

Environment and Natural Resources Trust Fund (ENRTF) 2010 Work Program

Date of Report: 11/24/2009
Date of Next Progress Report: January 2011
Date of Work Program Approval:
Project Completion Date: 6/30/2013

I. PROJECT TITLE: Science and Innovation from Soudan Underground Mine State Park

Project Manager: Jeffrey A. Gralnick
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Location: Ramsey County, St. Paul / Hennepin County, Minneapolis / St. Louis County, Soudan (Breitung Township)

Total ENRTF Project Budget:	ENRTF Appropriation	\$ 545,000
	Minus Amount Spent:	\$ 0
	Equal Balance:	\$ 545,000

Legal Citation: M.L. 2010, Chp. 362, Sec. 2, Subd. 3f

Appropriation Language:

\$545,000 is from the trust fund to the Board of Regents of the University of Minnesota to characterize unique microbes discovered in the Soudan Underground Mine State Park and investigate the potential application in bioenergy and bioremediation. This appropriation is available until June 30, 2013, by which time the project must be completed and final products delivered.

II. PROJECT SUMMARY AND RESULTS:

The Soudan Iron Mine near Ely, MN is home to an extreme environment where microorganisms are thriving 2300 feet below the surface in an ancient, salty brine. Though mining operations have been closed for almost 50 years, the mine is now a State Park managed by the DNR. Visitors can tour the mine, learning about the history of mining at Soudan and can also tour the state-of-the-art physics laboratory built at the bottom of the mine. Just a few hundred feet away from the physics laboratory, bubbling up from holes drilled in the last days of iron mining, is strange water – an incredibly salty brine that lacks any oxygen gas – and strange microorganisms (bacteria and other single-celled microbes) living in the water. This project has three broad goals: First, we will study the microbiology, chemistry and mineralogy of these brine ecosystems in the Soudan Iron Mine, which has never been thoroughly characterized scientifically. Second, we will explore potentially beneficial applications of organisms isolated from this extreme environment to identify novel antimicrobial and anticancer compounds and to test microbial isolates for potential bioenergy and bioremediation activities. The final goal of the

project will be to synthesize what we have learned about the brine environment into an interactive display for the park's Visitor Center and to help train park staff to discuss these environments with mine tourists and visitors. We will also use information gathered and expertise of the park staff to develop guidelines for protecting and preserving this unique environment.

III. PROGRESS SUMMARY AS OF:

IV. OUTLINE OF PROJECT RESULTS:

RESULT/ACTIVITY 1: Basic Science – Microbiology, Mineralogy and Geochemistry

Description: Result 1 will establish fundamental parameters for understanding the level 27 brine ecosystems. The microbiology section will focus on identifying and categorizing the microbial populations found in the mine. The mineralogy portion will focus on synchrotron-enabled analysis of both structure and composition of minerals found in the Level 27 brine and on samples from iron formations. The geochemistry analysis will focus on chemical and isotopic characterization of the Level 27 brine, in addition to heavy metal analysis on Level 10 and elsewhere in the mine as directed by mine staff.

Summary Budget Information for Result/Activity 1: ENRTF Budget: \$ 366,860
 Amount Spent: \$ 0
 Balance: \$ 366,860

Deliverable/Outcome	Completion Date	Budget
1. Molecular phylogentic analysis and isolation / characterization of microbes from the Soudan Iron Mine and publication of this work.	June 2013	\$157,750
2. Mineralogical, speciation and elemental analyses	June 2013	\$165,370
3. Isotope analysis, gas composition, geochemistry	June 2013	\$43,740

Result Completion Date:

Result Status as of (January 2011):

Result Status as of (July 2011):

Result Status as of (January 2012):

Result Status as of (July 2012):

Result Status as of (January 2013):

Final Report Summary:

RESULT/ACTIVITY 2: Innovative Applications – Novel Compound Discovery, Bioenergy and Bioremediation.

Description: In this section we will determine the feasibility of utilizing microorganisms isolated from the Soudan Iron Mine in three specific commercial areas. Our priority here is to identify potential commercial assets deriving from microbes cultivated from the Soudan Mine.

