LOI 2:

1) PROPOSAL TITLE: Analysis of soil-like materials in mine:

2) List of participants (partial):

Sookie Bang-SDSMT

Bruce Bleakley-SDSU

James Staley-University of Washington, Seattle

Mike Lehman-USDA-ARS

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3) Brief Description of proposed program:

Soil-like materials are present in many of the horizontal shafts in Homestake. These soil-like materials are probably in part soil that has been transported from the Earth's surface by human traffic, but much or most of it is probably secondary clay minerals, silt and/or sand produced by weathering of primary minerals in the mine.

Production of such materials below the Earth's surface makes them of interest because, unlike soils that develop at the Earth's surface, there is little or no green plant material growing in/decomposing in these subsurface areas, except for wooden materials such as railroad ties, etc. that were brought into the mine. Lack of abundant plant materials (such as plant roots) will limit the types of organic compounds introduced into these subsurface soils, and in turn greatly affect the types of microbial communities that are present in these soil materials.

The lowest level of the mine, with its elevated temperature and probably higher soil moisture content than levels nearer the surface, should lead to accelerated weathering of the soil materials compared to those formed nearer the Earth's surface.

Hypothesis: Chemical and physical properties of these subsurface soils will differ greatly from the surface soils that lie directly above the mine.

Hypothesis: Chemical and physical properties of these subsurface soils will be more similar at one given depth than at different depths in the mine.

Hypothesis: Subsurface soil materials formed in the deeper levels of the mine will be more highly weathered (and thus less fertile) than soil materials formed closer to the Earth's surface.

Chemical and physical analyses of the subsurface soils (and surface soils lying above and/or near the mine) will be conducted at South Dakota State University. Chemical analyses will include soil N,P,K levels; cation exchange capacity; organic matter; pH; selected metals; and soluble salts. Physical analyses will include soil particle size fractionation and textural analysis; aggregate stability analysis; particle density; and bulk density.

At several locations in the mine, standing and/or flowing water is present. Water samples will be obtained from selected areas and analyzed chemically (for pH, soluble salts, metals, organic carbon, etc.). Results will be compared to the subsurface soil nearest the water, to see if chemical properties of the soil and water are similar.

Hypothesis: Subsurface soil microbial communities will differ at different locations found at the same depth, due to gradients of moisture, temperature, and proximity to organic matter (especially lignocellulose/wood), and/or differences in soil texture.

Hypothesis: Soil microbial communities will differ at different depths in the mine, due to gradients of moisture, temperature, and proximity to organic matter (especially wood), and/or differences in soil texture.

Hypothesis: Soil microbial communities will differ most extensively between different depths, compared to microbial communities in different locations at the same depth.

Analyses of the subsurface soil microbial communities will include extraction of microbial community DNA from the soil materials, followed by analysis of PCR-amplified small subunit ribosomal RNA sequences via denaturing gradient gel electrophoresis (DGGE). Oligonucleotide primers specific for the Domains Bacteria and Archaea will be used to examine the prokaryotic microbial community. The DNA from discrete DGGE gel bands will be cloned into plasmid cloning vectors. Cloned fragments will be sequenced, and matches for the sequences will be sought via BLAST to determine identity of the major members of the subsurface microbial community (both culturable and non-culturable).
Also, a complete DNA clone library of DNA from both culturable and nonculturable microbes in the soil materials would be constructed and analyzed.

Carbon source utilization fingerprints of the subsurface soil microbial communities will be conducted using Biolog carbon source utilization plates. Direct microscopic counts to enumerate total bacterial numbers will be done using fluorescence

microscopy. Fluorescence *in situ* hybridization analysis (FISH) of soil samples will be compared to DGGE results to help describe the microbial community and the physical orientation of different microbial species at the microscopic level. Selected soil enzyme activities (cellulose, dehydrogenase, etc.) will be assayed using standard soil enzyme assays. Soil microbial biomass will be measured by chloroform fumigation technique. Soil respiration will be assayed using soil respirometer apparatus. Plate counts of culturable soil microbes will be conducted using selective culture media to count heterotrophic bacteria, fungi, and actinomycetes. Most probable number (MPN) estimates of selected physiologic groups such as nitrifiers, denitrifiers, nitrogen fixers, sulfur oxidizers, and sulfate reducers will be performed using appropriate broth culture media. Protozoans and nematodes will also be enumerated using standard methods. Assay of microbial processes in the subsurface soils such as denitrification, methanogenesis, and/or nitrogen fixation will use standard gas chromatographic methods.

At several locations in the mine, where there is standing and/or flowing water, water samples will be obtained from selected areas and subjected to the same microbiological analyses as for the subsurface soil. Results will be compared to the subsurface soil nearest the water, to see if chemical properties of the soil and water are similar.

4) Rough estimate of space requirements and specific or unusual technical issues involved in proposal: Some of the microbial work would be carried out at campuses and research facilities at a distance from the mine. If some microbiology facilities were available in the mine, some work could be conducted there, with the advantage of little or no storage time before sample processing.

5) Estimate of when access to underground facility will be required:

It would be desirable to have access to the facility sometime in 2006 , to be able to visit different sites and establish what specific sites would be best to concentrate studies on.

6) Any other general requirements or questions for the experiment, research, or outreach activities: None at present.