



Environment and Natural Resources Trust Fund (ENRTF) M.L. 2013 Work Plan

Date of Status Update Report: 10/02/2012

Date of Next Status Update Report: January 2014

Date of Work Plan Approval: June 11, 2013

Project Completion Date: Dec 30, 2016

Is this an amendment request? N

PROJECT TITLE: Harnessing Soudan Mine Microbes: Bioremediation, Bioenergy, and Biocontrol

Project Manager: Christine E. Salomon

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Location: Ramsey County, St. Paul / Hennepin County, Minneapolis / St. Louis County, Soudan (Breitung Township)

Total ENRTF Project Budget:

ENRTF Appropriation: \$838,000

Amount Spent: \$0

Balance: \$838,000

Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 03f

Appropriation Language:

\$838,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to continue the characterization of unique microbes discovered in the Soudan Underground Mine State Park that have potential applications for metal remediation in water resources, microbial electrofuels, and biocontrol of white-nose bat syndrome. This appropriation is available until June 30, 2016, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Harnessing Soudan Mine Microbes: Bioremediation, Bioenergy, and Biocontrol

II. PROJECT STATEMENT: The Soudan Iron Mine in northern Minnesota is the state's oldest and deepest iron mine. The mine was active from 1882 until 1962 when it was closed and developed into Soudan Mine Underground State Park. Although iron is no longer being extracted, the mine continues to provide valuable resources to the state of Minnesota, including access to fascinating microbial communities that may not exist anywhere else on the planet. The water seeping into the mine from exploratory holes drilled by the miners is highly unusual: It is extremely salty (2-3 times saltier than seawater), high in dissolved iron and other metals, and completely anoxic (without dissolved oxygen gas) until it mixes with the air in the tunnels. The Soudan Iron Mine provides a window into this unique subterranean world and direct access to microbes with special adaptations that can be harnessed for biotechnology.

This proposal builds on the success and findings of our current research program (funded by ENRTF 2010-2013) focused on characterizing microbes that live in this extreme environment and their unique metabolic capabilities. We propose to apply knowledge gained from our initial research project and harness these microbes to approach some of the most critical environmental challenges in Minnesota:

- 1. Removing metals from mine waters with microbes.** The park currently spends upwards of \$200k/year to remove metals from mine effluent. Bacteria and fungi are found thriving in areas of the mine that are heavily contaminated with copper, cobalt and other metals. These microbes are adapted to Soudan conditions, and could be developed for removal of metals from mine waters to meet water quality requirements. We propose to identify efficient metal binding bacteria and fungi with the goal of incorporating them into a bio-filter to treat the contaminated mine water on-site (bioremediation). This technology could be utilized by Soudan Park as well as other mines and contaminated environments.
- 2. Microbes and electrofuels.** Bacteria have the ability to eat and breathe iron. The iron-breathing bacteria can be used to generate electricity, an aspect that both Galnick and Bond have studied for over 10 years. Using electrodes, we can grow both kinds of bacteria, depending on how we poise the electrode (negative for iron breathers and positive for iron eaters). The Soudan Iron Mine is a unique environment that has novel populations of both kinds of bacteria. Iron breathing bacteria can be harnessed to generate electricity, while iron-eating bacteria will help create biofuels in a process called 'electrosynthesis.' We are enriching and culturing novel bacteria from the mine with our current ENRTF. In this new proposal we will test the best bacteria for our desired applications in electricity and electrofuels.
- 3. Inhibition of the White Nose Bat Syndrome fungus (Latin name: *Geomyces destructans*).** Since 2006, the fungal disease White Nose Syndrome (WNS), has decimated bat populations in the Northeastern US, incurring devastating economic and biodiversity losses. Although WNS has not yet reached Minnesota, the Soudan Mine serves as the largest hibernaculum in the upper Midwest and is threatened by the rapid westward spread of this deadly fungal disease (confirmed in Iowa and Ontario, Canada). We have tested microbial strains from the Soudan Mine against a panel of 10 different pathogenic bacteria and fungi, and found that ~40% of isolates inhibit the growth of fungi. We propose to identify strains (existing strain library and new isolates) that could potentially inhibit the WNS fungus. Our approach involves cultivating microbes from bats and bat roosts and testing them for inhibitory activity against at least three species of *Geomyces*. Because these anti-fungal isolates are already adapted to living in the extreme environment of the mine, they may be good candidates for potentially controlling or preventing WNS ("Biocontrol").