Summary Budget Information for Result/Activity 2: ENRTF Budget: \$ 168,140
 Amount Spent: \$ 0
 Balance: \$ 168,140

Deliverable/Outcome	Completion Date	Budget
1. Screen novel actinobacterial isolates for production of anticancer and antimicrobial drugs	June 2013	\$83,250
2. Test pure cultures and enrichments for the use of iron oxidizing bacteria in microbial fuel cells	June 2013	\$42,445
3. Isolate and characterize bacteria that promote oxidation / retention of copper and cobalt	June 2013	\$42,445

Result Completion Date:

Result Status as of *(January 2011):*

Result Status as of *(July 2011):*

Result Status as of *(January 2012):*

Result Status as of *(July 2012):*

Result Status as of *(January 2013):*

Final Report Summary:

RESULT/ACTIVITY 3: Public Outreach and Education

Description: We will collaborate to develop training for DNR tour guides to describe the features, microorganisms, biogenic mineralogy and geochemistry within the mine and collaborate with staff to generate educational displays and to develop best practices for protecting this unique environment.

Summary Budget Information for Result/Activity 3: ENRTF Budget: \$ 10,000
 Amount Spent: \$ 0
 Balance: \$ 10,000

Deliverable/Outcome	Completion Date	Budget
1. DNR microbiology training module	June 2013	\$ 500

2. Best practices assessment for level 27 ecosystems	June 2013	\$ 500
3. Visitor Center interactive display	June 2013	\$ 9,000

Result Completion Date:

Result Status as of (January 2011):

Result Status as of (July 2011):

Result Status as of (January 2012):

Result Status as of (July 2012):

Result Status as of (January 2013):

Final Report Summary:

V. TOTAL ENRTF PROJECT BUDGET:

Personnel: \$ 400,856

- \$0 – Jeffrey Gralnick (PI) 5% effort – Result 1.1, 2.2, 2.3, 3
- \$0 – E. Calvin Alexander (Co-PI) – Result 1.3, 3
- \$0 – Christine Salomon (Co-PI) – Result 2.1, 3
- \$35,280 - Brandy Toner (Co-PI) 8% effort – Result 1.1, 1.2, 3
- \$13,740 - Scott Alexander (Scientist) 8% effort – Result 1.3
- \$68,250 - Post-doc (Center for Drug Design) 50% effort – Result 2.1
- \$102,090 - Grad RA (Soil, Water, Climate) 50% effort – Result 1.2
- \$111,606 - Grad RA (Microbiology) 50% effort - Result 1.1
- \$69,890 - Grad RA (Microbial Engineering) 50% effort - Result 2.2, 2.3

Equipment/Tools/Supplies: \$ 71,344

- \$26,344 – Supplies for Microbiology PhD Student Molecular biology reagents required for this project (PCR reagents, DNA extraction kits, plasmid purification kits (\$250 ea, ~12/year), enzymes, chemicals, microbiology consumables (agar, media), general lab supplies (tubes, tips, gloves etc.), cultivation supplies, sterile sampling supplies.
- \$15,000 – Supplies for Soil, Water, Climate PhD Student supplies and consumables required for this project (chemicals / reagents, sample storage, sample preparation, general lab supplies - tips, tubes, pipettes, etc).
- \$15,000 – Supplies for Post-doc (Center for Drug Design) chemicals and glassware for culturing microbes, DNA isolation and sequencing for strain identification, solvents for compound isolation, HPLC and MS time for compound identification and structural characterization, laboratory consumables.
- \$15,000 – Supplies for Microbial Engineering MS Student Bioremediation experiments (heavy metal quantitation, pure and mixed culture screening for bioreduction, characterization of

strains, laboratory consumables) and bioenergy experiments (electrode maintenance, new reactor design for Fe oxidizers, media preparation) and laboratory consumables for MS student.

Travel: \$ 16,500

- \$6,000 - In-state travel to and from the Twin Cities to Soudan Underground Mine State Park. \$220 lodging per trip (2 hotel rooms) and \$180 for food and gas for a total of \$400 per trip (\$2000 total per year).
- \$10,500 - Toner and the SWC graduate student will attend beamtime (Argonne National Lab) to conduct mineralogical analyses three times yearly. Per year, we plan for two long trips (5 days of instrument time and 7 days of total travel) plus one shorter trip for micro-probe instrument time (2 days of instrument time and 3 days of total travel). This research requires travel by car from St. Paul to Argonne, IL (near Chicago, IL; car rental Enterprise \$405 × 3; gasoline \$127 × 3), and lodging at the Argonne Guest House (six nights per long trip, 3 nights per short trip \$63.60/night/person). Total: \$3500 per budget year. **Note: Minnesota does not have a facility like Advanced Photon Source at Argonne, which is unique in the United States. Work at the APS at Argonne is user-based (as it is a DOE National Lab), therefore travel to this facility is essential for the proposed work.**

