Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2016 Work Plan

Date of Report: January 19, 2016

Date of Next Status Update Report: January 1, 2017

Date of Work Plan Approval:

Project Completion Date: June 30, 2019

Does this submission include an amendment request? No

PROJECT TITLE: Reducing salt and metal removal costs with microbes

Project Manager: Daniel R. Bond

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Location: Statewide

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$596,000**
	Amount Spent:	**$0**
	Balance:	**$596,000**

Legal Citation: M.L. 2016, Chp. xx, Sec. xx, Subd. xx

Appropriation Language: $596,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to continue to research the potential of recently discovered microbes from Soudan Iron Mine in northern Minnesota for removal of salts and metals from ground and surface water resources. This appropriation is subject to Minnesota Statutes, section 116P.10. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Reducing salt and metal removal costs with microbes

II. PROJECT STATEMENT:

The removal of metals and salts from water remains a serious and costly issue affecting industries as diverse as mining, oil/gas recovery, and production of domestic drinking water. **Our research aims to enable new technologies that can use microorganisms for desalination and metal recovery, with a focus on passive or self-powered processes.** The Soudan Iron Mine contains exploratory boreholes where water saltier than seawater and high in heavy metals contains unique organisms able to thrive under extreme conditions. Our previous research revealed that some of these microbes can generate electricity, while others can remove metals from contaminated waters though direct precipitation and sorption. These discoveries could power many biological water remediation technologies. The first, termed 'microbial desalination cells', harnesses bacteria to create a 'push' of charged electrons to help 'pull' charged salts across membranes. Conditions in these devices can also aid collection and recovery of heavy metals such as Cu and Co by binding to electrodes. The second technology, 'metal removal reactors', relies upon fungi thriving in areas heavily contaminated with metals such as copper, cobalt, iron and manganese, that precipitate metal-containing solids and create high surface-area sorption sites. If maintained properly, bioreactors containing these fungi can efficiently attenuate the metal contaminants within the system, effectively removing them from water. Together, we propose these biological agents can drive bacterial-powered salt removal reactors that discharge into fungal bio-filters to treat contaminated effluents in areas of high salt or metal contamination. **These microorganisms will be able to address applications specific to the chemistry of Minnesota, where sulfates, chlorides, metals and other salts can be present in stormwater and industrial effluents.**

Our overall goal is to deliver microbes from extreme environments that are highly salt- and metal-resistant, yet still able to catalyze electrical reactions. The molecular basis for their high performance will be investigated, so we can track these organisms in functioning reactors and discover genetic traits that make an inoculum useful throughout the industry. These bacteria will be used to power model microbial desalination reactors under harsh conditions to reduce the overall cost of salt removal from waters. This project will also show how novel fungi discovered in the most contaminated areas of mines can remove metals that would normally clog desalination membranes and create environmental toxicity issues

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of: January 1, 2017
Project Status as of: July 1, 2017
Project Status as of: January 1, 2018
Project Status as of: July 1, 2018
Project Status as of: January 1, 2019

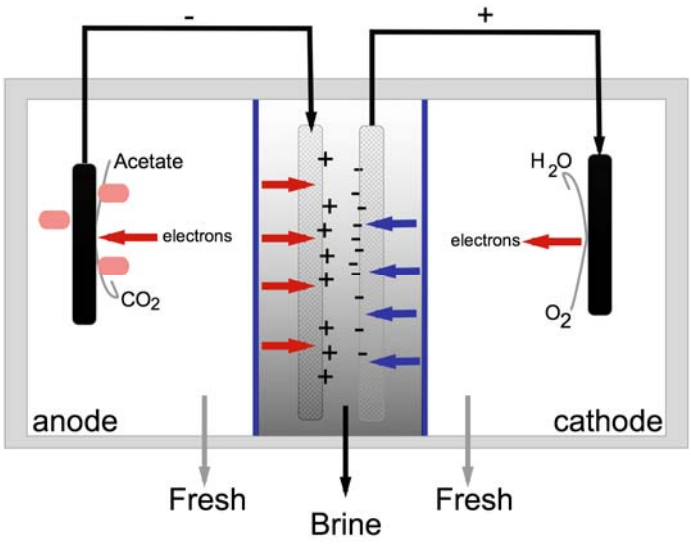
Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1:

Background: Microbe-powered desalination. Salt ions, from the sodium chloride in road salts to sulfates released from rocks and ores, can be the most difficult contaminants to remove from water. Energy must be invested to push salts across membranes in reverse osmosis desalination plants. Our team recently discovered microbes that have two unique skills: an ability to generate biological electricity, and the capacity to grow in extremely harsh environments. We will harness this rare combination in a new class of salt-removing reactors.