Some of the research being done in this project has potential intellectual property value and therefore Intellectual Property / Patent Strategies will be coordinated by the University of Minnesota Office of Technology Commercialization

Please note that, as part of the patent protection process, an Intellectual Property Disclosure will be filed with the Office of Technology Commercialization at the University of Minnesota on the technology proposed to be

developed through this project. As a result, some information pertaining to this project is confidential at this time. This work plan omits confidential information and provides lesser detail than it otherwise might.

III. PROJECT STATUS UPDATES:

Project Status as of (January 2014):

Project Status as of (July 2014):

Project Status as of (Jan 2015):

Project Status as of (July 2015):

Project Status as of (Jan 2016):

Project Status as of (July 2016):

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Removal of metals from mine waters with microbes

Description: The park currently spends approximately \$200k/year to remove metals from mine effluent. Fungi are found thriving in areas of the mine that are heavily contaminated with copper (Cu), cobalt (Co) and other metals (e.g. mercury, Hg). These microbes are adapted to Soudan conditions, and could be developed for removal of metals from mine waters to meet water quality requirements. We propose to identify efficient metal binding fungi with the goal of incorporating them into bio-filters or bioreactors to treat the contaminated mine water on-site (bioremediation). This technology could be utilized by Soudan Park as well as other mines and contaminated environments. *This project will be focused on isolating and characterizing fungi from the most contaminated areas of the mine and testing the best candidates for further development into a biofilter.*

Summary Budget Information for Activity 1:

ENRTF Budget: \$ 313,584
Amount Spent: \$ 0
Balance: \$ 313,584

Activity Completion Date:

Outcome	Completion Date	Budget
1. Complete culturing and characterization of fungi from high copper and cobalt areas of the mine	July 2014	\$104,528
2. Screen up to 20 cultures for metal binding capacity as function of pH	July 2015	\$104,528
3. Describe the metal binding capacity for most promising isolates as function of metal loading, ionic strength, and temperature.	Jan 2016	\$104,528

Activity Status as of (January 2014):

Activity Status as of (July 2014):

Activity Status as of (January 2015):

Activity Status as of (July 2015):

Activity Status as of (Jan 2016):

Activity Status as of (July 2016):

Final Report Summary:

ACTIVITY 2: Microbes and electricity

Description: The Soudan Iron Mine is a unique environment that has novel populations of bacteria that can eat or breathe iron. Iron breathing bacteria can be harnessed to generate electricity, while iron-eating bacteria will help create biofuels in a process called ‘electrosynthesis.’ We have enriched and cultured novel bacteria from the mine with our previously funded ENRTF project. This proposal will explore three key elements that are necessary to realize the potential of electrosynthesis: Test and prove the use of isolated Soudan bacteria interfaced with electrodes, molecular characterization of electron transfer in novel mine bacteria and optimization of electron transfer by bacteria associated with electrodes.

Summary Budget Information for Activity 2:

ENRTF Budget: \$ 317,646
Amount Spent: \$ 0
Balance: \$ 317,646

Activity Completion Date:

Outcome	Completion Date	Budget
1. Test isolated Soudan bacteria interfaced with electrodes: cathode enrichments in the lab and in the mine	July 2014	\$105,882
2. Illumina 16s rRNA sequencing and culturing of microbial communities on cathodes	July 2015	\$105,882
3. Screen microbes for iron oxidizers	July 2016	\$52,941
4. Initial molecular characterization of electron transfer in novel mine bacteria	July, 2016	\$52,941

Activity Status as of (January 2014):

Activity Status as of (July 2014):

Activity Status as of (January 2015):

Activity Status as of (July 2015):

Activity Status as of (Jan 2016):

Activity Status as of (July 2016):

Final Report Summary:

ACTIVITY 3: Biological control of White Nose Bat Syndrome

Description: Our approach for this proposal is focused on identifying inhibitory bacteria that live naturally in the Soudan Mine to utilize as biological control agents. Because these anti-fungal isolates are already adapted to living in the extreme environment of the mine, they may be good candidates for potentially controlling or

preventing WNS (“Biocontrol”). We propose to survey the microbial population (existing strain library and new isolates) for strains that could potentially inhibit the WNS fungus. *Bacteria and fungi will be cultivated from hibernaculum areas and bat surfaces to identify “native” species pre-adapted to the mine environment. Isolates will be tested for the ability to inhibit the growth of multiple species of Geomyces fungi.*

Summary Budget Information for Activity 3:

ENRTF Budget: \$ 206,770
Amount Spent: \$ 0
Balance: \$ 206,770

Activity Completion Date:

