Final Project Report: ***Characterizing the Biogenicity of Manganese Oxides in an Extreme Environment: Fort Stanton Cave as a Solar System Analog***

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Background and Overview of Expedition Objectives:
The recent discovery of manganese deposits on the Martian surface, at a concentration two orders of magnitude higher than previously observed, is a good indicator of the planet's aqueous history as well as the redox evolution of the environment. On Earth, manganese oxides are easily metabolized by primitive lithoautotrophs; therefore, manganese-rich environments on Mars serve as promising targets for astrobiology investigations.

Manganese oxide may be produced through both biotic and abiotic mechanisms, and is commonly found as a varnish coating on a host rock in arid environments. Most manganese oxide varnishes on Earth are biogenic, due to the kinetically favorable oxidation of these minerals by microorganisms compared with abiotic oxidation processes, however a biologic mechanism for the precipitation of iron and manganese varnish coatings on Mars has not yet been confirmed. Manganese oxide deposits in cave systems are generally rare, and can be the result of insoluble residue produced by the corrosion of carbonate bedrock. This observed corrosion residue could have been biologically influenced; the dissolution of manganese in the carbonate bedrock may be the result of the extraction of reduced manganese by microbes, which oxidize and deposit the manganese oxides at the surface. Reduced manganese may be oxidized by a microorganism (which may include bacteria, fungi, algae, and/or eukaryotes) to yield manganese oxide (Mn(II)O, $Mn(II,III)_3O_2$, or $Mn(IV)O_2$), which is then diffused to lower strata. Microbes that occupy these regions below the air-rock interface (and therefore have limited access to O_2) may reduce the oxidized manganese using organic carbon as a catalyst, producing Mn^{2+}. These microbes use the insoluble MnO_2 as an oxygen substitute, and their metabolic waste product is recycled as a redox reagent. The following equations depict the oxidation of manganese.

$$Mn^{2+} + \frac{1}{4}O_2 + \frac{3}{2}H_2O \rightarrow Mn(II)OOH + 2H^+$$

$$3Mn^{2+} + \frac{1}{2}O_2 + 3H_2O \rightarrow Mn(II,III)_3O_4 + 6H^+$$

$$Mn^{2+} + \frac{1}{2}O_2 + H_2O \rightarrow Mn(IV)O_2 + 2H^+$$

In order to confirm the biogenicity of such deposits on another planet, such as Mars, a suite of life detection instruments will first need to be tested on terrestrial field samples. The biosignatures associated with each instrument must also be characterized a priori.

Microbially-altered manganese oxides are present in Fort Stanton cave, and our previous work characterizing biosignatures from manganese deposits collected near Snowy River Passage suggests that Fort Stanton Cave is an excellent field site for these studies. The growth of cave manganese deposits is certainly enhanced by microbial activity; however the primary source of reduced manganese remains unknown, and therefore the extent of the influence manganese oxidizing bacteria have on these formations is uncertain. By measuring the concentration of these biosignatures in samples at various locations within the cave, we will ascertain whether manganese oxidizing bacteria predominantly leach reduced manganese by corroding bedrock, or whether reduced metals are transported to the cave wall via water sources, where microbial communities take advantage of abiotically precipitated varnish layers. If the latter is the dominant mechanism, we would expect manganese deposits close to water sources to be more frequently resupplied with reduced manganese, and therefore these communities of manganese oxidizing bacteria are expected to be more active. Collecting samples at variable distances to water sources would therefore provide us with varnishes containing a range of reduced manganese content, and thus a range of microbial activity.

Fort Stanton Cave Field Expedition:
We collected small rock samples containing manganese oxide deposits from inconspicuous locations within Fort Stanton Cave (entrance at 33° 30' 24.5844" N; 105° 29' 35.8506" W) at varying distances from water sources. A map of our path through the cave is presented in Figure 1. Samples are primarily categorized as manganese-coated clays and Liesegang Ring patterns (Figure 2), shown previously to be biologically mediated. Samples were acquired with sterile sample handling gloves, and placed in bulk sampling containers and DNA/RNA shield tubes.