The 'microbial desalination cell' contains electrodes colonized by bacteria, who generate electricity inside the device by breaking down waste organic matter at the anode. The electrical flow created by bacteria helps drive salts across membranes, leaving a concentrated brine for collection or sale. Bacteria can reduce the power needed for this kind of water purification by 50%. With over 20,000 large-scale plants worldwide, and millions of on-site salt treatment plants operating at natural gas hydraulic fracturing and industrial sites, even minor improvements to desalination technology will have a significant impact on energy usage and water recovery.



Principle of a Microbial Capacitive Desalination Cell: Electrons produced by bacteria oxidizing organic matter drives formation of a negative charge on one electrode in the central chamber, while exposure to oxygen creates a positive charge on a second. Cations and anions bind in response, and electrode potentials are reversed for discharge into an increasingly saline brine. In each cycle, the water circulating in electrode chambers becomes progressively less saline and lower in waste organic matter, and the waste brine increases in salinity. The cycling creates dramatic changes in electrode voltage that have never been investigated for their effect on the bacteria driving this process.

While Microbial Desalination has been demonstrated in preliminary reactors using bacteria obtained from freshwater habitats, there have been no attempts to enrich or evolve electricity-producing communities able to tolerate extreme conditions. Our experiments will obtain bacteria from naturally saline and metal-contaminated sites, track the bacteria in these enrichments to identify strains consistently powering our devices, and sequence their genomes to determine genetic elements common to these strains.

This Activity is divided into three subprojects:

1. We will perform direct comparisons of naturally obtained communities to mixtures of pure cultures, and test the ability of recently isolated cultures to invade established biofilms. Initial experiments demonstrating the value of our enrichment (measured by increases in current), and identification of strains from our culture collection that could be included in a final inoculum will be complete within one year. Sequencing of communities, enrichment of new organisms for possible isolation, comparisons with strain mixtures, and cathodic experiments are projected to be completed in years 2-3. An inoculum comprised of enriched, evolved, and/or isolated organisms for use in model microbial capacitive desalination cells will be assembled by year 3.

2. With the identification of bacteria and growth conditions that are able to provide higher current densities during voltage cycles and in the presence of harsh conditions, we will use genetic analysis to identify the basis for these adaptations. This will enable quick detection of new strains from other environments, and monitoring of community performance in future applications. We will aim to identify biological rate-limiting steps to reveal how future reactors can be constructed to harness the biological agents central to microbial desalination. Screening for genetic targets in each system will begin by year 2. For this objective, genes identified in these initial experiments will be deleted and verified to be involved in survival in desalination reactors by year 3.

3. For our final objective, we will construct model microbial capacitive desalination cells and inoculate them with our communities, for comparison with approaches practiced today which use organisms from freshwater or domestic sources. For these experiments, our primary objective will be reductions in start-up time, resistance to salt and metal shocks, and increases in current density compared to published data.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 376,000
Amount Spent: $ 0
Balance: $ 376,000

Outcome	Completion Date
1. Develop enrichment strategies able to identify robust salt- metal- or voltage-adapted strains capable of powering microbial desalination cells under harsh conditions.	July 1, 2017
2. Identify genetic basis for high performance under desalination conditions in key strains, complete two full genetic screens by year 2.	July 1, 2018
3. Treat real-world salt- or metal-contaminated effluents using microbial desalination cells and our improved inoculum. Operate at least 3 desalination cells.	July 1, 2019

Project Status as of: January 1, 2017
Project Status as of: July 1, 2017
Project Status as of: January 1, 2018
Project Status as of: July 1, 2018
Project Status as of: January 1, 2019

Final Activity Summary:

ACTIVITY 2:

Description: Fungi-mediated metal removal. Metals, from iron to copper, present special toxicity and solubility issues, and can clog devices designed for water purification. A number of different technologies currently exist for removing metals from water, but many of these technologies are expensive to operate (particularly for waters requiring long-term treatment) or are only partially effective and require other upstream treatments. Microbial metal-removal bioreactors are actively being developed to reduce treatment costs and provide alternative

mechanisms for remediation of complex waters. We will develop the strategies for fungi-mediated metal removal of a variety of metals of interest.