Outcome	Completion Date	Budget
1. Sample collections from bat roosts, substrates and bats (near end of hibernation period)	July 2015	\$34,461
2. Isolation and characterization of bacteria and fungi from bats and hibernaculum areas	July 2015	\$34,461
3. Characterization of <i>Geomyces</i> species susceptibility and resistance profiles against clinical antifungal compounds.	July 2014	\$34,461
4. Testing for presence of <i>Geomyces destructans</i> using RT-PCR	July 2016	\$10,000
5. Testing of bacterial and fungal antagonists against library of <i>Geomyces</i> species	July 2016	\$46,694
6. Fermentation and scale up of most promising antagonists.	July 2016	\$46,693

Activity Status as of (January 2014):

Activity Status as of (July 2014):

Activity Status as of (January 2015):

Activity Status as of (July 2015):

Activity Status as of (Jan 2016):

Activity Status as of (July 2016):

Final Report Summary:

V. 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 an interactive display at the Soudan Mine Visitor Center that will be developed as part of our current ENRTF project. We are also pursuing the

development of a satellite kiosk at the Minnesota Science Museum in St. Paul which will enable us to disseminate our research to an even greater audience.

Status as of (January 2014):

Status as of (July 2014):

Status as of (January 2015):

Status as of (July 2015):

Status as of (Jan 2016):

Status as of (July 2016):

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget (3 years total):

Budget category	Amount	Explanation
Personnel:		
Brandy Toner , Activity 1	\$7,916	2% FTE , requesting 1 week of summer salary per year for 3 years (74% salary, 26% fringe)
2 Graduate Research Students (Microbiology) Activity 2	\$257,646	50% FTE, (53% salary, 47% fringe)
1 Graduate Research Student (Soil,Water,Clim) Activity 1	\$116,845	50% FTE, (48% salary, 52% fringe)
1 technician (Plant Path) Activity 3	\$113,388	50% FTE (72% salary, 28% fringe)
1 undergraduate student worker (Plant Path.) Activity 3	\$15,435	25% FTE (100% salary)
1 Postdoctoral Research Associate (CDD) Activity 3 (82% salary, 18% fringe)	\$151,200	100% FTE (82% salary, 18% fringe)
Equipment/Tools/Supplies:		
Activity 1		
Microbiology supplies	\$24,000	Sterile sampling equipment, collection materials, growth media, culture reagents, sample tubes, pipette tips, plasticware, consumables) for two scientists x 3 years.
General lab supplies	\$24,000	glassware, vials, bottles, gloves, filters, filter cartridges for purified water (\$1000 per year), pH electrodes for field and lab measurements (4 x \$300, oxygen electrodes (2 x \$200), chemical reagents and buffers. For two scientists x 3 years.
Analytical chemistry analysis expenses & services	\$12,000	UMN Geochemistry core facility charges (\$50-75 per sample for

		metal analysis for ~85 samples over 3 years)
Activity 2		
Microbiology supplies	\$20,000	Sterile sampling equipment, collection materials, growth media, culture reagents, sample tubes, pipette tips, plasticware, consumables) for two scientists x 3 years.
Molecular biology supplies	\$20,000	PCR reagents, enzymes, DNA extraction kits, plasmid and gel purification kits (\$300 ea, ~12/yr), electrophoresis supplies (agarose, ladders, stains). For two scientists x 3 years
General lab supplies	\$20,000	glassware, vials, bottles, gloves, filters, filter cartridges for purified water (\$1000 per year), chemical reagents and buffers, for two scientists x 3 years
Activity 3		
Microbiology supplies for sampling and lab assays	\$12,000	Sterile sampling equipment, collection materials, growth media, culture reagents, sample tubes, pipette tips, plasticware, consumables
Molecular biology supplies	\$8,000	PCR reagents, enzymes, DNA extraction kits, cloning and gel purification kits (\$300 ea, ~6/yr), electrophoresis supplies (agarose, ladders, stains).
General lab supplies	\$10,000	glassware, vials, bottles, gloves, filters, chemical reagents and buffers
Sequencing for phylogenic analysis of microbial isolates	\$6,300	Sequencing fees (UMN core facility sample charge) for phylogenetic analysis of bacteria and fungi (all activities) \$3.50-\$7 per sample x ~500 samples over 3 years
Microscopy	\$2,500	Microscope hourly charges at UMN Imaging Core Facility (scanning electron, light, confocal)(\$37-45 per hour, est 15 hrs/yr x 3 yrs)
Publication fees	\$1,500	Page and color charges for publishing scientific manuscripts
Travel: In-state round trip travel between St. Paul and Soudan Mine Park	\$9,500	Travel, room/board for 4-8 researchers. \$250-500 lodging per

		trip, \$250 for food/gas, for a total of \$500-750 per trip x ~ 5 trips per year.
Soudan mine usage	\$5,770	Hoist trip charges (\$31.74) x 4 per average trip (~\$130). An 8 hour sampling trip will require 8 hours of park staff (Mine Hoist and maintenance personnel at \$35 per hour for a total of \$280) Total charges estimated to be \$1923 per year x 3 years
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND REQUEST =	\$838,000	

