Environment and Natural Resources Trust Fund
2019 Request for Proposals (RFP)

Project Title: **ENRTF ID: 053-B**

Stimulating Bacteria to Degrade Chlorinated Industrial Contaminants

Category: B. Water Resources

Sub-Category:

Total Project Budget: $ 252,884

Proposed Project Time Period for the Funding Requested: June 30, 2022 (3 yrs)

Summary:

Sites contaminated with chlorinated industrial pollutants are a significant problem in Minnesota. We will determine the best way to stimulate bacteria for faster and more complete pollutant dechlorination.

Name: Paige Novak

Sponsoring Organization: U of MN

Title: Professor

Department: Civil, Environmental, and Geo- Engineering

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Minneapolis MN 55455

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Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

The figure shows how different groups of dechlorinating bacteria work and how we plan to stimulate them.

_____ Funding Priorities _____ Multiple Benefits _____ Outcomes _____ Knowledge Base

_____ Extent of Impact _____ Innovation _____ Scientific/Tech Basis _____ Urgency

_____ Capacity Readiness _____ Leverage _______ TOTAL ______%

____ If under $200,000, waive presentation?

PROJECT TITLE: Stimulating bacteria to degrade chlorinated industrial contaminants

I. PROJECT STATEMENT

The goal of this project is to identify how the organisms that naturally cycle chlorine in uncontaminated Minnesota environments can best be deployed to detoxify chlorinated pollutants.

According to the Minnesota Pollution Control Agency's most recent report, there are:

- **92** contaminated **sites on the Minnesota "Superfund" List**,
- **$13.5M** was spent in FY 2015-16 alone on **clean-up tasks at these Superfund sites**,
- There are **621 additional contaminated sites in Minnesota**.

Of these sites, over half are **contaminated with chlorinated pollutants**, coming from dry cleaners and industrial processes, that are known or suspected to cause **serious human health effects**.

Research is needed to develop ways to affordably clean up chlorinated pollutants, safeguarding current and future human and economic health.

We know:

- **Bacteria can "breathe" toxic chlorinated pollutants** ("halorespirers"), but to survive, they require the presence of chlorinated pollutants.
- **Higher concentrations of chlorinated pollutants typically sustain these organisms more effectively**.
- During remediation **we want to remove chlorinated pollutants to very low concentrations**, which can make it difficult to sustain these "halorespiring" bacteria.
- **Natural chlorinated compounds (<u>not pollutants</u>) also exist in low concentrations** in uncontaminated sites as a natural part of soil.
- We have found that these **natural chlorinated compounds can stimulate pollutant dechlorination** in both "halorespiring" bacteria and other bacteria that use the dechlorinated carbon for growth.
- **We suspect that these other bacteria ("non-respiring dechlorinators") are able to dechlorinate pollutants to lower concentrations.**

In the proposed research we will **verify that "non-respiring dechlorinators" can dechlorinate pollutants to desired low concentrations**. We will determine **the best way to stimulate** both groups of bacteria with natural compounds **for pollutant dechlorination to low concentrations**, saving money and time, and reducing risk.

II. PROJECT ACTIVITIES AND OUTCOMES

We hypothesize that **amendments with different amounts of soil-based carbon versus natural (non-pollutant) chlorinated compounds will stimulate "halorespiring" and "non-respiring dechlorinators" differently**. This can be used to verify that "non-respiring dechlorinators" can dechlorinate pollutants to desired low concentrations and enable the addition of amendments to control the rate and extent of pollutant dechlorination based on the amount of pollutant present.

Activity 1: Determine how different amendments of natural compounds improve dechlorination

<u>The outcome of this activity will be understanding how to add amendments for rapid but complete pollutant degradation</u>. Experiments will be performed with the common pollutants trichloroethene (TCE) and

perchloroethene (PERC) and sediments from contaminated and uncontaminated sites containing different initial amounts of pollutants.

ENRTF BUDGET: $101,154

Outcome	Completion Date
*1. Measure the dechlorination of PCE and TCE in sediments with **high ratios** of soil-based carbon to natural chlorinated compounds when amended with stimulants of varying ratios of soil-based carbon to natural chlorinated compounds*	*6/30/21*
*2. Measure the dechlorination of PCE and TCE in sediments with **low ratios** of soil-based carbon to natural chlorinated compounds when amended with stimulants of varying ratios of soil-based carbon to natural chlorinated compounds*	*6/30/21*

Activity 2: Determine how the different groups of dechlorinating bacteria ("halorespiring" and "non-respiring dechlorinators") are affected by these amendments

The outcome of this activity will be a rapid and simple test to determine the best amendment for stimulating dechlorination at a particular site. Genes are the codes that "tell" organisms which functions to perform (such as breathing chlorinated compounds). By analyzing genes, we can understand which organisms dominate (and by what mechanism) in a given sample. Samples will be taken from the experiments described above and the genetic material will be extracted and analyzed over time. From this we will learn which genes are stimulated by the different amendments, which genes are responsible for different patterns of dechlorination, and which genes are initially present in different types of starting materials. By understanding how to "read" the genes used to dechlorinate pollutants, we will know the best amendment to add to stimulate dechlorination at a site without having to perform labor-intensive and expensive experiments.