Microorganisms can contribute to metal removal by promoting two different processes. The first process is through adsorption of metal to the cell biomass. Essentially, microorganisms can act as natural sponges, sorbing (i.e., removing) metals from the water. Microbial biomass produces surfaces on which these metal sorption processes occur. If the organisms continue to grow in the environment, they represent new surfaces for the metals to sorb. This continual regeneration of sorptive materials will likely drive down maintenance costs of any metal-removal technology.

In addition to metal sorption, some microorganisms can further accelerate removal of metals from water by promoting the formation of metal-containing minerals that are easily removed from water and are less hazardous to human and environmental health. This is a process known as biomineralization, in which an organism transforms a dissolved chemical compound into a solid mineral phase. Some microbially produced “biominerals” also possess sponge-like qualities and can sorb metals from water, thus representing a dual strategy for metal removal.

In our previous work at the Soudan Underground mine, we found microorganisms such as bacteria and fungi thriving in areas heavily contaminated with a variety of metals including copper, cobalt, nickel, iron and manganese. Furthermore, we have identified a diversity of fungal species, including many novel species, that are highly effective in adsorbing certain metals in complex waters (e.g., high salinity and high metals). Because of these observations, we have targeted the fungal microbial community for our metal removal strategy in this project. We will examine how these two naturally occurring processes (metal sorption and mineral formation) can be harnessed and applied to make efficient and cost-effective fungal bioreactors designed to treat metal-rich waters. This passive ‘upstream’ technology could also greatly improve the operation of 'downstream' membrane-based microbial salt removal technologies described in Activity 1.

This Activity is divided into three subprojects:

1. Our earlier work identified a number of organisms that effectively sorb certain metals, thus the first objective of the current project is to identify organisms that promote biomineral formation of manganese (Mn) as a strategy for increasing metal removal from contaminated waters. We will screen the nearly 100 fungal cultures we have from previous work to see which species are capable of Mn oxide biomineralization. We will also return to the Soudan Mine to try to identify and culture additional fungal species missed by the previous culture enrichment techniques. The field work and fungal screening will be started in the summer of 2016 and will be completed by the end of year 1.

2. The second objective is to determine if a single process (sorption or biomineralization) or both processes together is more effective in fungi-promoted metal removal from complex, metal-rich waters. We will compare the growth and metal removal capacity of several different fungal species for different metal contaminants (e.g., Mn and Ni). The culture(s) and processes deemed highly effective (those that reduce at least 50% of the initial dissolved metal concentrations) will be used for further bioreactor development. We will be conducting these experiments throughout the first and second years of the project, and will have all culture experiments completed by the end of year 2.

3. The last objective of Activity 2 is to build and develop prototype laboratory bioreactors to optimize metal removal from contaminated waters. We will build laboratory-scale reactors to simulate the physical and chemical conditions in an industrial metal bioremediation system. We will ensure that conditions remain constant and aerobic throughout the bioreactors. We will seed these bioreactors with fungal cultures and an inert, solid growth substrate in order to retain these cultures within the reactors. These bioreactors can be run independently and in conjunction with downstream salt removal technologies from Activity 1. We will start construction on the reactors in year 2 and optimization will be completed by the end of year 3.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 220,000
Amount Spent: $ 0
Balance: $ 220,000

Outcome	Completion Date
1. Identify fungal species from the Soudan Underground Mine that can promote formation of solid Mn oxide biominerals from metal-rich waters	July 2017
2. Identify the key fungal species and most effective metal removal pathways (sorption and/or biomineral formation) in batch laboratory experiments	July 2018
3. Build and operate laboratory scale reactors to optimize metal removal from complex, metal-rich waters	July 2019