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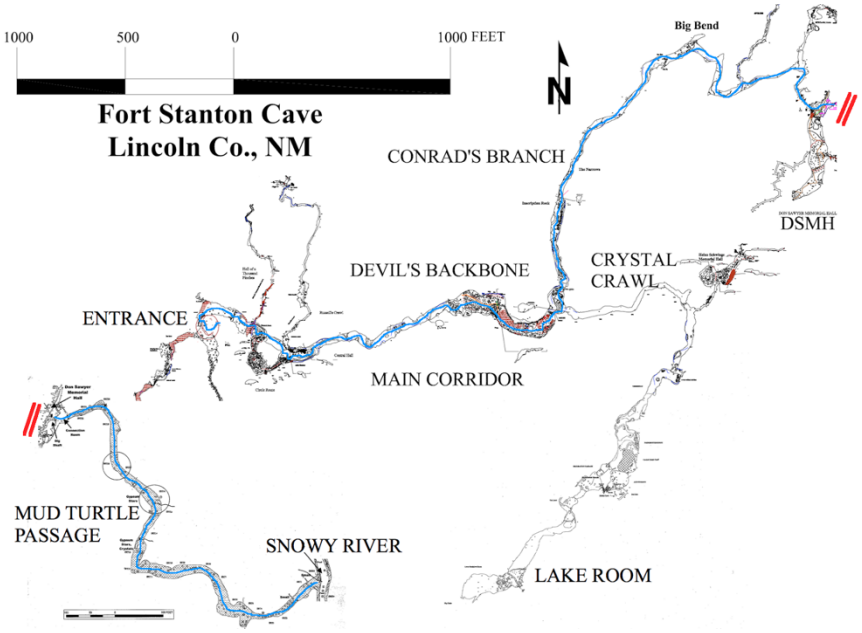

Figure 1: Approximate path through Fort Stanton Cave (blue path). This map has been truncated – mapped passages of the cave now exceed 50 km. Map courtesy of the Fort Stanton Cave Study Project.

Each sample was measured with the following three instruments to test their ability to characterize manganese oxide biosignatures:

1) Laser desorption time-of-flight mass spectrometer (LD-TOF-MS) (at NASA's Goddard Space Flight Center (GSFC)) identifies organic molecules and the molecular products associated with metabolization

2) Acoutso-optic tunable filter (AOTF) infrared (IR) reflectance spectrometer (at NMSU) identifies the crystal structure of the precipitated manganese oxides. Microbial mediation of manganese oxides produces more amorphous crystals compared with abiogenic precipitation

3) X-Ray power diffraction (XRD) (at NASA's GSFC) identifies the presence of manganate, a negatively charged MnO_4^{2-} uniquely precipitated by manganese oxidizing bacteria.

*Figure 2: **(Left)** Collection of Sample FSC305 by Kyle Uckert. Photo courtesy of Pete Lindsley. **(Right)** Liesegang Ring patterns on clay rocks just off the trail in Mud Turtle Passage.*

I divided each sample collected in half, inoculated half to act as a control (by heating to 90°C) and allowed the remaining half to continue growing in the lab under conditions designed to mimic the cave environment. I will measure each sample every 8 months with each instrument to

monitor the growth of the microbial population, as inferred by the detection of biosignatures. The estimated length of time to culture these samples is one year. Results of these studies will be prepared into a manuscript format for publishing.

Several LD-TOF-MS are available at the NASA GSFC Planetary Environments Laboratory, including commercial and prototype instruments, and can measure the molecular composition of a sample. Biosignatures may include the presence of high mass organic molecules and insoluble manganese oxides (Mn_2O_3, MnO(OH), Mn_3O_4, and MnO_2), especially oxides with high oxidation states (e.g. Mn(IV) oxides), which are indicative of biologically precipitated minerals.

The chemical and bulk organic composition of a sample may be probed by the IR spectrometer available at NMSU (PASA). The spectral range of PASA covers absorption bands of many of the basic organic compounds essential to life on Earth, including characteristic group frequencies of amino, hydroxyl, and methyl groups, and O-H, N-H, and C-H stretching fundamentals. An IR reflectance spectrum contains information about the mineral classes and surface reflectivity of a sample, which may be altered by the presence of microbial activity on rock surfaces

The bulk chemical composition of a sample is also revealed by XRD. Microbial precipitation of manganese oxides produces manganate (MnO_4^{2-}) more frequently than abiotic precipitation mechanisms. A higher concentration of manganate relative to control samples may indicate the active production of manganese oxides.