Additional Budget Items: \$ 56,300

- \$1,500 - Printing (publication fees to publish scientific research)
- \$6,000 - Soudan Mine Usage: Sampling trips (5 per year) will be scheduled in advance with the Park Manager during scheduled operation hours and during regularly scheduled shift schedules. Hoist trip charges are \$30.74 per one way trip. A typical sampling trip will require 4 hoist operations (\$122.96). An 8 hour sampling trip will require 8 hours of personnel time (Mine Hoist & Maintenance Lead at \$33.36 per hour) for a total of \$266.88. A single 8 hour sampling trip is estimated to cost \$389.84 (approx \$400 per trip or \$2000 per year).
- \$2,500 – Microscopy - Scanning electron microscopy (\$37/hour) Light microscopy (\$48/hour) - User fees at CBS Biological Imaging Facility, St. Paul - estimate 9-10 hours SEM and 9-10 hours Light per year.
- \$6,300 – Sequencing - for phylogenetic analysis of microbial communities - bacteria, fungi and archaea (AGAC Sequencing facility on UM campus \$3.50 / reaction, estimate 600 reactions / year over 3 years)
- \$30,000 – Chemical, isotopic and gas analysis - ICP/OES Measurements 135 @ \$20 (\$2700 total), Field Measurements 135 @ \$12 (\$2100 total), IC Measurements 135 @ \$20 (\$2700 total), ICP/MS Measurements 135 @ \$20 (\$2700 total), D and 18O isotope Measurements 105 @ \$20 (\$2100 total), C, S and Sr isotope Measurements 72 @ \$100 (\$7200 total), Radiometric Dating Measurements 75 @ \$100 (\$7500 total). Data logger (\$1500) and Sensors (\$1500) will also be purchased.
- \$10,000 - Outreach development (display, content development, education, implementation, content updates)

TOTAL ENRTF PROJECT BUDGET: \$545,000

Explanation of Capital Expenditures Greater Than \$3,500: None

VI. PROJECT STRATEGY:

A. Project Partners:

James Essig (DNR, Park Manager – Soudan Underground Mine State Park) will help coordinate research trips to the mine, outreach activities on site, training modules for park staff and future commercialization possibilities.

Dr. Daniel Bond (University of Minnesota) is an Assistant Professor of Microbiology and member of the BioTechnology Institute. Dr. Bond is an expert in microbial fuel cell technology and metal reduction and will co-advise the student working on the bioenergy portion of the project in years 2 and 3.

B. Project Impact and Long-term Strategy:

This project seeks to explore a novel resource of Minnesota: the extreme microbes found at the bottom of the Soudan Iron Mine. Deep terrestrial brine environments have not been studied extensively anywhere and this particular brine environment might be unique on our planet, because it is located within an ancient iron deposit. The Soudan Underground State Park is uniquely equipped to facilitate studies of this environment, where experiments and samples can be processed in the physics laboratory located just minutes away from the sample sites. We will explore potential applications of microorganisms found in the mine for applications in bioenergy, bioremediation and production of novel antimicrobial and anticancer compounds. Because environments such as this have never been sampled for such applications, we believe the chances of success are high, positioning the University of Minnesota and the DNR for potential revenue-generating discoveries.

The basic science portion of this project will be used to generate a fundamental understanding of the microbiology, geochemistry and mineralogy of these brine ecosystems. This information will be critical to determine how best to protect and preserve these unique ecosystems, while still accessing them scientifically and displaying them to the public tours. It is our long-term goal to develop this site for additional research and outreach with funding from the National Science Foundation and NASA.

C. Other Funds Proposed to be Spent during the Project Period:

University of Minnesota is contributing the salaries of Profs. Gralnick, Alexander and Salomon towards this project (estimated \$45,000). In kind contribution of Soudan Underground State Park staff time towards protection planning, display development and potential commercial application development (estimated \$1000).