Explanation of Use of Classified Staff: n/a

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

Number of Full-time Equivalent (FTE) funded with this ENRTF appropriation: 9.81

Number of Full-time Equivalent (FTE) estimated to be funded through contracts with this ENRTF appropriation: none

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
Non-state			
1 month of salaries + fringe for each investigator (Salomon, Gralnick, Toner, Bond and Blanchette) annually x 3 years	\$161,691	\$0	In-kind support of effort for PIs from each department (Center for Drug Design, Microbiology/Biotechnology Institute, Soil, Water, Climate and Plant Pathology)
State			
	\$	\$	
TOTAL OTHER FUNDS:	\$161,691	\$0	

Add or remove rows as needed

VII. PROJECT STRATEGY:

A. Project Partners: Additional partners include **Jim Essig** (DNR Park Manager of Soudan Mine State Park) who will help coordinate research activities and **Dr. David Blehert** (USGS, WI) who will provide advice and future assistance with testing microbial isolates against the WNS fungus *Geomyces destructans*.

B. Project Impact and Long-term Strategy:

Our long term goal is to develop practical and valuable applications using the unique microbes that we have identified in the Soudan Mine. The metabolic capabilities of these microbes have real potential for applied and novel biotechnologies. The proposed work will contribute essential information towards our understanding of the roles and capacities of fungi in bioremediation, the development of electrofuels, and the potential for

biological control of the fungal pathogen involved in White Nose bat Syndrome. In addition to the expected knowledge and increased understanding in these areas, we also expect to make progress towards tangible products harnessing these biotechnologies such as a bio-filter to remove dissolved metals, electrofuels, and biological control strains of bacteria that inhibit *Geomyces destructans*.

C. Spending History:

Funding Source	M.L. 2007 or FY08	M.L. 2008 or FY09	M.L. 2009 or FY10	M.L. 2010 or FY11	M.L. 2011 or FY12-13
ENRTF				545,451 Subd. 3(f)	

VIII. ACQUISITION/RESTORATION LIST:

IX. MAP(S): graphic attached

X. RESEARCH ADDENDUM: Due to the patent protection being sought for the technology being developed with this project, in consultation with LCCMR staff it was determined that ENRTF research project requirements for a research addendum and peer review could be satisfied through an internal University of Minnesota peer review process that meets standard University of Minnesota protocols for a patent seeking situation. As a result the research addendum produced for the project and potentially revised through the peer review process will remain confidential while patent protection is pending.

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted not later than [January 2014], [July 2014], [January 2015], [July 2015], [Jan 2016] and [July 2016]. A final report and associated products will be submitted between Jan 30 and August 15, 2017 as requested by the LCCMR.

Attachment A: Budget Detail for M.L. 2013 Environment and Natural Resources Trust Fund Projects

Project Title: *Harnessing Soudan Mine Microbes: Bioremediation, Bioenergy and Biocontrol*

Legal Citation: *M.L. 2013, Chp. 52, Sec. 2, Subd. 03f*

Project Manager: *Christine E. Salomon*

M.L. 2013 ENRTF Appropriation: \$ 838,000

Project Length and Completion Date: 3 years (completion and final report Dec 2016)