ENRTF BUDGET: $151,730

Outcome	Completion Date
1. Analyze the genes in initial starting material for the experiments	*1/31/20*
2. Analyze the types and quantities of genes in the experiments described in Activity 1 over time	*5/1/22*
3. Correlate type and number of genes with dechlorination patterns/rates/extents	*6/20/22*

III. PROJECT PARTNERS:
None.

IV. LONG-TERM- IMPLEMENTATION AND FUNDING:
Minnesota has impressive environmental resources but also a large number of sites that need to be remediated at a large cost. Novak has worked on the organohalide respiration of chlorinated pollutants for about 20 years. She is the first to perform research on the existence of halorespiring bacteria in uncontaminated environments and the first to show that pollutant degradation can be stimulated through the addition of uncontaminated soil extracts to the bacteria present. The goal of this project is to identify how the organisms that naturally cycle chlorine in uncontaminated Minnesota environments can best be deployed to detoxify chlorinated pollutants. This research should enable the development of new remediation technologies that are more effective and less expensive than those currently used, cleaning more sites and improving Minnesota's environment.

V. TIME LINE REQUIREMENTS:
The proposed project will be completed in the allotted three-year period.

2019 Proposal Budget Spreadsheet

Project Title: Stimulating bacteria to degrade chlorinated industrial contaminants

IV. TOTAL ENRTF REQUEST BUDGET: 3 years

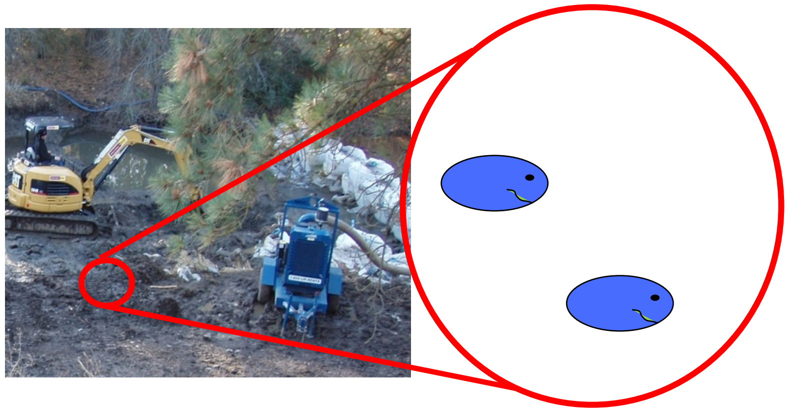
BUDGET ITEM	AMOUNT
Personnel:	$ 205,684
Novak (PI, 4% time per year for three years, salary 75% of cost, fringe benefits 25% of cost). Project supervision and resporting, provide direction to the graduate student. Total estimated cost is $33,161.	
Graduate student (50% time per year for three years, 57% salary, 32% tuition, 11% fringe benefits). Conducting laboratory experiments and analyzing samples for different dechlorinating genes. Total estimated cost is $142,523.	
Undergraduate student (13 weeks (i.e., summer), full time per year for three years). Assisting with routine analyses (chloride, TCE, PCE measurements) and laboratory experiments. Total estimated	
Equipment/Tools/Supplies: Funds for laboratory supplies are requested ($11,000/year). This includes, but is not limited to: DNA soil extraction kits, materials for quantifying genes present, primers for deep genetic sequencing, pipette tips, eppendorf tubes, glassware, chemicals for standards and experiments, analytical consumables, analytical fees, solvents, reagents, and gloves. Funds ($8,000 total) are also requested for sequencing via Illumina sequencing. One sequencing run is approximately $2,000-2,200. It is anticipated that the Illumina sequencing on the samples can be sufficiently multiplexed to perform all the needed sequencing with 4 runs. Additional funds budgeted for equipment repair and maintenance ($6,000).	$ 47,000
Travel: Mileage charges to sites for sample collection. Mileage will be reimbursed $0.55 per mile or current U of M compensation plan.	$ 200
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 252,884