D. Spending History:

Preliminary studies have been conducted five different times during the past two years on site through the donation of staff time and hoist operation by the Soudan Underground State Park (estimated ~ \$2,000). The University of Minnesota has contributed to preliminary studies by funding undergraduate summer students working in the labs of Profs. Gralnick and Alexander (estimated ~ \$10,000). Profs. Gralnick, Salomon and Toner have used startup funds provided by the University of Minnesota to fund initial microbiological, mineralogical and novel compound studies (estimated ~ \$20,000)

VII. DISSEMINATION:

- Publications to primary scientific journals will be submitted covering all aspects of this proposal. Strains of interest will be made available through the American Type Culture Collection (ATCC, with appropriate usage restrictions agreed to by the University of Minnesota, LCCMR and the DNR).

- Intellectual Property / Patent Strategies will be coordinated by the University of Minnesota Office of Technology Commercialization, LCCMR and the DNR.

- Results will also be communicated to the general public through the interactive display to be developed as a part of Result 3 in this proposal, serving the public of the State of Minnesota and visitors to the Soudan Underground Mine State Park.

VIII. REPORTING REQUIREMENTS: Periodic work program progress reports will be submitted not later than Jan / July 2011, Jan / July 2012 and Jan 2013. A final work program report and associated products will be submitted between June 30 and August 1, 2011 as requested by the LCCMR.

