Procedures for laboratory sample bulk chemical determination*. Sandia National Laboratory Technical Procedures 61: 72 pp.

Jones, B. & Kahle, C.F. (1993). Morphology, relationship, and origin of fiber and dendrite calcite crystals. *Journal of Sedimentary Petrology 63*: 1018-1031.

Kolthoff, I.M. & Sandell, E.B. (1952). *Textbook of quantitative inorganic analysis*. *3rd ed*. New York. Macmillan: 759 pp.

Kopczynski, E.D., Bateson, M.M. & Ward, D.M. (1994). Recognition of chimeric all-subunit ribosomal DNAs composed of genes from uncultivated microorganisms. *Applied and Environmental Microbiology 60*: 746-748.

Kuske, C. R., Barns, S.M. & Busch, J.D. (1997). Diverse uncultivated bacterial groups from soils of the arid southwestern United States that are present in many geographic regions. *Applied and Environmental Microbiology 63*: 3614-3621.

Liesack, W., Weyland, H. & Stackebrandt, E. (1991). Potential risks of gene amplification by PCR as determined by 16S rDNA analysis of a mixed-culture of strict barophilic bacteria. *Microbial Ecology 21*: 191-198.

Little, B.J., Wagner, P. & Gerchakov, S.M. (1986a). A quantitative investigation of mechanisms for microbial corrosion. In Dexter, S.C. (ed.) *Biologically induced corrosion*. National Association of Corrosion Engineers: 209-214.

Little, B.J., Wagner, P. Gerchakov, S.M., Walch, M. & Mitchell, R. (1986b). The involvement of a thermophilic bacterium in corrosion processes. *Corrosion 42*: 533-536.

Melim, L.A. (1991). *The origin of dolomite in the Permian (Guadalupian) Capitan Formation, Delaware Basin, west Texas and New Mexico: Implications for dolomitization models*. Unpublished PhD dissertation, Southern Methodist University, Dallas, TX: 215 pp.

Northup, D.E., Carr, D.L., Crocker, M.T., Hawkins, L.K., Leonard, P. & Welbourn, W.C. (1995). Biological investigations in Lechuguilla Cave. *NSS Bulletin 56*: 54-63.

Northup, D.E., Reysenbach, A.L. & Pace, N.R. (1997). Microorganisms and speleothems. In Hill, C.A. & Forti, P. (eds.) *Cave Minerals of the World 2nd ed*. National Speleological Society, Huntsville, Alabama: 261-266.

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

Pentecost, A. & Bauld, J . (1988). Nucleation of calcite on the sheaths of cyanobacteria using a simple diffusion cell. *Geomicrobiology Journal 6*: 129-135.

Polyak, V.J. & Güven, N. (2000). Clays in caves of the Guadalupe Mountains, New Mexico. *Journal of Cave and Karst Studies 62*(2): 120-126.

Queen, J.M. (1994). Influence of thermal atmospheric convection on the nature and distribution of microbiota in cave environments. In Sasowsky, I.D. & Palmer, M.V. (eds.) *Breakthroughs in karst geomicrobiology and redox chemistry*, Karst Waters Institute Special Publication 1: 62-64.

Reysenbach, A.-L., Wickham, G.S. & Pace, N.R. (1994). Phylogenetic analysis of the hyperthermophilic pink filament community in Octopus Spring, Yellowstone National Park. *Applied and Environmental Microbiology 60*: 2113-2119.

Rusterholtz, K. & Mallory, L.M. (1994). Density, activity and diversity of bacteria indigenous to a karstic aquifer. *Microbial Ecology 28*: 79-99.

Ward, D.M., Bateson, M.M., Weller, R. & Ruff-Roberts, A.L. (1992). Ribosomal RNA analysis of microorganisms as they occur in nature . *Advances in Microbial Ecology 12*: 219-286.

Went, F.W. (1969). Fungi associated with stalactite growth. *Science 166*: 385-386.