Project Status as of: January 1, 2017
Project Status as of: July 1, 2017
Project Status as of: January 1, 2018
Project Status as of: July 1, 2018
Project Status as of: January 1, 2019

Final Activity Summary:

V. DISSEMINATION:

The primary mode of dissemination will be via peer-reviewed articles and status reports. We will also convene at least one joint meeting with our entire LCCMR project group and potential collaborators/partners from UMN-Duluth, NRRI, DNR and other interested parties who may be able to adopt our technology as we begin construction of pilot-scale reactors.

Description:

Project Status as of: January 1, 2017
Project Status as of: July 1, 2017
Project Status as of: January 1, 2018
Project Status as of: July 1, 2018
Project Status as of: January 1, 2019

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Overview Explanation
Personnel:	$ 430,900	1 postdoc (~$157,000) for 3 years, and 1 graduate student (~$114,000) for 3 years, to lead enrichment, isolation, and analysis of bacteria essential to microbial desalination, along with construction and operation of desalination reactors. 1 graduate student for 2 years ($~84,000) , plus summer salary support for two faculty for 3 years ($70,000) leading isolation and characterization of new fungi able to bind and precipitate metals from water.
Professional/Technical/Service Contracts:	$ 0	
Equipment/Tools/Supplies:	$ 154,600	Parts for construction of new reactors (~$11,100), operation of larger reactors ($28,000) reagents for molecular biology (~$30,200), DNA sequencing and analysis, geochemical analysis such as water and XRD measurements (~$18,200), electrochemistry supplies such as membranes and electrodes ($19,500), laboratory consumables (~$27,200).
Travel Expenses in MN:	$ 6,000	Routine sampling of Soudan and other areas, obtaining water for desalination and metal recovery
Other:	$ 4,500	Peer-reviewed journal costs.
TOTAL ENRTF BUDGET:	**$** 596000	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:
b

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: N/A

B. Other Funds: The University of Minnesota will provide administration, laboratory space, and maintenance at no cost, estimated value below.

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
	$	$	
State			

No indirect cost recovery is being claimed on this proposal. Based on UMN policy of 52% indirect costs on all non-graduate student fringe and non-equipment funds, approximately $273,000 in administrative and overhead support is being provided by the University of Minnesota	$ 273,000	$ 0	Salaries for Bond and Gralnick, University administration and maintenance, etc.
TOTAL OTHER FUNDS:	**$ 273,000**	**$ 0**	

VII. PROJECT STRATEGY:

A. Project Partners:

Team Leader: Dr. Daniel Bond (UMN) is an Associate Professor of Microbiology and the BioTechnology Institute. He performed many of the original experiments discovering microbial electricity production by metal-reducing bacteria and will direct construction of microbial desalination reactors.

Dr. Jeff Gralnick (UMN) Associate Professor in the Department of Microbiology and the BioTechnology Institute is an expert in electron transfer by bacteria, and led the LCCMR project that discovered the bacteria tolerant of extreme conditions used in this proposal, and pioneered the genetic analysis of these organisms using high-throughput methods.

Dr. Brandy Toner (UMN) Associate Professor in the Soil, Water and Climate Department is an expert in geomicrobiology and toxic metals, and responsible for all mineralogical and metal analyses. Dr. Toner conducted the original experiments to document metal binding by Soudan Mine fungi.

Dr. Robert Blanchette (UMN) is an expert in fungal biology, and discovered the fungi able to adsorb metals in our previous LCCMR project.

Dr. Cara Santelli (UMN) is an expert in fungi active in mining and metal-impacted sites, and discovered fungal strains able to directly precipitate metals on their surfaces. She will be responsible for new fungal discovery and reactors operated upstream of microbial desalination cells.

Jim Essig (DNR Park Manager of Soudan Mine State Park) is an additional partner (not funded by ENRTF) include who will help coordinate research activities.