IX. RESEARCH PROJECTS:

Attachment A: Budget Detail for 2010 Projects - Budget per year by R/Cumulative Budget July 1, 2010-June 30, 2013												
Project Title: Science and Innovation from the Soudan Underground Mine State Park #216-G												
Project Manager Name: Jeffrey Gralnick												
Trust Fund Appropriation: \$545,000												
2010 Trust Fund Budget	Result 1 Budget:	Amount Spent (date)	Balance (date)	Result 2 Budget:	Amount Spent (date)	Balance (date)	Result 3 Budget:	Amount Spent (date)	Balance (date)	TOTAL BUDGET	TOTAL REMAINING	
	Basic Science - Microbiology, Mineralogy and Geochemistry			Innovative Applications - Drug Discovery, Bioenergy and Bioremediation			Public Outreach and Education					
BUDGET ITEM												
PERSONNEL: Wages												
Jeffrey Gralnick PI 5% effort no salary requested												
	Brandy Toner Co-PI 8% effort	26,667	0	26,667						26,667	26,667	
	Scott Alexander Scientist 8% effort	10,353	0	10,353						10,353	10,353	
	TBN Post-doc Assoc (Center for Drug Design) 50% effort				57,000	0	57,000			57,000	57,000	
	TBN Grad RA (Soil,Water,Climate) 50% effort	57,690	0	57,690						57,690	57,690	
	TBN Grad RA (Microbiology) 50% effort	73,506	0	73,506						73,506	73,506	
	TBN Grad RA (Microbial Engineering) 50% effort				40,000	0	40,000			40,000	40,000	
PERSONNEL: Fringe Benefits												
	Brandy Toner Co-PI 32.3% fringe (\$3546 retirement & \$2832 health insurance)	8,613	0	8,613						8,613	8,613	
	Scott Alexander Scientist 32.7% fringe (\$516 retirement & \$1689 health insurance)	3,387	0	3,387						3,387	3,387	
	TBN Post-doc Assoc (Center for Drug Design) 19.75% fringe (\$0 retirement & \$6054 health insurance)				11,250	0	11,250			11,250	11,250	
	TBN Grad RA (Soil,Water,Climate) 77% fringe (\$0 retirement & \$9714 health insurance)	44,400	0	44,400						44,400	44,400	
	TBN Grad RA (Microbiology) 64.5% fringe (\$0 retirement & \$12378 health insurance)	38,100	0	38,100						38,100	38,100	
	TBN Grad RA (Microbial Engineering) 74.7% fringe (\$0 retirement & \$6736 health insurance)				29,890	0	29,890			29,890	29,890	
Supplies												
	Molecular biology reagents required for this project (PCR reagents, DNA extraction kits, plasmid purification kits (\$250 ea., ~12/year), enzymes, chemicals, microbiology consumables (agar, media), general lab supplies (tubes, tips, gloves etc.), cultivation supplies, sterile sampling supplies - Micro PhD Student - Years 1-3	26,344	0	26,344						26,344	26,344	
	Laboratory supplies and consumables required for this project (chemicals / reagents, sample storage, sample preparation, general lab supplies - tips, tubes, pipets, etc) - SWC PhD Student - Years 1-3	15,000	0	15,000						15,000	15,000	
	Lab supplies for Postdoc (Center for Drug Design) - specifically required for this project: chemicals and glassware for culturing microbes, DNA isolation and sequencing for strain identification, solvents for compound isolation, HPLC and MS time for compound identification and structural characterization, laboratory consumables for Postdoc (50% time) - Years 1-3				15,000	0	15,000			15,000	15,000	
	Bioremediation experiments - Heavy metal quantitation, pure and mixed culture screening for bioreduction, characterization of strains, laboratory consumables.				15,000	0	15,000			15,000	15,000	
	Bioenergy experiments - electrode maintenance new reactor design for Fe oxidizers, media preparation, laboratory consumables for MS student - Years 2-3											
Other Direct Costs												
	Microscopy - Scanning electron microscopy (\$37/hour) Light microscopy (\$48/hour) - User fees at CBS Biological Imaging Facility, St. Paul - estimate 9-10 hours SEM and 9-10 hours Light per year.	2,500	0	2,500						2,500	2,500	
	Sequencing for phylogenetic analysis of microbial communities - bacteria, fungi and archaea (AGAC Sequencing facility on UM campus \$3.50 / reaction, estimate 600 reactions / year over 3 years)	6,300	0	6,300						6,300	6,300	
	Chemical, isotopic and gas analysis - ICP/OES Measurements 135 @ \$20 (\$2700 total), Field Measurements 135 @ \$12 (\$2100 total), IC Measurements 135 @ \$20 (\$2700 total), ICP/MS Measurements 135 @ \$20 (\$2700 total), D and 18O isotope Measurements 105 @ \$20 (\$2100 total), C, S and Sr isotope Measurements 72 @ \$100 (\$7200 total), Radiometric Dating Measurements 75 @ \$100 (\$7500 total). Data logger (\$1500) and Sensors (\$1500) will also be purchased.	30,000	0	30,000						30,000	30,000	
	Soudan Mine Usage: Sampling trips (5 per year) will be scheduled in advance with the Park Manager during scheduled operation hours and during regularly scheduled shift schedules. Hoist trip charges are \$30.74 per one way trip. A typical sampling trip will require 4 hoist operations (\$122.96). An 8 hour sampling trip will require 8 hours of personnel time (Mine Hoist & Maintenance Lead at \$33.36 per hour) for a total of \$266.88. A single 8 hour sampling trip is estimated to cost \$389.84 (approx \$400 per trip or	6,000	0	6,000						6,000	6,000	
Printing												
	Publication fees (~ 3 total, \$500/publication - page charges required to make scientific discoveries available to other scientists and the public)	1,500	0	1,500						1,500	1,500	
Travel expenses in Minnesota												
	Travel: In-state travel to and from the Twin Cities to Soudan Underground Mine State Park. \$220 lodging per trip (2 hotel rooms) and \$180 for food and gas for a total of \$400 per trip (\$2000 total per year).	6,000	0	6,000						6,000	6,000	
Travel outside Minnesota												
	Travel: Toner and the SWC graduate student will attend beamtime (Argonne National Lab) to conduct mineralogical analyses three times yearly. Per year, we plan for two long trips (5 days of instrument time and 7 days of total travel) plus one shorter trip for micro-probe instrument time (2 days of instrument time and 3 days of total travel). This research requires travel by car from St. Paul to Argonne, IL (near Chicago, IL, car rental Enterprise \$405 x 3; gasoline \$127 x 3), and lodging at the Argonne Guest House (six nights per long trip, 3 nights per short trip \$63.60/night/person). Total: \$3500 per budget year. **Note: Minnesota does not have a facility like Advanced Photon Source at Argonne, which is unique in the United States. Work at the APS at Argonne is user-based (as it is a DOE National Lab), therefore travel to this facility is essential for the proposed work.**	10,500	0	10,500						10,500	10,500	
Other												
	Outreach development (display, content development, education, implementation, content updates)						10,000	0	10,000	10,000	10,000	
COLUMN TOTAL		\$366,860	\$0	\$366,860	\$168,140	\$0	\$168,140	\$10,000	\$0	\$10,000	\$545,000	\$545,000