V. OTHER FUNDS *(This entire section must be filled out. Do not delete rows. Indicate "N/A" if row is not applicable.)*

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period:	N/A	
Other State $ To Be Applied To Project During Project Period:	N/A	
In-kind Services To Be Applied To Project During Project Period: Novak will provide unpaid time to the project (including 2% cost-share). Because the project is overhead-free, laboratory space, electricity, and other overhead costs are provided in kind. The University of Minnesota overhead rate is 54%.	$ -	
Past and Current ENRTF Appropriation: No past projects are related to this proposal; the PI, however, has successfully led or contributed to 6 past LCCMR projects.	N/A	
Other Funding History:	N/A	

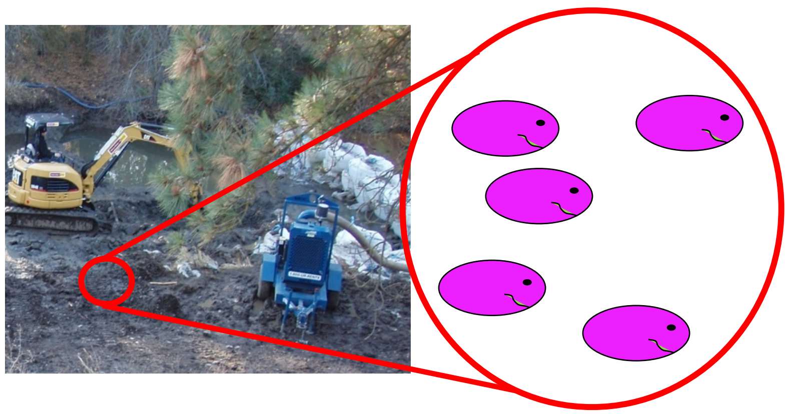
Stimulating bacteria to degrade chlorinated industrial contaminants

The problem



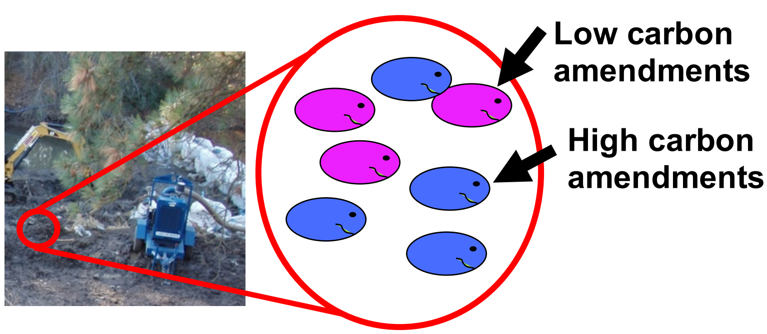
- Bacteria exist that "breathe" chlorinated contaminants (**blue bacteria**)
- Bacteria need higher concentrations of contaminants to thrive
- There is often too little contamination to support the bacteria, but too much to consider the site safe

A potential solution



- Other bacteria exist that dechlorinate compounds but don't "breathe" these compounds (**pink bacteria**)
- They work more slowly, but may degrade the contaminants to lower concentrations, cleaning the site to a greater extent

What we need to know



- How do we best stimulate both types of bacteria for fast and complete dechlorination?
- How can we monitor their progress without expensive and time-consuming experiments?

Project Manager Qualifications and Organization Description

Dr. Paige J. Novak
Professor, Department of Civil, Environmental, and Geo- Engineering and Resident Fellow of the Institute on the Environment, University of Minnesota

B.S., Chemical Engineering, 1992, The University of Virginia, Charlottesville, VA.
M.S., Environmental Engineering, 1994, The University of Iowa, Iowa City, IA.
Ph.D., Environmental Engineering, 1997, The University of Iowa, Iowa City, IA.

Dr. Novak will be responsible for overall project coordination, planning, and reporting. She is an expert in applied environmental microbiology and has been studying biological dechlorination and anaerobic biological processes for over 20 years. She is the first person to study organohalide respirers in uncontaminated ecosystems and to study these organisms in the upper Midwestern United States. She has also developed remediation technologies for the cleanup of chlorinated pollutants and has studied the addition of organohalide respirers to environments to enable the dechlorination of polychlorinated biphenyls, very persistent and now banned, pollutants. She has successfully managed multiple LCCMR projects.

Organization Description
The University of Minnesota is one of the largest, most comprehensive, and most prestigious public universities in the United States (http://www1.umn.edu/twincities/01_about.php). The laboratories and offices of the PI and co-PI contain all of the necessary fixed and moveable equipment and facilities needed for the proposed studies.