B. Project Impact and Long-term Strategy:

The proposed work is based on a discovery from our current LCCMR program, which first discovered a range of exotic life in the abandoned mine, then explored the unique microbiology of the Soudan Mine in search of new drugs, bacteria, and remediation strategies (LCCMR 2010-2013 and 2013-2016). We are now prepared to demonstrate an application of these newly discovered bacteria as some of these microbes can be used in industrial and environmental settings. This funding is essential to the scale-up and demonstrations that protect the Intellectual Property of the microbial component, while we will obtain federal funding (National Science Foundation, Department of Energy, United States Department of Agriculture) to support future work understanding the biology enabling this technology. We will share our reactors and organisms with collaborators at NRRI and the University of Minnesota-Duluth for implementation studies related to bioremediation.

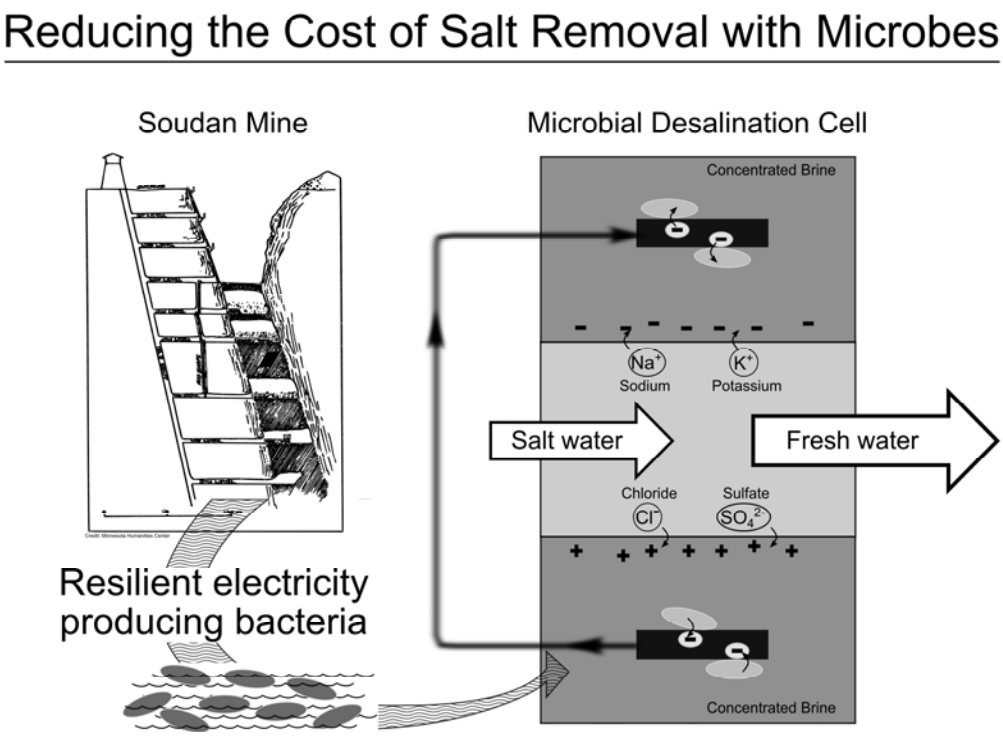
C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
ENRTF, ML-2010-"Science and Innovation from Soudan Underground Mine State Park", The first LCCMR project to	2010-2013	$545,000

support visits to the Soudan Mine with bacterial sampling, led to discovery of bacteria on different levels of Soudan Iron Mine.		
ENRTF ML 2013-03f "Harnessing Soudan Mine Microbes: Bioremediation, Bioenergy, and Biocontrol", This project focused on isolation of bacteria producing antibiotics and agents for control of White Nose syndrome, as well as bacteria that could possibly produce electricity at electrodes. Two strains discovered in 2013-03f will be utilized in the project proposed here.	2013-2016	$838,000

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S):



X. RESEARCH ADDENDUM: Submitted in a separate document.

XI. REPORTING REQUIREMENTS:

Work plan status update reports will be submitted no later than January 1, 2017, July 1, 2017, January 1, 2018, and July 1, 2018. A final report and associated products will be submitted by August 15, 2019.