Date of Update: 12/17/2012

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET (3 years total)	Activity 1 Budget	Amount Spent	Balance	Activity 2 Budget	Amount Spent	Balance	Activity 3 Budget	Amount Spent	Balance	TOTAL BUDGET	TOTAL BALANCE
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BUDGET ITEM	Result 1. Removal of metals			Microbes and electricity			Biological control of White				
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Personnel (Wages and Benefits) overall	253,584	0	253,584	257,646		257,646	151,200	0	151,200	662,430	662,430
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Brandy Toner, Co-PI, 2% effort (74% salary, 26% fringe) (Estimated \$7916)											
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TBN Grad RA (Soil, Water, Climate), 50% effort (48% salary, 52% fringe) (Est. \$116,845)											
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Robert Blanchette, Co-PI, 10% effort, no salary requested											
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TBN technician (Plant Pathology), 50% effort (72% salary, 28% fringe) (est. \$113,388)											
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TBN undergraduate student worker (Plant Pathology), 25% effort (100% salary) (est.\$ 15,435)											
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Jeff Gralnick, Co-PI, 10% effort, no salary requested											
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Daniel Bond, Co-PI, 10% effort, no salary requested											
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(2) TBN Grad RA (Microbiology), 50% effort, (53% salary, 47% fringe) (Est. \$257,646)											
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Christine Salomon, PI, 10% effort, no salary requested											
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TBN, Postdoctoral associate, 100% effort, 82% salary, 18% fringe (Est. \$151,200)											
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Equipment/Tools/Supplies											
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Activity 1: Microbiology supplies: Sterile sampling equipment, collection materials, growth media, culture reagents, sample tubes, pipette tips, plasticware, consumables) For two Grad RA scientists x 3 years	24,000	0	24,000							24,000	24,000
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Activity 1. General lab supplies: glassware, vials, bottles, gloves, filters, filter cartridges for purified water (\$1000 per year), pH electrodes for field and lab measurements (4 x \$300, oxygen electrodes (2 x \$200), chemical reagents and buffers. For two Grad RA scientists x 3 years.	24,000	0	24,000							24,000	24,000
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Activity 1: Analytical chemistry analysis (Geochem core facility fee for service)	12,000	0	12,000							12,000	12,000
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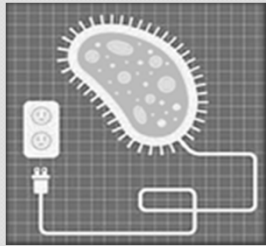
Activity 2: Microbiology supplies: Sterile sampling equipment, collection materials, growth media, culture reagents, sample tubes, pipette tips, plasticware, consumables) for two scientists x 3 years.				20,000	0	20,000				20,000	20,000
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Activity 2. Molecular biology supplies: PCR reagents, enzymes, DNA extraction kits, plasmid and gel purification kits (\$300 ea, ~12/yr), electrophoresis supplies (agarose, ladders, stains). For two scientists x 3 years				20,000	0	20,000				20,000	20,000
Activity 2. General lab supplies: glassware, vials, bottles, gloves, filters, filter cartridges for purified water (\$1000 per year), chemical reagents and buffers, for two scientists x 3 years				20,000	0	20,000				20,000	20,000
Activity 3: Microbiology and assay supplies: Sterile sampling equipment, collection materials, growth media, culture reagents, sample tubes, pipette tips, plasticware, consumables							12,000	0	12,000	12,000	12,000
Activity 3. Molecular biology supplies: PCR reagents, enzymes, DNA extraction kits, cloning and gel purification kits (\$300 ea, ~6/yr), electrophoresis supplies (agarose, ladders, stains).							8,000	0	8,000	8,000	8,000
Activity 3. General lab supplies: glassware, vials, bottles, gloves, filters, chemical reagents and buffers							10,000	0	10,000	10,000	10,000
Other Direct Costs											
Sequencing fees (UMN core facility sample charge) for phylogenetic analysis of bacteria and fungi (all activities) \$3.50-\$7 per sample x ~500 samples over 3 years, all activities							6,300	0	6,300	6,300	6,300
Microscope hourly charges at UMN Imaging Core Facility (scanning electron, light, confocal)(\$37-45 per hour, est 15 hrs/yr x 1.5 yrs) All activities							2,500	0	2,500	2,500	2,500
Printing publication and color page fees to publish work in scientific manuscripts							1,500	0	1,500	1,500	1,500
Travel expenses in Minnesota In-state round trip travel between St. Paul and Soudan Mine Park: room/board for 4-8 researchers, mileage, plus mine hoist charges, est 5-6 trips/yr (1-3 days each trip) for 3 years. Expense base on U of M employee expense reimbursement plan.							9,500	0	9,500	9,500	9,500
Soudan mine usage costs Hoist trip charges (\$31.74) x 4 per average trip (~\$130). An 8 hour sampling trip will require 8 hours of park staff (Mine Hoist and maintenance personnel at \$35 per hour for a total of \$280) Total charges estimated to be \$1923 per year x 3 years							5,770	0	5,770	5,770	5,770
COLUMN TOTAL	\$313,584	\$0	\$313,584	\$317,646	\$0	\$317,646	\$206,770	\$0	\$206,770	\$838,000	\$838,000

copper
mercury
cobalt

Bioremediation:
Toxic Metal Removal

Harnessing Soudan Iron Mine Microbes



Bioenergy:
Microbes and
Electrofuels



STOP

Biocontrol:
Invasive White
Nose Bat fungus

Confirmed White Nose Bat Syndrome (WNS) Areas. WNS was first detected in upstate New York in 2006 and is rapidly spreading westward across North America.