Environment and Natural Resources Trust Fund
M.L. 2016 Project Budget

Project Title:Reducing salt and metal removal costs with microbes
Legal Citation: *Fill in your project's legal citation from the appropriation language - this will occur after the 2016 legislative session.*
Project Manager: *Daniel R. Bond*
Organization: *University of Minnesota*
M.L. 2016 ENRTF Appropriation: $596,000
Project Length and Completion Date: 3 ***Years, June 30, 2019***
Date of Report: 12/4/2015

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	***Microbe-powered desalination***			***Fungi-mediated metal removal***				
Personnel (Wages and Benefits), Overall Costs:	$270,958	$0	$270,958	$159,942	$0	$159,942	$430,900	$430,900
Postdoc *#1 : Design, build, operate microbial desalanation cells. (83% salary, 17% benefits), 1 FTE/y for 3 years, estimated at $157,109*		$0						
Graduate Student *#1 : Cultivate, test, and provide bacteria capable of electricity production and metal precipitation (81% salary, 19% benefits) 1 FTE/y for 3 years, estimated at $113,849*		$0						
Graduate Student *#2 : Cultivate, test, and provide fungi capable of metal recovery (81% salary, 19% benefits) 1 FTE/y for 2 years, estimated at $84,356*					$0			
Assistant Professor Cara Santell *: mentor undergraduate research and graduate student #2, assist with field work and all activity 2 objectives (1 month summer salary, fringe benefits, 3 years). One month of summer salary is $8,716 in the first academic year with 3% salary increase for the following project years. The fringe benefit for Nine -Month B -term faculty is 33.7% based on the University regulation, estimated at $36,022*					$0			
Associate Professor Brady Toner *: mentor undergraduate research and #2, assist with field work, mentor and assist with geochemistry analyses (1 month summer salary, fringe benefits, 3 years). One month of summer salary is $12,800 (in the first academic year with 3% salary increase for the following project years. The fringe benefit for Nine -Month B -term faculty is 33.7% based on the University regulation, estimated $39,564*					$0			
Equipment/Tools/Supplies: Overall Costs:	$98,400	$0	$98,400	$56,200	$0	$56,200	$154,600	$154,600
Machine shop charges for desalination and metal removal reactors at lab scale, including membranes, ports and gaskets ~$1500/each, estimated total $11,100		$0			$0			
Bioreactors: power supplies, peristalitic pumps, gas controllers, temperature, salinity and pH monitoring sensors to document water quality, estimated total $28,000		$0			$0			
Laboratory consumables; gloves, sterile pipet tips, tubes, syringes, stoppers, glassware for cultivating bacteria in the absence of oxygen, ~$3200/y per active researcher, estimated total $27,200 based on historical average		$0			$0			
Molecular biology reagents: polymerase chain reaction enzymes, restriction enzymes, plasmid and PCR miniprep kits, cloning reagents, sequence verification, DNA synthesis, ~$3800/y per researcher, estimated costs $30,200		$0			$0			
Electrochemical consumables: reference electrodes, wire and electrodes, anaerobic grade gasses and catalysts to remove oxygen, membranes. ~$3500/y per researcher (2 researchers), estimarted costs $19,500		$0						
DNA sequencing (Mayo clinic or UMN sequencing center, $1500/genome or metagenome sample) Imaging of electrodes ($40/h), software licenses ($300/y), estimated costs $20,400		$0			$0			
Geochemical analyses for measuring concentrations of metals in batch experiments and laboratory bioreactors ($32/sample; 500 samples = $16,000); X-ray diffraction mineral analyses $32/hour; 20 hours = $640; columns and detectors for HPLC/GC/IC analysis ($500/y), estimated costs $18,200		$0			$0			
Travel expenses in Minnesota								
Routine sampling and research trips to Soudan Mine (~4x/y), includes one night lodging and vehicle costs according to University of Minnesota reimbursement rates	$4,000	$0	$4,000	$2,000	$0	$2,000	$6,000	$6,000
Other							$0	
Publications and dissemination of results in Open Access (non-restricted) journals	$3,000	$0	$3,000	$1,500	$0	$1,500	$4,500	$4,500
COLUMN TOTAL	**$376,358**	$0	**$376,358**	**$219,642**	$0	**$219,642**	**$596,000**	**$596,000**